

**Theracurmin: An Antioxidant and its Protective Effect on Hippocampal
Neurogenesis**

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Abstract

Alcoholism is responsible for numerous harmful physiological changes to the body and brain. The neuropathological consequences that become apparent are the increases of oxidative burden, release of superoxide radicals, uncontrollable peroxidation leading to defective neurotransmission and cell death. Chronic neuroinflammation results from the damage accrued from the oxidative stress. Neuroinflammatory pathways are mediated by brain cytokines such as TNF- α (tumor necrosis factor- alpha). Dysregulation of cytokines like TNF- α causes a cascade of cellular activity that ultimately leads to increases in reactive oxygen species (ROS). The increase of neuroinflammation and oxidative stress results in harmful neurological damage, such as the impairment of neurogenesis in the dentate gyrus of the hippocampus. There has been evidence that curcumin, one of three curcuminoids found in turmeric root, contains various characteristics, such as anti-inflammatory, immune-regulatory, and antioxidant properties, that make it a promising candidate for therapeutic treatment. Our goal is to determine the efficacy of curcumin in preventing damage to the newborn neurons of the hippocampus caused by chronic ethanol consumption, using a rat model of alcoholism, the Long- Evans Rat.

Background

Alcohol Abuse

Alcohol is one of the most abused substances in the United States. According to the National Institute on Alcoholism Abuse and Alcoholism, about 87% of the population over the age of 18 reported drinking alcohol some time in their life. About 15 million individuals have been diagnosed with Alcohol Use Disorder (AUD), with 9.8 million men and 5.3 million women affected [1]. On a societal level, it costs the government billions of dollars, with 3/4th of the costs being attributed to binge drinking. Behaviorally, it has been linked to loss of productivity at work and in academia, engaging in negative behaviors, and increase in consumption of the toxic substance to achieve the same effect. Physiologically, it reduces white and grey matter throughout the brain, especially in the hippocampus. The hippocampus is important for memory consolidation and spatial cognition, but more importantly, it is one of two areas in the brain where adult neurogenesis, or birth of newborn neurons in the adult brain, occurs. Despite the numerous studies examining the deleterious effects of alcohol drinking on the brain, there are few studies that have assessed a safe, natural compound that can act as a protective agent. **This research experiment utilized a rat model of alcoholism for curcumin to act as a neuroprotective agent for newborn neurons in the hippocampus during chronic ethanol consumption.** If the experiment is successful, then the use of curcumin as a neuroprotective agent for drug addiction may be extended to studies involving other classes of drugs of abuse; furthermore future translational studies may explore the potential benefits of this compound in other species, and eventually, in humans.

Neurocircuitry and Addiction

A percentage of people who routinely consume alcohol will begin exhibiting the classical characteristics of drug addiction: compulsion to take a drug, increases in negative emotional state when drug access is limited, and preoccupation to participate in drug use [2]. The compulsion to take the drug is known as the binge/intoxication stage, where the addict participates in the drug use for pleasure. Drugs of abuse interact with reward circuits of the brain, such as the mesocorticolimbic dopamine system and the medial forebrain bundle, including other brain regions like the VTA and hypothalamus [2]. One of the main neurotransmitter systems involved with alcohol abuse is GABA_A, an inhibitory neurotransmitter. Acute reinforcement of the drug's effects is established due to the limbic information that is being transmitted from the hippocampus, amygdala, and frontal cortex to the nucleus accumbens.

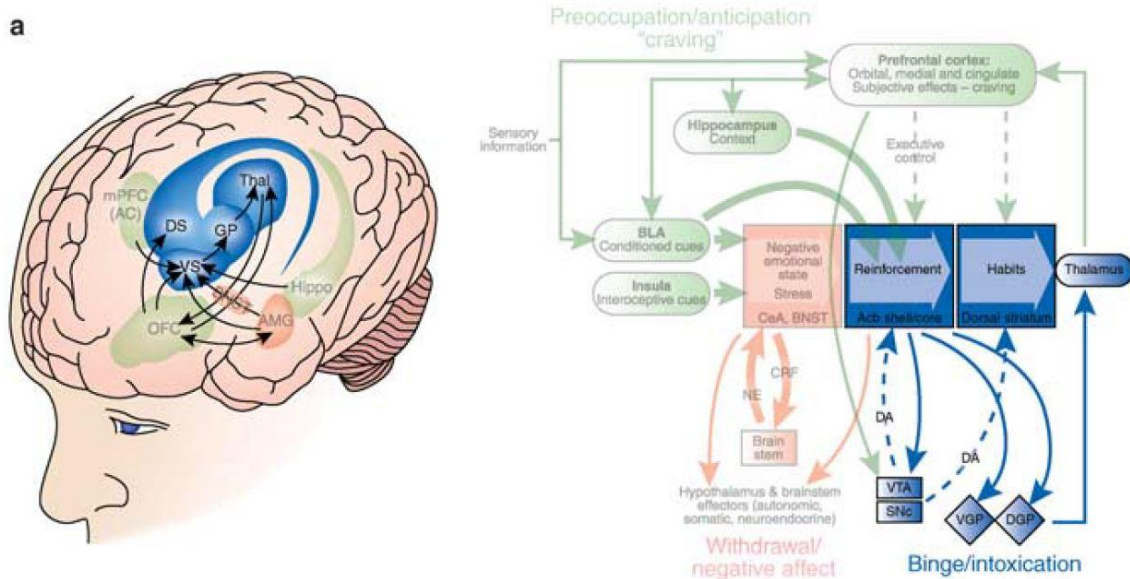


Figure 1: Binge/ Intoxication Stage. (Koob and Volkow 2002)

When individuals stop taking the drug, they may experience anxiety, negative emotional states, and discomfort; these negative states and behaviors are known as withdrawal/negative affect [2]. An important brain structure in the consolidation of the negative states is the extended amygdala, “composed of the central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST), and a transition zone in the medial (shell) subregion of the nucleus accumbens.” [2]

This overarching brain structure is crucial in receiving limbic, afferent information and projecting the interpreted information to efferent projections, where it is passed on to the extrapyramidal motor system. Three key elements are important during the intoxication stage: (1) a decrease in functioning of the neurotransmitter systems related to reward processing, (2) neuroadaptation of various neuromodulatory systems related to stress and homeostasis, (3) development of anxiety- like behavioral responses. Withdrawal causes noticeable decreases in activity of both the mesolimbic dopamine system and the serotonergic system associated with the nucleus accumbens. Drug withdrawal is correlated with an increase in brain reward threshold in rodents, suggesting a decrease in reward sensitivity and an increase in desire to self-administer the drug of abuse in humans. The negative state of the individual activates the brain stress- arousal system, leading to negative reinforcement of drug consumption. The reward- related neurotransmitter systems are desensitized while the stress response during drug withdrawal over-activates the hypothalamic- pituitary- adrenal axis (HPA axis), resulting in elevated levels of corticotropin- releasing factor (CRF).

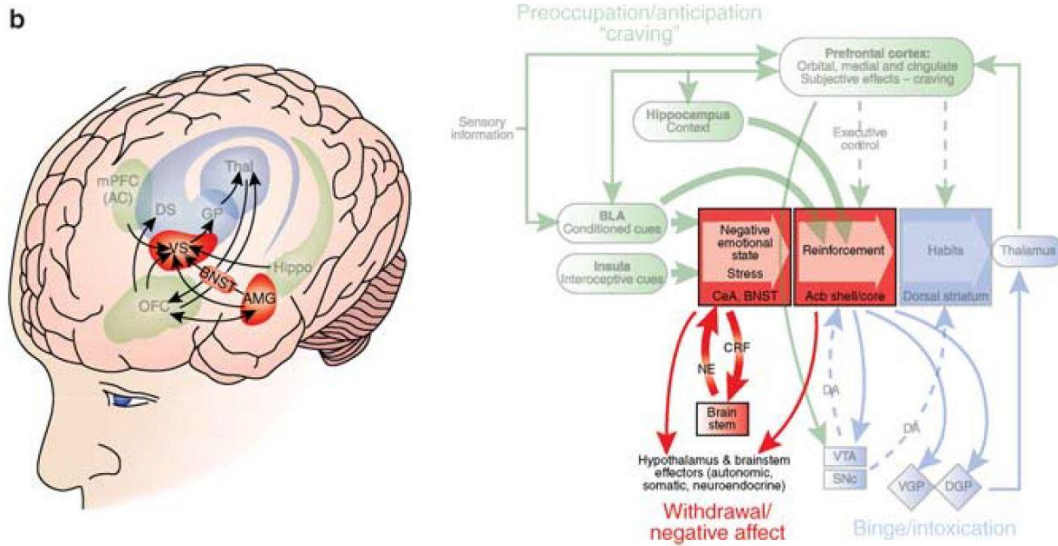


Figure 2: Withdrawal/ negative affect stage (Koob and Volkow 2002).

Preoccupation/ anticipation is when the individual reinstates the drug of abuse triggered by drug-related stimuli or acute stressors. It has been hypothesized that there are two distinct instances of drug seeking: through drug consumption itself and through acute stressor or negative emotional state. The former has been mediated by glutamate and localized to the medial prefrontal cortex [2]. Acute stressors have been linked to the extended amygdala via molecules including CRF and norepinephrine. In regard to alcohol addictions, there are suggestions that the extended amygdala is the instigator of the aberrant increase of the CRF and glutamatergic systems. As the cycle of addiction strengthens, areas such as the hippocampus begin to show deficiencies in such tasks as short term memory.

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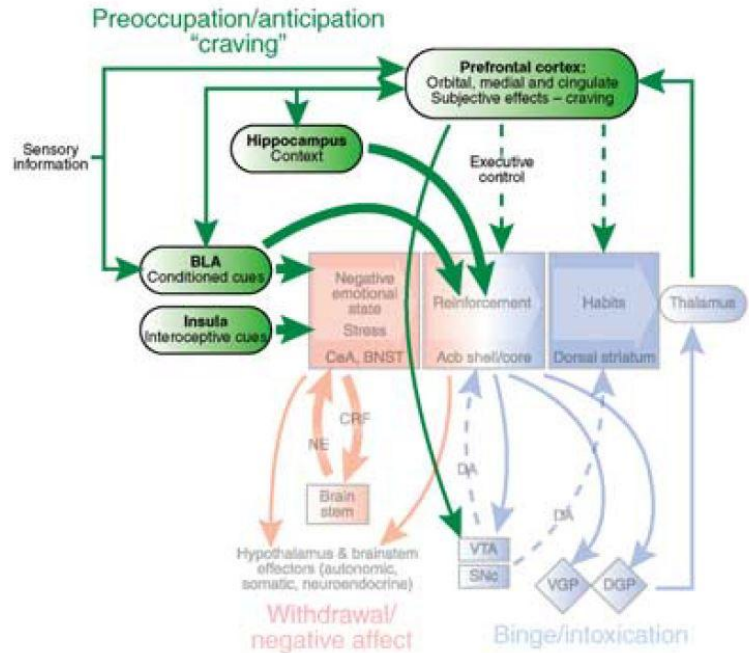
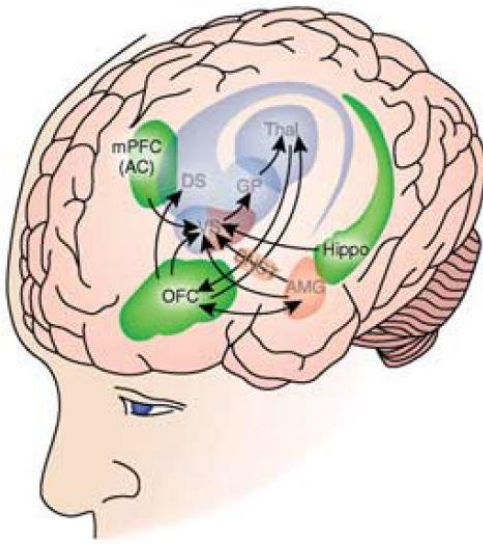


Figure 3: Preoccupation/ anticipation (Koob and Volkow 2002).

Hippocampus and Neurogenesis

In mammals, there are only two sites of neurogenesis known: the olfactory bulb and the dentate gyrus. The hippocampus an area of the brain believed to participate in the consolidation of new memories, contextualization, and retrieval of memory. New research suggests that the dentate gyrus may perform pattern separation [3]. Pattern separation is possible because of the dentate gyrus' unique characteristic of being a site of neurogenesis because of the neuronal population being comprised of immature, hyperexcitable neurons that can respond to extreme range of inputs [4]. The dentate gyrus consists of a neuronal population, specifically known as neuronal progenitor cells (NPC) that mature into excitatory granule cells; the granule cells project from the dentate gyrus to CA3 [5]. Initially, the NPCs undergo an intensive maturation process that will allow them to grow from NPCs to granule cells. The maturation process is

broken down into several steps, with the first step taking place in a short period of time. During this step, new granule cells initially are “functionally silent” due to their independent connection and lack of action potentials. The immature granule cells first projections are inhibitory GABAergic inputs, followed by the creation of a dendritic spine and mossy fiber axons, allowing the granule cells to develop a connection to its surrounding network; neurons exhibit incredible and highly variable physiologically characteristics in their activation thresholds, capacitance, membrane resistance, ending in robust synaptic plasticity [5]. These new cells integrate into the granule cell layer, by expanding dendritic connections to other granule cells and establishing axonal connections with the CA3 [6]. The neuroplastic nature of these neurons during adulthood give credence to the potential for vast organizational changes in the dentate gyrus as the result of learning and memory formation.

Metabolic Effect of Alcohol on the Brain

Chronic alcoholism is associated with loss of brain mass, neuronal death, cognitive defects, alteration and degradation of neurotransmitter systems of the brain, including GABAergic and dopaminergic systems. Early ethanol binge drinking study on rats utilized BrdU (analog of thymidine) to tag with proliferating cells in the dentate gyrus; BrdU integrates into the DNA during the S phase of the cell cycle. Results found that rats who were in the binge drinking cohort had lower levels of BrdU tagged cells in the dentate gyrus in than the control. This is hypothesized to be a result of a decrease in the neuronal progenitor cell proliferation (NPCs) via slower cell cycles or a decrease in population of cell cycling [6].

There is strong evidence supporting that binge drinking is mediated by drastic fluctuations between inhibitory/ excitatory amino acids and/ or the release of monoamines. Glutamate, an excitatory amino acid, is responsible for binding to such receptors like NMDA, AMPA, mGluR, which have been connected to learning and memory tasks. Binge drinking studies have identified that higher levels of glutamate levels and the establishment of nitric oxide molecules via calcium/ calmodulin pathways and nitric oxide synthesis [7]. The high levels of glutamate invoke excitotoxicity by the release of calcium, causing a cascade in which nitric oxide is released, leading to depolarization of the mitochondria and increased influx of Na⁺ into the cell. This in turn increases the demand of ATP [7]. Calcium interacts with phospholipases, which results in the generation of reactive oxygen species (ROS).

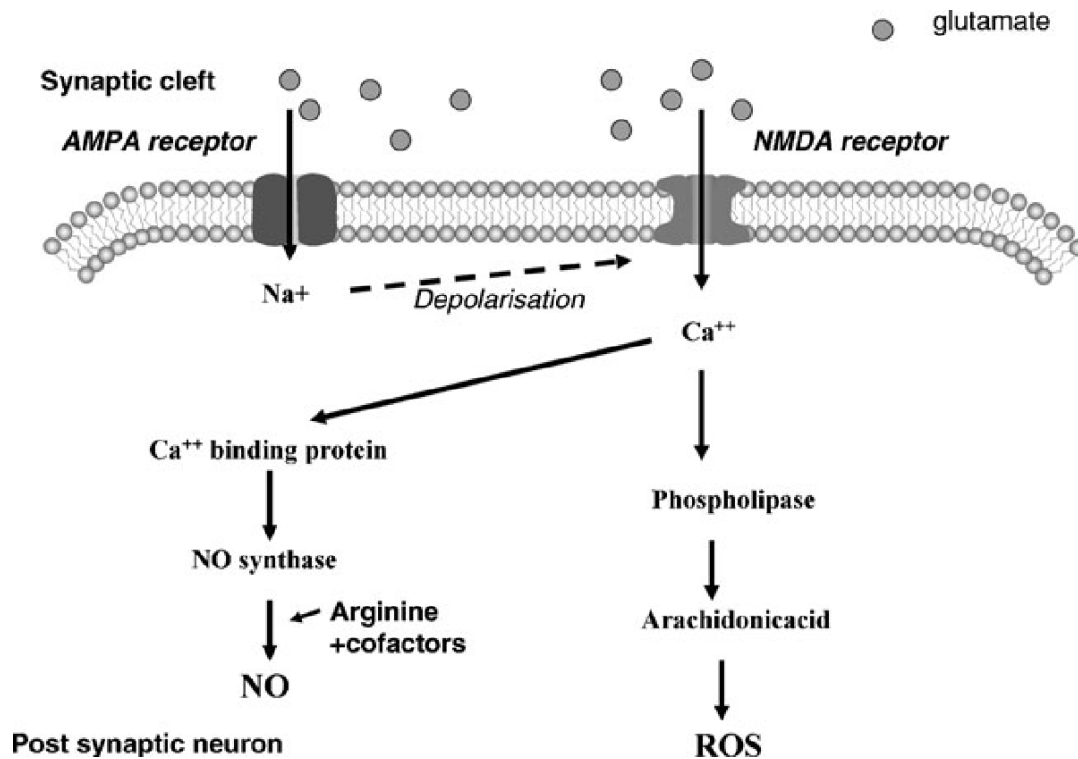
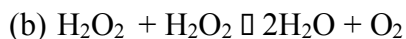
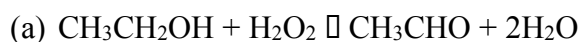


Figure 4. Glutamate interaction with AMPA and NMDA receptors.

Reactive oxygen species are extremely reactive molecules with short- life spans, generated from molecules such as H_2O_2 (hydrogen peroxide), $\text{O}_2^{\cdot-}$ (superoxide anion) and $\text{OH}\cdot$ (hydroxyl radical). These reactive species are crucial at low levels as they regulate normal physiological functions like proliferations, migration, cell death, etc. [8] Equilibrium is established between ROS with the use of antioxidants like glutathione peroxidase (glutathione), catalase, and vitamins. Catalase, for example, is in one of the body's natural antioxidants responsible for the removal of H_2O_2 (hydrogen peroxide, a toxic intermediate of ethanol) and for the oxidation of ethanol itself. [8]



When this equilibrium is disrupted, and there are excessive levels of ROS present, oxidative stress is triggered, resulting in the damage to proteins, lipids, membranes, organelles, nucleic acids, and DNA [9]. Environmental stress, caused by the toxic nature of alcohol for example, reacts with cytochrome P450 reductase, eventually leading to the creation of a free radical that reacts with oxygen to create ROS or will dismutate to create the H_2O_2 via superoxide dismutase. H_2O_2 is detoxified either by catalase (mentioned earlier) or glutathione peroxidase (generating the antioxidant glutathione). In some instances, H_2O_2 will react with Fenton to create $\text{OH}\cdot$, one of the ROS species that will damage the macromolecules. The damage caused to the macromolecules leads to cell death via apoptosis and necrosis.

Tumor necrosis factor (TNF) is involved in the glutamate- cytokine neurotoxic pathway in the brain by activating the transcription factor NF- κ B (nuclear factor kappa- B), which is involved with oxidative stress and inflammation [10]. TNF receptors, TNFR1 and TNFR2, are classified as membrane glycoprotein receptors that bind specifically to TNF. TNFR2 is linked with immune responses, but more importantly TNFR1 is linked in the cytoplasmic death domain, otherwise known as the TNFR1- induced activation of stress- induced kinase signaling. When the TNFR1 complex is assembled first by the disassembly of SODD (silencer of death domains) and TRADD (TNF receptor associated death domain) [11]. When binding occurs at the TRADD site, adaptor proteins like RIP (receptor interacting protein) and TRAF2 (TNF receptor associated factor 2) are gathered. The newly associated complex initiates a “RIP-dependent activation of NF- κ B signaling to initiate pro-survival signaling, cellular proliferation, and cytokine production” [11]. Along with pro- survival proteins, the complex also gathers cIAP 1,2 (apoptosis proteins 1 and 2), which signal the apoptotic pathways like JNK and ERK. Separation of the TRADD/ TRAF2/ RIP complex, along with the association of the FADD (Fas- association DD), leads another complex to be assemble: DISC (death- induced signaling complex). The complex utilizes caspase- 8, ultimately activating an intrinsic and extrinsic apoptosis pathway. TNFR1 signaling complex nature (apoptosis, proliferation, activation) plays a variety of roles in maintain homeostasis in the cell.

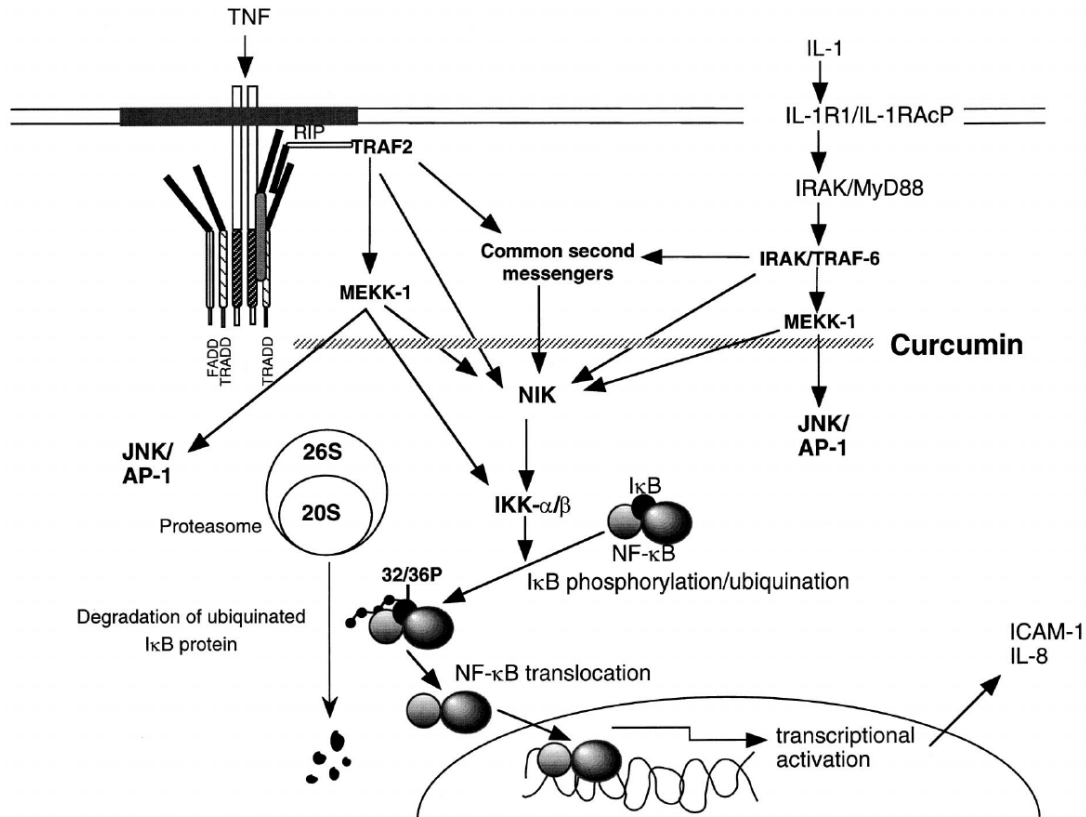


Figure 5. Mechanism of action that curcumin has on the NF- κ B. (Source: Jobin et al. 1999)

Antioxidants Protective Effects

The prominent pathological mechanism thought to be related between chronic alcoholism and the brain is oxidative stress of the liver and many other types of cells. According to Herrera et. al., “Selective impairment of hippocampal neurogenesis by chronic alcoholism: Protective effects of an antioxidant”, they studied effects that ebselen, a synthetic antioxidant, and its efficacy on protecting the brain from the deleterious effects of alcohol. Four cohorts of rats were used, separated by diet and treatment of ebselen in their diet. BrdU was used to track the existence of newborn cells in the dentate gyrus of the hippocampus. The data showed that ebselen did not have a promoting effect on the population of cells in the dentate, but it extremely

potent in its protective properties in preventing damage from alcoholism. “Here, administration of ebselen results in a decrease in ethanol-induced cell death in the DG and particularly in protection of newly born neurons” [12]. Ebselen carries much promise, as it is almost 100% effective in protecting the brain further damage under their experimental parameters, but the chemical makeup of ebselen complicates the issue. It is a synthetic, organo- selenium compound, and this study is based on chronic alcohol paradigms. There is promise in ebselen, though it is preferred to find a more naturalistic compound.

Jumping from chronic alcohol research to the relevancy of binge alcohol research, Crews et al. has continued to support the role of antioxidants as protective agents from the deleterious effects of alcohol. Supporting the findings from the Herrera et al. study, Crews utilizes four promising antioxidants in a rat-based study of ethanol binge drinking to test their efficacy. Four cohorts of rats were paired with the four antioxidants: butylated hydroxytoluene (BHT), blueberry extract (BB), ebselen (EB), and vitamin E (VE). The cohorts were given the antioxidants before and during the ethanol treatments. Their findings support that antioxidants indeed have protective properties, but their properties differed greatly. BHT was effective in reducing the glutamate TNF- α neurotoxic pathway activation by preventing transcription factor, NF- κ B, from starting the biochemical cascade, reducing the levels of ROS present; ebselen as shown to prevent the formation of oxidative radical via inflammatory enzymes, but was poor in scavenging ROS; BB promotes antioxidant activity, increase cognitive effects, decrease striatum ROS, and improving motor performance; Vitamin E was effective in the oxidation of lipids but not in protecting the brain from the binge drinking of ethanol [10]. BHT was unique in its ability to reverse the brain damage that occurred during binge drinking and in its anti-

neuroinflammatory and reduction of oxidative stress in the hippocampus, suggesting a oxidative-inflammation mechanism of neurotoxicity and lowered levels of neurogenesis. “Brain damage is associated with activation of NF- κ B–DNA binding, induction of COX2 and morphological signs of microglial activation, i.e., OX42, suggesting that neuroinflammation contributes to binge ethanol-induced neurodegeneration. Nuclear factor κ B is a key proinflammatory transcription factor that can increase expression of COX2, activate microglia, and promote the formation of other proinflammatory factors. Butylated hydroxytoluene reduced binge ethanol-induced increases in NF- κ B–DNA binding and COX2 expression, consistent with blocking binge ethanol induced neuroinflammation” [10].

In conclusion, both BHT and ebselen strongly support the notion that antioxidants can play a protective role in providing the brain with protective countermeasures from the neurotoxic effects of chronic and/or binge drinking alcohol treatments. Ebselen is shown to have a promoting antioxidant effects in the brain and reversing the inhibition of ethanol on neurogenesis but no noted effects on the promotion of neurogenesis itself. BHT was unique in preventing the binge drinking induced damage to the brain through various biochemical pathways and mechanisms and in blocking the inhibition of ethanol on neurogenesis). Unfortunately for both compounds, they are synthetic compounds. It is important to find a compound that is more naturalistic in origin that needs more studies to confirm its safety and that does not require finesse in administration.

Curcumin

Turmeric has long been used as a spice in the preparation of foods. There are three major curcuminoids: bidehydroxycurcumin, desmethoxycurcumin, and curcumin.

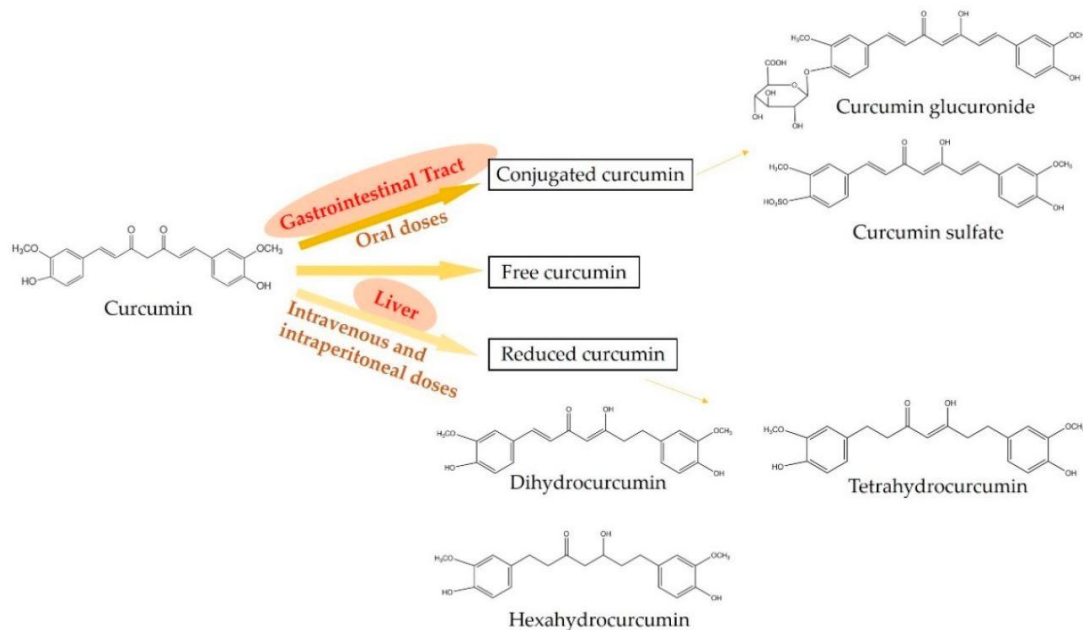


Figure 6. Metabolic pathway of curcumin. (Xu et al. 2018)

Of the three structures, curcumin and its metabolites have been shown to have a powerful and positive effects in many areas of bioactivity. In terms of antioxidant activity, the phenolic groups of curcumin are believed to drive the activity, resulting in reduction of oxidative stress while increasing the levels of antioxidant enzymes like glutathione peroxidase and catalase [13]. It has also been shown to have anti-inflammatory properties by suppressing cytokines like TNF- α and interleukin- 2β ; it has the potential to regulate pathways like the NF- κ B, a known inflammatory pathway [13]. It has the potential to be used to as an immune- regulatory treatment, anti- cancer treatments, offers hepatoprotection, cardiovascular protection, and neuroprotective effects. Clinical trials have highlighted the promise of curcumin due to its diverse ability to alleviate pain

and treat patients; as more research is conducted on the value of curcumin, so validity for therapeutic treatment will continue to grow [14]. The neuroprotective effects have promise in the treatment of neurodegenerative diseases and inflammation, and research has supported this theory.

Early research has suggested that one of the curcuminoids of turmeric, curcumin, is a scavenger of free radicals in the body. It is a potent scavenger of superoxide anions, nitrogen dioxides, and nitric oxide [15]. As interest began to grow in the potential benefits of naturalistic options for the treatment of pathology, so has the interest of researchers in their interest in curcumin. In the study done by Jobin et al., curcumin was used to study the inflammation pathways in the intestinal lining. Research suggests that curcumin can inhibit the activation of NF- κ B activation by stopping the IKK activity from being initiated [16]. When inflammation is detected, a signaling cascade begins. It is a complex cascade that involves multiple types of proteins, kinases and subsequent activity. Curcumin has the ability to inhibit the signaling activation of the NIK pathway and the TRAF/ RIP signaling of the IL- 1β and TNF- α pathways, respectively. Importantly, the signaling is can be stopped in various points in the cascade. Activation of the TNF- α cascade is halted, which has been connected to the start of apoptosis of many cells by the activation of TRAF-1 and various associated proteins. Adding onto the promise of curcumin, it is shown to be a nontoxic natural compound that is nonmutagenic. It is hypothesized that the inflammation pathways of the intestinal lining are like those found in the brain and central nervous system. Curcumin's effectiveness in various domains of safe, naturalistic treatments was beginning to become well- documented. This concept is extended in Xiao et al. study of curcumin as a treatment to reduce pain in lumbar radiculopathy. Data

strongly supports that it was successful in an overall attenuation of the neuroinflammation TNF- α pathway, protection against apoptosis, alleviated primary neurons from inflammation, heavily reduced the production of ROS, and lowered neuropeptide released from the TNF- α pathway [17]. This evidence suggests that curcumin is a viable antioxidant that could be included as part of a daily diet or even as a supplement when it comes to the treatment of spinal pain. To strengthen the position of curcumin as a potential for neuroprotective treatment, Baj and Seth study of ‘Role of Curcumin in Regulation of TNF- α Mediated Brain Inflammatory Responses’ detailed how the curcumin lowers cytokine and TNF- α levels, preventing the activation of NF- κ B, which leads to the production of ROS while scavenging the ROS that exists [18]. An extremely promising result from this study showcased that curcumin was safe to be administered in a large number of human trials. Lower numbers of side effects were recorded in comparison to steroid/ non- steroid anti- inflammatory medications [18].

It has been a running fact that curcumin has low chemical stability, making the administration of the curcumin as a viable treatment in various systems a complicated matter due to its low bioavailability[12]. Fortunately, the Theravalues Corporation was able to develop a highly absorptive version of curcumin utilizing colloidal nano- particles, named “Theracurmin” [19]. This formulation of curcumin increased the efficacy of bioavailability in rats by 40x and in humans by 27x. Theracurmin was tested in a human based alcohol study, with findings demonstrating in reduction of acetaldehyde levels in the blood [19]. This finding, paired with the history of curcumin as a viable therapeutic treatment for various domains of health, makes it a viable candidate for our attempts to validate this antioxidant as a protective treatment for preserving neurogenesis in the hippocampus, which has not been looked examined before.

Materials and Methods

Animal Model of Binge Drinking: Drinking in the Dark Paradigm

Animals

40 Adult- male Long- Evans rats are received at the age of 6 weeks. The rats are single housed upon arrival and allowed to acclimate to the new environment for the first week. Rats are always given access to food (rat chow) and water. 44 rats are used in the study; 4 groups of rats created: ethanol + curcumin, control + curcumin, ethanol + sucrose, control + sucrose. The study is conducted in accordance to the guidelines from the University of California San Diego's Institutional Animal Care and Use Committee.

Model of Binge Drinking: Drinking in the Dark Paradigm

According to Thiele and Navarro's "Drinking in the Dark' (DID) Procedures: A Model of Binge- Like Ethanol Drinking in Non- Dependent Mice", the DID model is advantageous because it is able to help spur binge- like drinking in mice with minimal access to ethanol, little training to encourage their drinking, comparatively short length of experiment, and the removal of powerful, addictive, confounding compounds like sweeteners to the alcohol [20]. This approach is more effective than other models such as the intermittent access 2 bottle choice as higher rates of consumption of ethanol overall were noted in the DID/ intermittent comparative study. [21]. This is a valid model to use in our experiment as it can be modified to fit our ethanol binge drinking paradigm in rats while allowing us to study addictive- like behaviors from excessive alcohol consumptions and make comparisons and connections to the human model of binge drinking.

When the rats are received, they undergo a phenotyping period during their acclimation period. Water is provided and recorded over 2 days. Rats are given 5% ethanol during the first day, followed by access to 10% ethanol on the second day. The amount of ethanol consumed is recorded (in mL) for both days. A preference score is calculated for each rat that takes (for each day):

Alongside the ethanol phenotyping, rats are given the curcumin treatment + 10% sucrose(6mL) over the phenotyping period. Rats that consumed curcumin consistently were used for the treatment groups. The preference score is utilized to organize the rats into 4 groups: ethanol + curcumin, control + curcumin, ethanol + sucrose, control + sucrose.

During the 1st 5 weeks, the control group is given 10% sucrose solution (2g/ kg) in a 15mL Falcon tube. The rats are given a curcumin treatment (50mg/ kg), which also contains the 10% sucrose. 10% ethanol is used during the drinking period. The same measurements are given to the rats in the last 5 weeks of the period with the expectation that 20% ethanol is given.

Rats are administered the treatment at 2pm in the afternoon, 8 hours prior to the drinking in the dark paradigm. Ethanol is given at 10pm, three hours after lights are out [during the dark cycle]. The rats are allowed free access to ethanol for the subsequent 4 hours [water is removed during this 4-hour period]. Ethanol measurements are taken at 3 time points during the drinking period: 30 min, 2 hours, and 4 hours [recorded from a 50mL Falcon tube].

In a 24 hour period

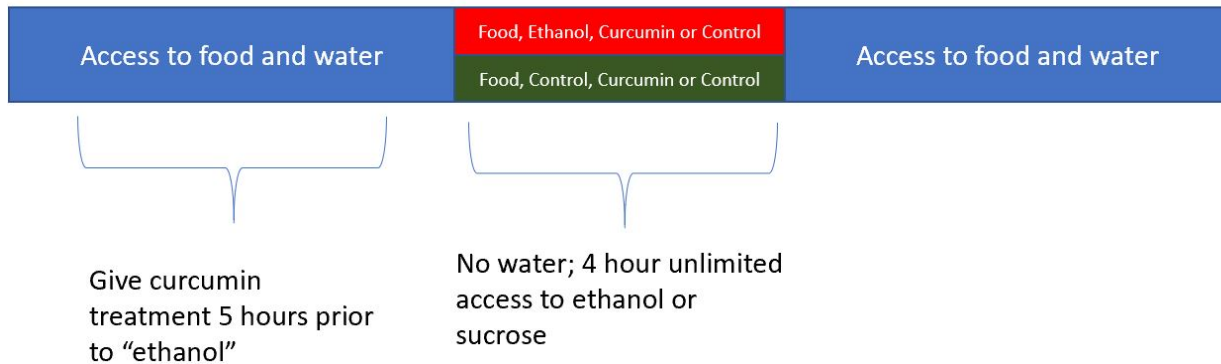


Figure 7. Daily timeline of DID model.

Method of Targeting Newborn Cells in the Hippocampus

IdU preparations and Injections

The newborn neuronal populations are labeled at the start and end of the study IdU [230gmg/kg] (iododeoxyuridine) are known analogs of BrdU (bromodeoxyuridine), and antibodies can be selected to target the analogs of choice. IdU is injected after the first session of DID.

Perfusion and Histology

Rats are euthanized two days after the experiment ends, followed by transcardial perfusion. The brain is sectioned and then is stored in cryoprotectant. For the immunohistostaining process, antibodies to IdU (BrdU analog), Ki67, and NeuN are used to stain the cells.

Results

The effects of Theracurmin as a viable treatment for a chronic alcoholism model was experimented utilizing a drinking-in-the-dark alcohol paradigm can by using hippocampal cells of the rat. Alcohol are notoriously difficult studies to conduct because it takes time to see the results expected and the administration of alcohol varies between studies with their pros and cons. Ethanol is a bitter solution that is aversive to rats prior to the acquisition of consistent drinking., so previous studies usually administered ethanol via gavage or injection. Self-administration conducted in an attempt to create a more naturalistic approach to ethanol administration. Phenotyping the rats and calculating an alcohol preference score was important in helping us balance the groups by having rats with low preference score in the same group with those rats with high preference scores. This was extended to the remaining three groups. Our DID paradigm was modified to include a pre- treatment (Theracurmin + sucrose) hours before the inclusion of the 4-hour drinking period of ethanol. For the first five weeks, the rats could drink 10% ethanol during the drinking period while the second half of the experiment allowed them to drink 20% ethanol during the drinking period. During both drinking periods, data containing daily amounts of the fluid consumption were collected, like water and ethanol. Ethanol drinking was collected at three different time periods: thirty minutes, two hours, and four hours.

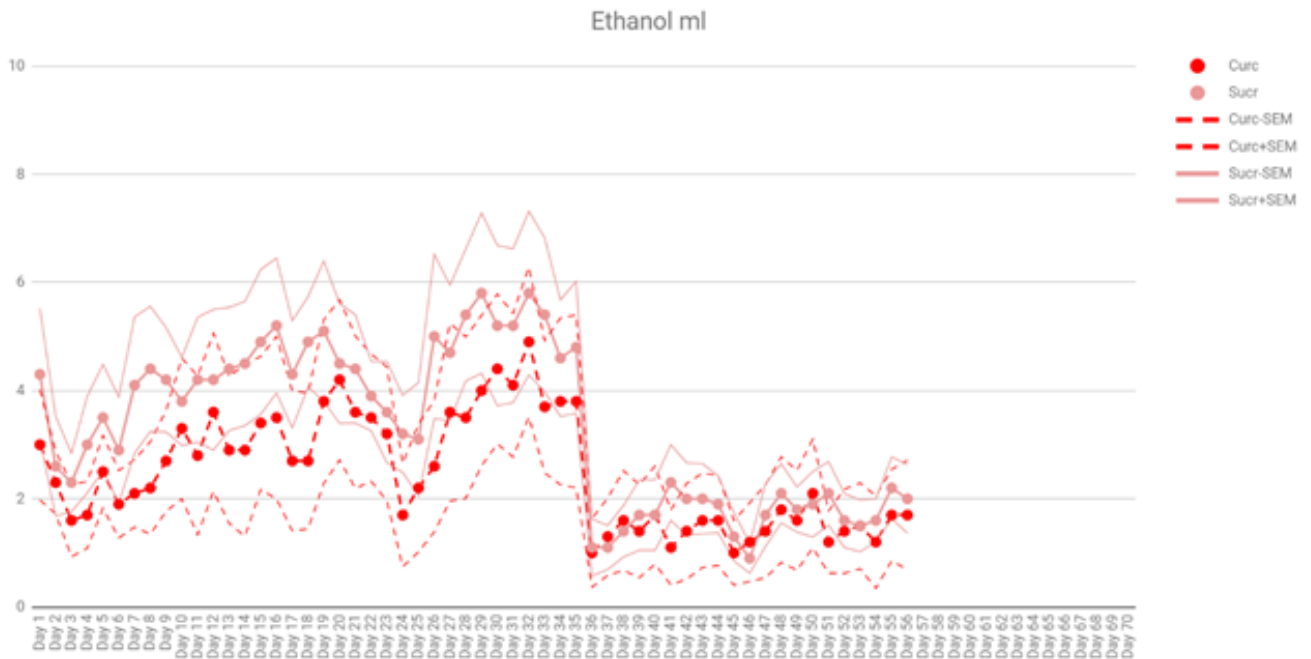


Figure 8. Daily average of ethanol consumed by treatment group and non- treatment group of rats in mL. At day 35, there was a switch between 10% and 20% ethanol.

In order to determine whether or not our modified DID paradigm was successful in inducing escalation in ethanol consumption, the average amount of mLs of ethanol consumed between the treatment group and the non- treatment group over the 10 weeks. Figure 8 represents the average mLs consumed, displaying slight increases in consumption in the first 35 days. At the switch from the 10% ethanol to the 20%, there was a large decrease, with little increases in

the escalation of the consumption.

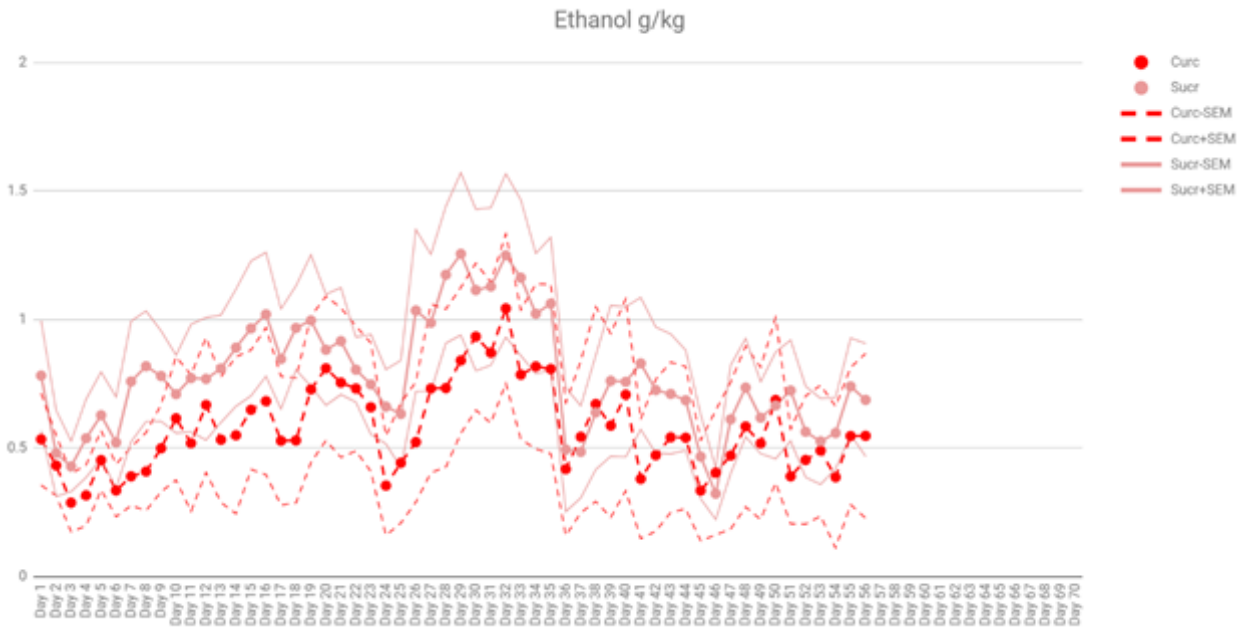


Figure 9. Daily average of ethanol consumed by treatment group and non- treatment group in respect to g/kg of group of rats.

Figure 9 takes the daily average ethanol consumption in grams per kilogram the treatment group and non- treatment group over the 10 weeks. Rats in the treatment group consumed less than per kilogram than those in the non- treatment group in the first 35 days of drinking. This is a consistent feature seen in the both the average mLs of ethanol consumed and in the g/kg data. Figure 9 confirms that there is no real escalation in the drinking. It is hypothesized that this is possible due to the addictive reward of sucrose in the daytime; normally there is no other reward used in the drinking paradigm. We believe more time may be needed for escalation in drinking to occur. A unique feature in the data set is that the non- treatment groups have about double the number of consistent drinkers in comparison to the treatment groups. Even where there was a

transition from the 10% ethanol to 20% ethanol, which had a drop in consumption in all groups, the non- treatment group still had higher consistency of drinking. This may push us to let the experiment run longer and possible differences at the end of the data collection cycle.

Control Animals Tend to Escalate Drinking More Than Curcumin Treated Animals

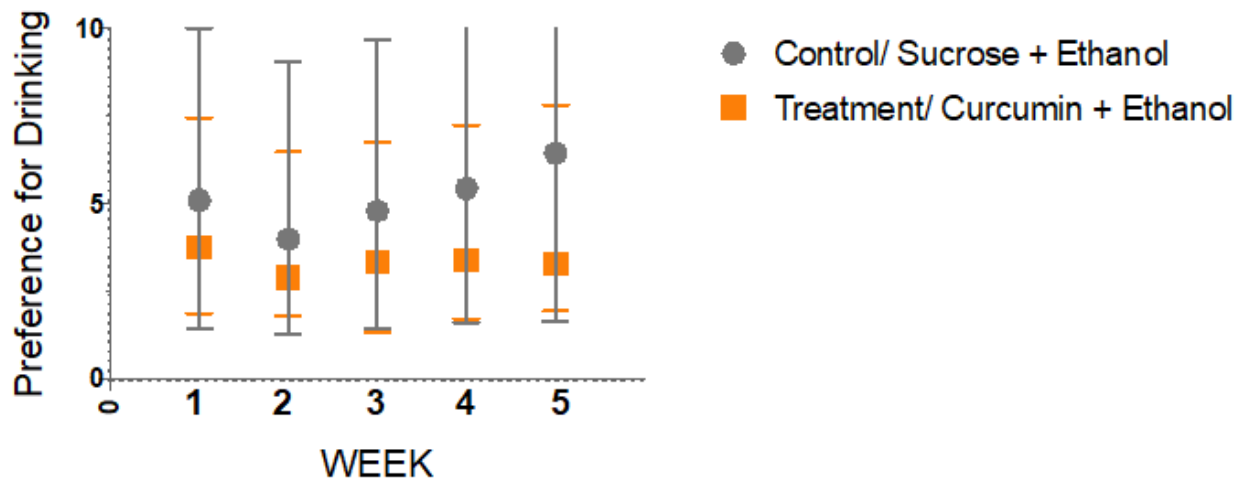


Figure 10. Alcohol preference score for rats on a weekly basis. Rats 1-10 are the ethanol + curcumin group. Rats 11-20 are the ethanol + sucrose group. This score is calculated for the first five weeks of the experiment (10% ethanol only) as the second half is still ongoing (20% ethanol).

A weekly preference score was calculated for both the treatment and non- treatment groups, for each individual rat, labeled from 1- 20, respectively. Figure 10 shows the preference of alcohol consumption for each individual rat in respect to total fluids consumed during the week. The preference score was calculated by taking the average of ethanol consumed in a one-week period and divided it over total amounts of fluid consumed in that week (water consumed + treatment consumed), regardless of the individual weight of the rats. This figure graphically show an increase in ethanol preference (or lack of) for the individual rat. As the rat begins to

consume more ethanol in respect to the other fluids, the preference score begins to increase as well. As mentioned earlier, the non- treatment group had notable increases in their preference score than those in the treatment group. This extends the idea from the Figure 9, where maybe there is the beginning of ethanol escalation consumption in the non- treatment group, which would prompt more time for the experiment to run. The high variability of drinking was tested with a two way repeated ANOVA measure (1 within weeks and 1 between treatment groups) and revealed no significant interaction. There is a notable trend so more time is needed to confirm this observation with confidence.

_____ Data concerning the immunostaining of the brain cells was not possible at the time of reporting because the experiment is still on- going. More results concerning this area of the experiment will be added as the experiment reaches its end.

Discussion

Note: As of writing, the experiment is still ongoing. Immunostaining and cell counting conducted at a further date. As such, it will be explained as expected results.

This study has been important in establishing efficacy of the rat model DID paradigm with a potent antioxidant dissolved in sucrose. The results have shown that there is some effect of the DID paradigm in inducing escalation in ethanol consumption in both the treatment and non- treatment groups. The first five weeks of the experiment had promising results for the non- treatment group. The results were promising as the rats self- administered alcohol, unlike many other alcohol studies where the ingestion of alcohol is done by gavage or other oral means. The non- treatment group displayed some key features of Koob and Volkow's model of addiction: self administration of the drug of abuse and the need for an abstinence period existing to strengthen the need to indulge in the drug activity [2]. More heavy drinkers in the non- treatment group is strengthened by the results from Figure 10, where more rats have an escalating preference for alcohol consumption over the five weeks in comparison to the treatment group, where there is one rat that clearly shows an escalating preference for alcohol while some show small to moderate increases in preference. The experiment was extended to include another five week period with an increase from 10% ethanol to 20% ethanol. In the transition period, there is a noted decrease in the average of both the mLs consumed and the average g/kg of ethanol consumed in the treatment and non- treatment group. This transition period skews the data as the increase in alcohol percentage also means an increase in the bitter taste of alcohol. The increase in bitterness is believed to have caused the rats to develop a taste aversion to alcohol but there is a return to drinking noted in the subsequent days. Increases in the 20% ethanol consumption

again pushed us to extend the drinking period to allow escalation to occur. The increases in time is needed to confirm whether or not escalation will occur in the model, though the results have been promising.

Expected results: the immunostaining will utilize three stains, IdU, NeuN, and Ki67. IdU is labeled at the beginning of the experiment and will stain for newborn cells in the dentate gyrus of the hippocampus. In order to ensure that the stained IdU cells are neurons and not another type of cell, it is double labeled with NeuN. If the cell stained responds to both the IdU and NeuN, it will confirm that it is a neuron of the dentate gyrus. Ki67 is an endogenous marker that can be used to tag cells at a certain time point without the need of an exogenous marker. The Ki67 marker is used to label cells of the dentate gyrus at the time of death. This sectioned will be expanded upon when the immunostaining analysis is completed.

Conclusion

_____Alcoholism is responsible for numerous neuropathological deficits. Alcohol abuse is becoming a great threat to society, with it starting to affect not only those of drinking age, but also adolescents. Alcoholism's neuropathological effects have been traced to increases in oxidative stress and production of volatile reactive oxygen species (ROS), depletion of the body's natural antioxidants like glutathione, and the overactivation of inflammation pathways like the TNF- α pathway. Research in the realms of antioxidants have been promising but there has negative concerns of justifying the use of certain potent antioxidants. Some compounds like ebselen mimics the function of glutathione and replace the depleted levels of the antioxidant, though the safety of the synthetic compound has not been quantified and the results from Herrera et al. have been hard to replicate, as detailed by Crew et al. in their study of multiple antioxidants like ebselen and butylated hydroxytoluene (BHT). BHT is another promising compound, but rat based research studies have shown that it has fatal effects to organ systems in relatively small doses, despite being FDA approved. Curcumin has beneficial properties from both compounds: it has a wide breadth of research showing to have promising effects in the hepatoprotection, antioxidant activity, and even neuroprotection [13]. The development of Theracurmin has solved the problem of curcumin's low bioavailability, increasing its efficacy for absorption in the human body. We developed a rat model of alcoholism to assess the possibility of curcumin as an intervention for preserving neurogenesis in the brain. We expect that our research will show that the active compound of theracurmin, curcumin, will protect the hippocampus (e.g. dentate gyrus) from the neurotoxic effects of alcohol. Though there were fluctuations of ethanol consumed, both treatment and non- treatment groups showed promising behaviors in drinking. This is

supported by the preference scores of rats in the first 5 weeks (overall weekly ethanol preference score will be calculated once the drinking paradigm is over). Our modified DID paradigm worked to a moderate degree despite the introduction of an addictive substance like sucrose prior to the ethanol drinking period. The next step would be using immunostaining techniques to quantify the number of cells in the dentate gyrus. The promise of curcumin is not to act as a wonder drug for those suffering from the addictive nature of alcohol but rather to act as a preventative approach to diminishing the deleterious effects of ethanol. Curcumin exists in the turmeric root, which is commonly used around the world as a spice. Future studies would be to test the effectiveness of curcumin in behavioral studies, expanding upon its safety and validity for preventative measures on a national scale.

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