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ETHYL ALCOHOL METABOLISM AND NEUROBIOLOGY IN HUMANS: FACTORS CONTRIBUTING TO ALCOHOLISM AND NEUROTOXICITY

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ABSTRACT

The consumption of ethanol has profound effects on human metabolism and central nervous system function. The oxidation of ethanol by alcohol dehydrogenase results in the production of toxic acetaldehyde and excess NADH, causing the disruption of normal metabolic processes and organ function. The risk for tissue damage and alcoholism is influenced by genetic polymorphisms in alcohol dehydrogenase and acetaldehyde dehydrogenase. Ethanol modulates the release of most major neurotransmitters in the brain including GABA, acetylcholine, glutamate, dopamine, serotonin, and opioid peptides. Changes in neurotransmission produce ethanol's characteristic psychoactive properties. Genetic variation in neurotransmitter receptors is correlated with susceptibility to the effects of alcohol and the risk of alcoholism. With chronic consumption, ethanol can cause irreversible brain damage through thiamine depletion and glutamate excitotoxicity. Ideas for further research are proposed.

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I. Introduction

Ethanol's effects on human metabolism and neurotransmission are complex and remain an area of intense research. This review attempts to combine the limited current knowledge on alcohol metabolism and neurobiology, in addition to describing two pathological conditions associated with chronic alcohol consumption: Wernicke-Korsakoff syndrome and glutamate excitotoxicity.

Ethanol is a simple and ubiquitous substance, unique among drugs in its ability to be used as an energy source. In order to participate in basic energy pathways, alcohol must first be converted to acetate. This process is catalyzed by alcohol dehydrogenase and acetaldehyde dehydrogenase. The function of these enzymes is responsible for the damaging effects of alcohol and partially mediates its addictive properties. Their efficiency is highly influenced by genetics. Alcohol's interference with energy metabolism results in a wide array of pathological conditions. In the CNS, chronic disruption of thiamine metabolism causes largely irreversible neural degradation that commonly manifests itself as Wernicke-Korsakoff syndrome.

Alcohol's modulation of neurotransmission is not easily characterized, as it involves virtually all major neurotransmitter systems and is subject to a great deal of genetic variability. Alcohol's depressant effects stem from its interaction with GABA and NMDA receptors, while its addictive qualities are governed by dopamine and serotonin. Chronic consumption leads to CNS habituation and the possibility of neurotoxic withdrawal symptoms as a consequence of glutamate receptor over-activation.

II. Alcohol Metabolism and Alcoholism

A. Introduction

Over 90% of consumed ethanol is metabolized by the body; the rest is eliminated in its unaltered form by the kidneys and lungs. Due to its solubility in both aqueous and lipid solutions, alcohol diffuses throughout the body (Lieber, 1997). It is metabolized in a variety of locations including the brain (Zimatkin and Buben, 2007), but the majority of alcohol is oxidized in the liver and stomach (Lieber, 1997). Alcohol interferes with some of the most basic cellular pathways, so its effects on homeostasis and metabolic processes are complex. Polymorphisms in the enzymes of alcohol metabolism have been correlated with the risk of developing alcoholism.

In general, alcohol is oxidized in a two-step process, first producing acetaldehyde. This reaction is primarily catalyzed by the enzyme alcohol dehydrogenase (ADH), which uses NAD+ as a coenzyme and hydrogen receptor, per the following reaction (Dolega, 2010):

$$CH_3CH_2OH + NAD^+ = CH_3CHO + NADH + H^+$$

Acetaldehyde is a toxic molecule that plays a part in the neurotoxicity (Harper, 2007) and addiction potential of alcohol. It is also a recognized teratogen (Tong et al., 2011). Acetaldehyde's toxicity necessitates its oxidation to acetyl-CoA by acetaldehyde dehydrogenase (ALDH) in a NAD+-dependent mechanism similar to that of ADH:

$$CH_3CHO + NAD^+ + CoA = \text{Acetyl-CoA} + NADH + H^+$$

The net result of this reaction is the production of harmless acetic acid. This simple mechanism belies the complexity of alcohol metabolism, as there are many forms of ADH/ALDH and genetic variability in those enzymes can greatly affect the speed and efficacy of alcohol clearance. There are also other enzymes involved in alcohol metabolism besides this primary "cytosolic" dehydrogenase pathway, such as cytochrome P-450 of the microsomal ethanol oxidizing system (MEOS) and catalase; non-oxidative metabolism has been shown to occur as well. The pathway used and genetic polymorphisms can have important metabolic and behavioral ramifications. Accordingly, their biochemical variations and the role of genetics are discussed in more detail below.

B. ADH/ALDH Pathway

i. ADH

Human alcohol dehydrogenase (Figure 1) is a dimeric metalloenzyme consisting of amino acid residues and a zinc ion. ADH is concentrated in the cytosol (Lieber, 1997).

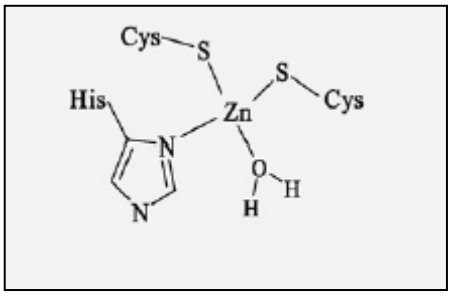

Figure 1: Subunit of Human Alcohol Dehydrogenase. From Dolega, 2010.

While seven genetically distinct classes of alcohol dehydrogenase occur in nature, only five are present in humans (Duester et al., 1999); seven genes encode for these five classes (Edenberg, 2007). ADH classes I, II, and IV metabolize ethanol (Crabb et al., 2004). ADH class I enzymes oxidize the majority of orally ingested ethanol and exist in multiple isoforms: AHD1A, B, and C (Duester et al., 1999). These isoforms were originally known as ADH1, 2, and 3, and are referenced in that way in much of the literature (Edenberg, 2007).

ADH class I is the most prevalent form in the liver and has a low K_m for ethanol, binding it strongly even at low concentrations (Crabb et al., 2004). ADH class II has a higher K_m and is active at higher BACs (Edenberg, 2007). ADH class III has a very low affinity for ethanol and instead binds ω-hydroxy-fatty acids and larger alcohols, in addition to functioning as a formaldehyde dehydrogenase (Crabb et al., 2004; Dolega, 2010). It is also the only ADH that has been shown to have a non-xenobiotic substrate (Dolega, 2010). Table 1 provides a summary of the types of ADH.

Gene Name	Old Name	Protein	Class
ADH1A	ADH1	α	I
ADH1B	ADH2	β	I
ADH1C	ADH3	γ	I
ADH4	ADH4	π	II
ADH5	ADH5	χ	III
ADH6	ADH6	ADH6	V
ADH7	ADH7	σ	IV

Table 1: Genes, classes, and protein subunit nomenclature of human ADH forms. Adapted from Edenburg, 2007.

Each subunit of the ADH dimer has an alcohol-binding catalytic domain and a coenzyme domain which binds to NAD+. The binding of NAD+ induces a conformational change allowing alcohol to bind to the zinc ion, replacing water. A hydride is transferred from the alcohol to NAD+. The alcohol is then released as an aldehyde along with NADH. NAD+ and a water molecule re-bind to the enzyme (Dolega, 2010).

This mechanism results in excess NADH production, responsible for some of the metabolic changes brought about by alcohol intoxication. In the presence of excess NADH, pyruvate is converted to lactate by the NADH-dependent enzyme lactate dehydrogenase. The build-up of lactate causes hyperlactemia and acidosis. An acidic environment interferes with numerous body functions. The kidney's ability to eliminate uric acid is inhibited; the lack of NAD+ slows the citric acid cycle and oxidation of fatty acids is decreased, which in part causes triglyceride accumulation in the liver and eventual fatty liver disease. In individuals with depleted glucose levels, the high NADH/NAD+ ratio and lack of pyruvate may lead to dangerous hypoglycemia, but under other physiological conditions may instead cause hyperglycemia (Lieber, 1997). Due to the very basic cellular role of NADH, the metabolic effects of alcohol are complex and still not fully understood.

The subunits of ADH may hybridize between types of the same class but not between classes (Lieber, 1997). ADH1B and ADH1C, which consist of β and γ subunits, respectively, have polymorphic forms resulting from single nucleotide polymorphisms (SNPs) in coding regions (Edenberg, 2007). The common alleles for ADH1B are ADH1B*1, 2, and 3; for ADH1C, ADH1C*1 and 2. These alleles code for specific subunits, such as β1, β2, γ1, etc. (Lee et al., 2006). β1 has a 70- to 80- times slower turnover rate than the other two variants, while the γ1γ1 ADHC homodimer has a 70 percent greater turnover rate than the γ2γ2 form. Caucasians

and East Asians typically have the ADH1B*1 or ADHB*2 alleles. ADHB*3 is found primarily in those of African or American Indian descent. ADHC*1 is more common in East Asian and African populations than in the other two groups (Lee et al., 2006). Variation in these alleles has been correlated with the risk for alcoholism and liver disease.

The ADH1B*2 and ADHC*1 alleles were found to be protective against alcoholism in a study on Taiwanese men (Thomasson et al., 1991). These two alleles are often inherited together, and it is suspected that the protective effect of ADHC*1 may be exaggerated. In Caucasians, ADHC*1 homozygotes have less than a 20% improvement in ethanol oxidative capacity than their ADHC*2/ADHC*2 counterparts (Lee et al., 2006). These results have been generalized to other Asian populations (Whitfield, 1997). The ADH1B*2 allele is less common in people of European descent, but seems to protect against alcoholism in that population as well (Borras et al., 2000). The ADH1B*3 allele is associated with a lower risk of developing alcoholism in African-Americans and some Native Americans (Edenberg, 2007). The ADH1B*3 allele is exclusive to those populations and does not seem to exert as significant of a protective effect as ADH1B*2 (Lee et al., 2006).

It is hypothesized that the higher clearance rates of these enzymes results in a more rapid increase in acetaldehyde, making further drinking uncomfortable. Table 2 illustrates the effect of genotype on ethanol oxidation rate. Of course, having a more effective ADH enzyme does not preclude one from becoming an alcoholic. Individuals with the ADH1B*2 allele that become alcoholics may have a greater risk of developing liver disease due to rapid increases in acetaldehyde, however (Whitfield, 1997). SNPs in non-coding regions of ADH genes also affect the risk of alcoholism; the reasons for this are unclear (Edenberg, 2007).

Genotype	K_m (mM)	V_{max} (mmol/min/1.4 kg)
ADH1B*1 + ADH1C*1	0.13 ± 0.04	2.1 ±0.1
ADH1B*1 + ADH1C*2	0.11 ± 0.04	1.7 ± 0.1
ADH1B*2 + ADH1C*1	1.6 ± 0.1	20 ± 1
ADH1B*2 + ADH1C*2	1.7 ± 0.1	19 ± 1
ADH1B*3 + ADH1C*1	9.8 ± 3.7	7.6 ± 1.3
ADH1B*3 + ADH1C*2	15 ± 5	8.2 ± 1.4

Table 2: Effect of genotype on K_m and V_{max}. All genotypes were homozygotic for the given alleles. Data adapted from Lee et al, 2006.

ADH is involved in so-called "first-pass" metabolism (FPM), a phenomenon also exhibiting genetic variability (Dolega, 2010; Crabb et al., 2004; Lee et al., 2006). FPM begins in the stomach, where a portion of ingested alcohol is oxidized in the stomach lining, and continues in the liver. FPM eliminates ethanol before its interaction with other organs, thus preventing an increase in the blood concentration of alcohol and subsequent tissue damage. It appears to be most efficient when a low level of alcohol is consumed, about one drink, and the stomach contains food (Crabb et al., 2004; Dipadova, 1987). The effect can still be observed at two drinks, but at higher doses FPM is insignificant. Organ damage associated with alcoholism also reduces the ability to eliminate alcohol by FPM. The presence of food may increase alcohol oxidation by increasing the time in which it can be absorbed by the stomach or small intestine (Dipadova, 1987), or by increasing the speed of NADH reoxidation (Meijer et al., 1975).

The significance of the stomach and small intestine in first-pass metabolism is debated. Asians lacking the ADH7 enzyme, predominant in the stomach, do show a decrease in FPM

(Crabb et al., 2004), but the liver is considered the primary site of FPM in most studies. The stomach probably has a small accessory role. The liver is far more efficient at oxidizing first-passed ethanol when compared to the stomach. Oxidation rates at very low ethanol concentrations are 95% for the liver versus 20-30% in the stomach (Lee et al., 2004).

Gender differences in alcohol tolerance are partially attributable to FPM. ADH is actually more active in women, as it is inhibited by androgens. Gastric ADH, however, is less active. Body composition is an even more important factor. Women, on average, have 12% less water because of their higher body fat percentages. Blood alcohol concentration will therefore rise faster and reach a greater final level from the same dose of alcohol. At higher alcohol doses, FPM will have a negligible effect on peak blood alcohol concentration in either gender (Lieber, 1998).

Regardless of the site of FPM, the type of ADH enzyme expressed will mediate its effect. Individuals with an ADH1C*1/ADH1C*1 genotype oxidize about 10% more alcohol from FPM than ADH1C*2 homozygotes (Lee et al., 2006). ADH1C is the only class I ADH present in the stomach (Crabb et al., 2004). FPM efficiency in the liver follows trends similar to that mentioned previously, for example livers expressing ADH1B*2 would be more efficient at FPM than ADH1B*1 livers.

ii. ALDH

ALDH is a tetrameric enzyme, with 10 genes encoding 10 separate ALDH enzymes identified in humans (Crabb et al., 2004). Of these, only two encode for enzymes that metabolize acetaldehyde, ALDH1A1 and ALDH2. Their corresponding enzymes are ALDH1 and ALDH2. ALDH1 is found in the cytosol and has a high K_m for acetaldehyde; its primary

substrate is retinal. ALDH2 is located in the mitochondria and has a high affinity for acetaldehyde. Both enzymes are relatively ubiquitous but are concentrated in the liver and stomach (Hsu et al., 1985; Yoshida et al., 1998).

ALDH2 is polymorphic with two recognized alleles, ALDH2*1 and ALDH2*2. ALDH2*2 is an inactive form caused by a G→A point substitution in a coding region (Yoshida et al., 1998). The ALDH2*2 allele is virtually dominant, with heterozygotes possessing a very limited ability to oxidize acetaldehyde (Edenberg, 2007; Thomasson et al., 1991). The absence of a functional ALDH2 enzyme causes a rapid build-up of acetaldehyde after alcohol consumption. Accumulation of this toxin causes nausea, light-headedness, tachycardia, and distinctive facial flushing (Edenberg, 2007; Thomasson et al., 1991). In individuals that exhibit a flushing reaction, it was found that acetaldehyde levels can rise 10-20 times higher than in non-flushers (Crabb et al., 2004). The inactive isozyme is especially common in Asian populations, with a frequency of up to 50% (Edenberg, 2007; Quertemont, 2004). Possession of this allele is associated with a marked decrease in the risk of alcoholism, as demonstrated by many studies. Among Japanese men, only 2 to 5% of alcoholics possess the ALDH2*2 allele; in Taiwan, only 6% of alcoholics (Crabb et al., 2004). ALDH2*2 alcoholics are most likely heterozygotes, as alcoholics with an ALDH2*2/ALDH2*2 genotype are almost non-existent (Edenberg, 2007). Table 3 demonstrates this effect in Chinese males.

	Genotype Frequency			**Allele Frequency**	
	ALDH2*1/*1	ALDH2*1/*2	ALDH2*2/*2	ALDH2*1	ALDH2*2
Non-alcoholics (50)	.52	.36	.12	.70	.30
Alcoholics (50)	.88	.12	.00	.94	.06

Table 3: Genotype and allele frequencies of ALDH in Chinese men. Adapted from Thomasson et al, 1991.

People with the ALDH2*2 allele that continue to drink substantially despite their aversive reaction to alcohol have a greater chance of damaging their organs. The resulting high concentration of acetaldehyde means ALDH2*2 alcoholics are more likely to develop liver cirrhosis, cancer, and asthma (Crabb et al., 2004).

The unpleasant reaction caused by an elevation in acetaldehyde levels is the reason for the effectiveness of disulfiram, a drug used to treat alcoholism. Administration of this drug causes symptoms including flushing, nausea, and respiratory distress after just a small amount of alcohol is consumed. Disulfiram binds to and inhibits ALDH1, which is sufficient to cause a response severe enough to likely discourage further drinking (Hsu et al., 1985).

Chronic alcohol consumption causes a decrease in the efficiency of ALDH while ADH is relatively unaffected; causing an exceptionally high concentration of acetaldehyde to build up in the livers of alcoholics (Lieber, 1997). This contributes to the development of liver cirrhosis.

C. Microsomal Ethanol Oxidizing System

It was discovered around 40 years ago that another metabolic pathway for alcohol existed, the microsomal ethanol oxidizing system or MEOS (Lieber, 1998). This pathway was discovered based on the observation that the smooth endoplasmic reticulum (SER) proliferates after chronic alcohol consumption (Lieber, 2004). The SER was found to contain the components of the MEOS, which is concentrated in the liver (Lieber, 2004). The MEOS uses multiple types of cytochrome P450 to metabolize ethanol into acetaldehyde.

While ADH is an essential component of alcohol oxidation, the MEOS has numerous unique characteristics that make it equally as important. Under normal conditions, the MEOS

only metabolizes a moderate portion of the alcohol that enters the body; in deer mice, around 10% of consumed alcohol. However, MEOS activity is increased with chronic consumption. ADH is not inducible, and therefore does not allow the body to build up a metabolic tolerance to alcohol. The MEOS, however, is highly inducible by chronic alcohol consumption and has a higher K_m, making it especially significant when large amounts of alcohol are consumed for long periods of time (Lieber 1997). The MEOS is responsible for the metabolism of a variety of xenobiotic substances other than alcohol. Its increased efficiency as a result of chronic alcohol consumption causes a tolerance to numerous drugs, and it is responsible for some alcohol-drug interactions when the substances are ingested simultaneously. One element of the pathway specifically induced by alcohol, the cytochrome P450 known as CYP2E1, produces toxic metabolites and free radicals through the breakdown of substances other than alcohol (Lieber, 1998).

The ability for MEOS to be induced is an important part of metabolic ethanol tolerance in alcoholics, independent of neural tolerance. The induction of MEOS causes alcoholics to clear ethanol and other drugs from their blood at a faster rate. CYP2E1 in particular is up-regulated in response to ethanol consumption. CYP2E1 was found to be 4 to 10 times more prevalent in the livers of subjects who had been drinking recently (Lieber, 1998). The mechanism for induction is unknown, but may be due to protein stabilization; RNA stabilization and increased transcription have been discounted (Millonig et al., 2011).

Ethanol is only one of many substrates metabolized by the MEOS, so induction increases tolerance to a variety of substances including but not limited to antipyrine, warfarin, pentobarbital, and diazepam (Lieber, 1998). Warfarin is metabolized twice as fast in recovering

alcoholics; thus the dosage has to be modified for the drug to remain effective (Lieber 1997). Tolerance can remain in place for weeks after alcohol consumption is stopped (Lieber, 1998).

A synergistic effect is observed when drugs metabolized by the MEOS are taken at the same time as alcohol. Alcohol and the other substrate must compete for a limited number of enzyme active sites. Due to the partial saturation of MEOS, blood levels of a drug may rise to much higher concentrations than when the drug is taken without alcohol. Combining alcohol with tranquilizers, barbiturates, and opiates can be deadly for this reason (Lieber, 1998).

Particularly dangerous is CYP2E1's ability to break down xenobiotics into toxic substances and free radicals. Some of the xenobiotics metabolized by CYP2E1 would be commonly ingested by alcoholics, including cocaine, acetaminophen, and a few anesthetics and industrial solvents. Damage due to acetaminophen is especially prevalent as it is typically taken during times of withdrawal to ease the symptoms of a hangover. Under these circumstances, ethanol and other substrates are not competing for CYP2E1. Acetaminophen is rapidly metabolized into harmful by-products (Lieber, 1998).

Finally, CYP2E1 is associated with the production of free radicals and active oxygen species, leading to liver injury and an increased cancer risk. The combination of alcohol, fasting, and acetaminophen depletes glutathione, a substance that binds free radicals, while cirrhosis leads to lower levels of the antioxidant α-tocopherol (Lieber, 2000).

D. Catalase and Non-oxidative Metabolism

The main function of the enzyme catalase is to break down hydrogen peroxide to water and oxygen; however, it can oxidize alcohols and other substances when sufficient hydrogen

peroxide is present. Initial studies erroneously concluded that catalase played only a very minor role in alcohol oxidation, but the rat livers used were not treated with a physiologically normal quantity of fatty acids. Fatty acids are required for hydrogen peroxide production and the stimulation of catalase. Methanol, a common substrate for catalase, is taken up by the liver four times faster with an infusion of fatty acids (Handler and Thurman, 1990). Methanol is oxidized by catalase but not ADH so it is often used as a marker of catalase activity.

In a study of ADH-deficient deer mice, it was found that alcohol metabolism was decreased 75% by the catalase inhibitor aminotriazole. By comparing the rate of alcohol elimination in ADH-positive and -negative mice, it was concluded that catalase-mediated ethanol oxidation may account for up to 50% of alcohol metabolism at low levels, and nearly all ethanol oxidation at high blood concentrations in ADH-positive mice. Treatment with fructose, an inhibitor of H_2O_2 production and thus catalase, caused significant decreases in methanol and ethanol metabolism in ADH positive mice at high doses; this result is shown in Table 4 (Bradford et al., 1993). Fructose stimulates the oxidation of NADH and therefore boosts ADH function, but this effect seems to be outweighed by the inhibition of catalase at high fructose doses.

Alcohol	Fructose (g/kg)	Rates of Alcohol Metabolism (mmol/kg/h)
Ethanol	0	7.5 ± 0.5
	2	9.9 ± 0.5
	10	5.4 ± 0.5
Methanol	0	5.2 ± 0.3
	2	5.3 ± 0.4
	10	2.1 ± 0.3

Table 4: Effects of Fructose on Rates of Alcohol Metabolism in ADH positive mice. Adapted from Bradford et al., 1993.

Catalase is the main pathway for ethanol oxidation in the brain. In one experiment, rat brains were perfused with ethanol and the concentration measured over time. Baseline levels were established for different perfusion rates. Ethanol concentrations rose beyond baseline levels when the rats were treated with aminotrizole, a catalase inhibitor (Zimatkin and Buben, 2007).

All previous pathways have involved the oxidation of ethanol into acetaldehyde, but this is not the only reaction in which ethanol can participate *in vivo*. Ethanol can also undergo non-oxidative reactions to form fatty acid ethyl esters (FAEE). Such reactions occur most commonly in the liver and pancreas and have also been observed in the heart and brain (Laposta and Lange, 1986). The formation of FAEEs has been implicated in alcoholic pancreatitis in rats, and is suspected to cause damage to all organs in which they accumulate. The inhibition of oxidative pathways like ADH, the MEOS, and catalase stimulates non-oxidative alcohol metabolism. (Werner et al., 2002).

III. Alcohol's Modification of Neurotransmission and the Neurological Basis of Alcoholism

A. Introduction

Ethanol is a central nervous system depressant and has effects similar to anesthetics, benzodiazepines, and barbiturates. The acute psychological effects of alcohol include behavioral dis-inhibition, changes in mood, impaired memory, and decreased cognitive abilities. Alcohol also has negative effects on fine motor skills and muscular coordination in a dose-dependent manner. At progressively higher doses, alcohol can be dangerously toxic by causing nausea, vomiting, sedation, coma, and eventually respiratory depression and death.

Alcohol's specific pharmacological propertie are considerably more complex than that of most drugs. It was once thought that alcohol and volatile anesthetics exerted their effects primarily by changes to the fluidity of the lipid bilayer of cells. The hydrophobic nature of alcohols seemed to be a mediating factor, with the most hydrophobic alcohols producing deeper anesthesia (Lovinger, 1997). Ethanol's effect on the phospholipid bilayer is actually no more significant than the changes brought about by daily temperature changes in the brain, however (Eckardt et al., 1998). Instead, alcohol produces changes in neural transmission by primarily inhibitory effects on ligand-gated ion channels.

Ethanol modulates the GABA, serotonin, dopamine, acetylcholine, NMDA, and opioid neurotransmitter systems, among others (Eckardt et al., 1998). As such, it is a very non-specific drug and its mechanisms are still not fully understood. Genetic variations in neurotransmitter receptors further complicate matters by influencing the acute effects of ethanol and the risk of

addiction. To adequately explain the neurological properties of ethanol, each of these pathways must be examined individually.

B. GABA

i. Pharmacology

Gamma-aminobutyric acid, or GABA, is a major inhibitory neurotransmitter in the central nervous system (Figure 2).

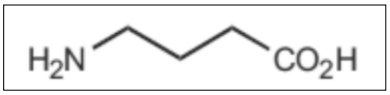

Figure 2: Gamma-aminobutyric acid (GABA)

There are three types of GABA receptors in the brain: $GABA_A$, $GABA_B$, and $GABA_C$. $GABA_A$ and $GABA_B$ receptors are fairly ubiquitous in the human CNS. $GABA_C$ receptors, on the other hand, are found mostly in localized areas during development. One exception in adults is the retina.

$GABA_A$ and $GABA_C$ are ionotropic ligand-gated receptors. In general, members of this large class of receptors control ion permeability by opening or closing a centrally-located ion channel in response to the binding of a ligand. Ionotropic GABA receptors are heteromeric proteins comprised of five subunits, structurally homologous to glycine, acetylcholine, and $5HT_3$ receptors (Figure 3).

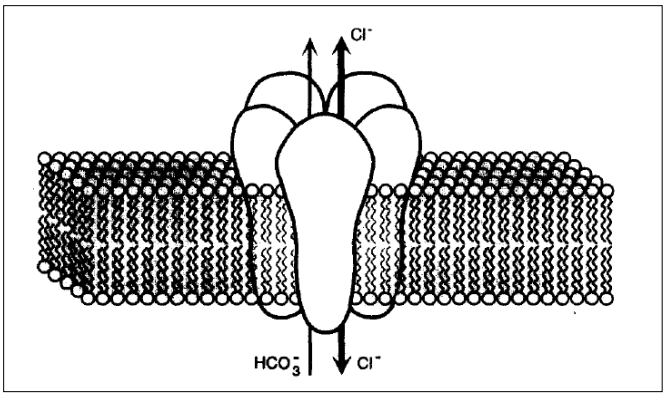

Figure 3: Diagram of a $GABA_A$ receptor heterodimer, consisting of five subunits and a central ion pore. From Kaila, 1994.

Eight different subunits classes have been identified so far: α, β, γ, π, δ, ε, θ, and ρ, with further variations existing in the form of six α, three β, three γ, and three ρ subunits (Weiner and Valenzuela, 2006). ρ subunits are found only in, and are the sole subunits of $GABA_C$ receptors (Lobo and Harris, 2008). Each subunit has a terminal cysteine-cysteine loop and four hydrophobic transmembrane domains known as TM1, 2, 3, and 4. TM2 faces the inside of the structure and forms the ion pore or channel (Liljequist and Engel, 1982).

Most $GABA_A$ receptors consist of two α, two β, and one of the other types of subunits (Lovinger, 1997). The most common $GABA_A$ receptor is formed by two α_1, two β_2, and one γ_2 subunit. Due to the wide variety of subunits, synaptic $GABA_A$ receptors are very diverse in their function and distribution. Additionally, some $GABA_A$ receptors are locating extrasynaptically where they participate in slow, "tonic" inhibition. These receptors commonly have a δ subunit (Weiner and Valenzuela, 2006). These unique receptors may be affected by ethanol as well (Lobo and Harris, 2008; Weiner and Valenzuela, 2006). The multiple subunits of the $GABA_A$ receptor are responsible for its ability to bind a variety of drugs. In the most common type of $GABA_A$ receptor, $\alpha_1\beta_2\gamma_2$, agonists and antagonists bind between the α and β subunits. Benzodiazepines have a high affinity for an allosteric region at the α and γ subunits. At

physiological concentrations, benzodiazepines will not bind to $GABA_A$ receptors with α_4 or α_6 subunits (Liljequist and Engel, 1982).

$GABA_B$ is a metabotropic receptor with considerably less variation in its structure. It is a Class C G-protein-coupled receptor (GPCR) (Enna and Mohler, 2007). $GABA_B$ belongs to the same family as metabotropic glutamate receptors and exists as a combination of GT1 and GT2 subunits, which are homologs. $GABA_B$ exerts long-term changes by activating a GTP-protein. $GABA_A$ is the most common GABA receptor and the most relevant when discussing ethanol, although contemporary research has shown that $GABA_B$ receptors' role has been underestimated (Weiner and Valenzuela, 2006).

When GABA or another agonist binds to a postsynaptic $GABA_A$ receptor, a chlorine ion channel opens in the cell membrane. Chlorine flows into the cell down its electrochemical gradient, causing the interior of the cell to become more negatively charged. The negative charge reduces the resting potential of the neuron and decreases the chance that the neuron will fire an action potential. Action potentials occur when a neuron is depolarized to a threshold charge, causing the opening of sodium channels and a large influx of positively-charge sodium ions. Action potentials are the basis for much of neural signaling, and typically cause a neuron to release neurotransmitters into a synapse. An action potential in a presynaptic GABAergic neuron will cause the release of GABA into a synapse, where it will bind to receptors on the postsynaptic cell. The postsynaptic neuron is rapidly inhibited.

$GABA_B$ receptors are more complex and less direct. Postsynaptic $GABA_B$ receptors activate potassium channels, causing an efflux of positive charge and hyperpolarization of the neuron. Presynaptic receptors cause inhibition through inactivation of voltage-gated calcium channels, which limits neurotransmitter release. The coupled G-proteins also inhibit adenyl

cyclase (Weiner and Valenzuela, 2006). Ethanol's effect on GABA transmission involves both types of receptors. Figure 4 below is a simple diagram showing the function of the $GABA_A$ and $GABA_B$ receptors.

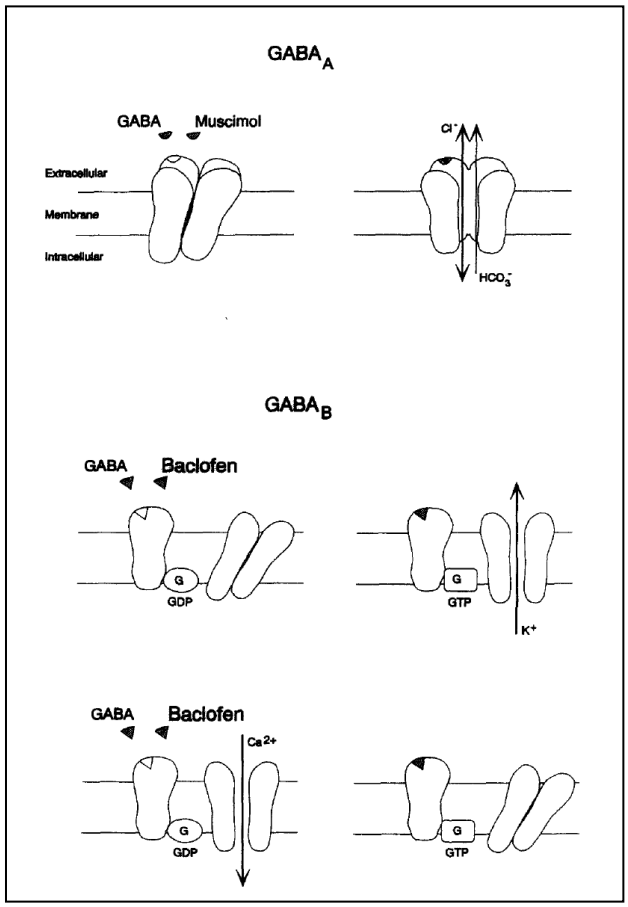

Figure 4: Diagram of $GABA_A$ and $GABA_B$ function. Ionotropic $GABA_A$ receptors increase cell permeability to chlorine through an integrated protein channel. $GABA_B$ receptors act through a G-protein and open a K^+ channel or inactivate a Ca^{2+} channel. Muscimol and baclofen are GABA agonists. From Kaila, 1994.

The first direct evidence for ethanol's effect on GABA transmission was gained from experiments using known GABA agonists and antagonists. Muscimol, a GABA agonist, increases the sedative properties of ethanol, while bicuculline, a GABA antagonist, reduces

them. Ethanol also prevents convulsions normally caused by the administration of bicuculline. Picrotoxin, another GABA antagonist, produces symptoms similar to alcohol withdrawal.

From a more clinically relevant perspective, it was found that ethanol potentiated the effects of benzodiazepines and barbiturates. Consuming ethanol and benzodiazepines or barbiturates simultaneously greatly increases the potency of these drugs. Chronic consumption of ethanol, however, will reduce their effectiveness when taken alone; tolerance results as a consequence of GABA receptor desensitization (Weiner and Valenzuela, 2006). It was initially hypothesized that ethanol was a simple allosteric activator of GABA receptors, but subsequent research has unveiled a more nuanced mechanism. Ethanol's potentiation of GABAergic transmission is facilitated by a complex series of pre- and postsynaptic actions that differ by brain region.

Post-synaptically, ethanol binds to an allosteric region on the transmembrane domain of the $GABA_A$ receptor (Weiner and Valenzuela, 2006) (Figure 5).

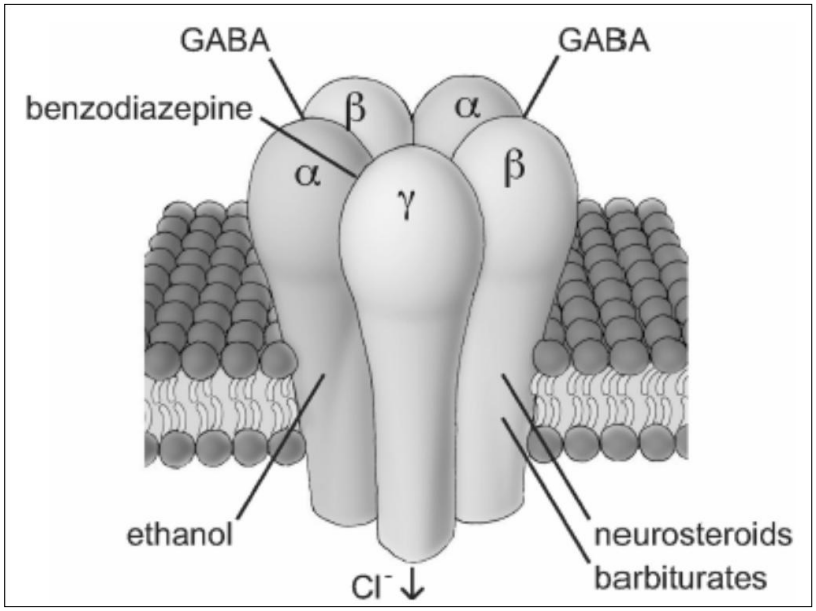

Figure 5: Binding sites of ethanol and other drugs on the $GABA_A$ receptor. Ethanol binds to the transmembrane domain. From Uusi-Oukari and Korpi, 2010.

Amino acid substitutions in the TM2 and TM3 transmembrane domains significantly reduce the effects of ethanol, so these domains are likely essential for the allosteric binding (Lobo and Harris, 2008). The $GABA_A$ receptor's affinity for ethanol is determined by its subunit composition, with receptors containing α_4, α_6, and δ subunits displaying the highest affinity for ethanol. α_1 subunits seem to be responsible for the anxiolytic effects of alcohol. The δ subunit is most commonly incorporated into extrasynaptic $GABA_A$ receptors (Korpi and Sinkkonen, 2006). These subunits are not necessarily required for ethanol binding, however, and the affinity of the δ subunit is controversial (Uusi-Oukari and Korpi, 2010). Due to the many possible subunit combinations, the subunit binding phenomenon has not been entirely explored to date.

It is suspected that certain receptor types will produce specific behavioral changes when ethanol activates them. For example, mice with hyperactive α_1 receptors were less anxious than the wild type when alcohol was consumed. This receptor-behavior specificity is true in the case of benzodiazepines. The α_2, α_3, and α_5 subunits are linked to the anxiolytic, muscle relaxant, and tolerance properties of benzodiazepines, respectively (Lobo and Harris, 2008).

Pre-synaptic actions involve multiple receptors and the mechanism is poorly understood, but clearly involves G-protein coupled receptors (GPCRs). Surprisingly, presynaptic $GABA_B$ activation by ethanol manifests itself as the inhibition rather than potentiation of GABAergic effects. Blocking $GABA_B$ potentiates the effect of ethanol, but ethanol does not seem to bind to $GABA_B$ directly. Ethanol most likely acts upstream of a G-protein pathway, ultimately increasing the amount of GABA released at the synapse. This process may involve CB1, CRF1, and/or $5\text{-}HT_{2C}$ receptors. Treating CB1 with an antagonist increases GABA release, lending credence to the hypothesis that it regulates ethanol-induced GABA release in a way similar to $GABA_B$; both are linked to the $G_{\alpha i}$ G-protein. Most GPCRs linked to this protein, when

inactivated, enhanced GABA release. CRF1 and 5-HT_{2C}, in contrast, blocked GABA release when treated with an antagonist. These GPCRs are associated with the $G_{\alpha s}$ G-protein. Further complicating matters, pre-synaptic potentiation does not seem to occur at low ethanol concentrations. A blood alcohol level of approximately 0.1% seems to be required to induce increased GABA release (Ariwodola and Weiner, 2004; Kelm, Criswell, and Breese, 2011).

Ethanol also may mediate GABAergic transmission by increasing the concentrations of certain neurosteroids, such as allopregnanolone. These compounds function as allosteric and presynaptic GABA activators. Their production probably lengthens the duration of ethanol's acute effects. Blocking the synthesis of these steroids limits some of the symptoms of ethanol intoxication (Weiner and Valenzuela, 2006).

Ethanol's influence on GABAergic transmission is complex and still under intense scrutiny, but is very likely responsible for some of its inhibitory effects on the CNS. Ethanol binds directly as an allosteric activator of $GABA_A$ based on receptor subtype, and also seems to enhance pre-synaptic GABA release through G-proteins. Due to the diversity of GABA receptors, ethanol's activity is largely dependent on brain region.

ii. Locations of Modified GABA Transmission in the CNS

GABAergic neurons are relatively ubiquitous in the mammalian CNS, and function as the primary inhibitory force in the brain. 20-30% of synapses are GABAergic (Kaila, 1994). While "inhibition" may seem to imply that these neurons simply depress neural function, their actual integrated function is far more diverse. In certain neural circuits, activation of a GABAergic neuron may actually lead to a net excitation of neurons, for example by inhibiting an inhibitory interneuron. GABAergic neurons are instrumental in shaping signals and ensuring that neurons

do not fire at the wrong time, as evidenced by the seizures caused by general GABA antagonists (Enna and Mohler, 2007).

The brain areas that are likely affected the most significantly by increased GABAergic transmission via ethanol are the hippocampus, cerebellum, amygdala, and parts of the neocortex. Postsynaptic GABA activation via ethanol has inhibitory effects in the hippocampus, the nucleus accumbens, and the central amygdala (Weiner and Valenzuela, 2006).

Ethanol presynaptically facilitates GABA release in the hippocampus, medium spiny neurons in the nucleus accumbens, and Purkinje fibers of the cerebellum. Ethanol may inhibit granule cells of the cerebellum through extrasynaptic receptors (Weiner and Valenzuela, 2006).

iii. Physiological/Behavioral Changes

Linking changes in GABAergic transmission to specific behavior is difficult, but some assumptions can be made based on key evidence: The effects of GABA agonists and antagonists when used in conjunction with alcohol, alcohol's interactions with other drugs, and the effects of alcohol on transgenic animals.

Ethanol-induced sedation and suppression of motor activity were potentiated by the GABA agonist muscimol and reversed by the GABA antagonist picrotoxin. The severity and frequency of convulsions produced by picrotoxin and bicuculline, another antagonist, were reduced by high doses of ethanol (Liljequist and Engel, 1982).

Alcohol increases the potency of other depressant drugs, including drugs that act primarily on GABA receptors. Alcohol's sedative, anxiolytic, amnesic, and hypnotic effects are similar to those produced by benzodiazepines and barbiturates, and are increased by these drugs.

Alcohol's effect on GABAergic transmission in the hippocampus and cerebellum contributes to alcohol's effect on memory and coordination, respectively. High doses of alcohol can prevent the formation of long-term memories, producing a form of anterograde amnesia. These can be "en bloc," in which no memories are recalled despite cues, or "fragmentary," where the subject remembers some but not all details. Formation of long-term memories requires proper functioning of the CA1 pyrimidal cells of the hippocampus. The CA1 neurons integrate information from other brain areas such as the neocortex and encode it as a long-term memory (White, 2003). Ethanol increases the effect of GABA on these cells through presynaptic, postsynaptic, and neurosteroid mechanisms. It is important to note that GABA is not the only neurotransmitter affecting this area; NMDA receptors are even more essential in long-term memory formation.

Alcohol binds with a high affinity to certain GABA receptors on cerebellar granule cells, which are important for motor control. In the short-term, this leads to loss of coordination and ataxic gait; with long-term heavy consumption, these neurons can actually be permanently damaged (Hanchar et al., 2005).

Mice or rats bred to lack a certain receptor type show different reactions to alcohol than the wild type. Deletion of previously mentioned alcohol-sensitive GABA receptor subunits such as α_1 blunts some of the behavioral effects of alcohol (Lobo and Harris, 2008).

GABA has also been implicated in the risk for developing alcoholism, a property also subject to genetic influence. Two lines of alcohol-preferring rats have a greater density of GABA receptors in the nucleus accumbens when compared to normal rats. $GABA_A$ antagonists decrease drinking behavior in these animals (McBride et al., 1990).

C. Acetylcholine

i. Pharmacology

Acetylcholine was the first recognized neurotransmitter, and is one of the most studied (Figure 6). It is found not only in the CNS but also in the peripheral nervous system, notably at neuromuscular junctions.

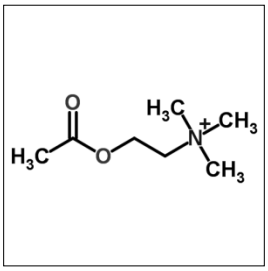

Figure 6: Acetylcholine

Like GABA, acetylcholine acts at both ligand-gated ion channel and metabotropic receptors. The most important site for ethanol action is the ligand-gated nicotinic acetylcholine receptor, or nAChR. The nAChR, as its name applies, is activated by nicotine (Figure 7).

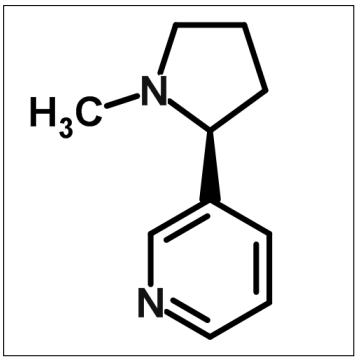

Figure 7: Nicotine, a major ligand of nAChRs.

nAChRs in the brain are permeable to sodium, calcium, or both depending on their subtype composition. The diffusion of these positively-charged ions into the cell induces

membrane depolarization and increases the chance that the neuron will fire an action potential. Acetylcholine therefore produces a postsynaptic excitatory response.

nAChRs belong to the same family of receptors as $GABA_A$ and are composed of five subunits. Unlike $GABA_A$ receptors, however, there are only α and β subtypes in the brain. The known subunits are α_2 to α_{10} and β_2 to β_4. The most common nAChRs in the CNS are the α_7 homodimer, consisting of five α_7 subunits, and the $\alpha_4\beta_2$ heterodimer, with two α_4 and three β_2 subunits. The α_7 subtype is permeable to only Ca^{2+} while the $\alpha_4\beta_2$ subtype is permeable to both Ca^{2+} and Na^+ (Figure 8) (Davis and de Fiebre, 2006).

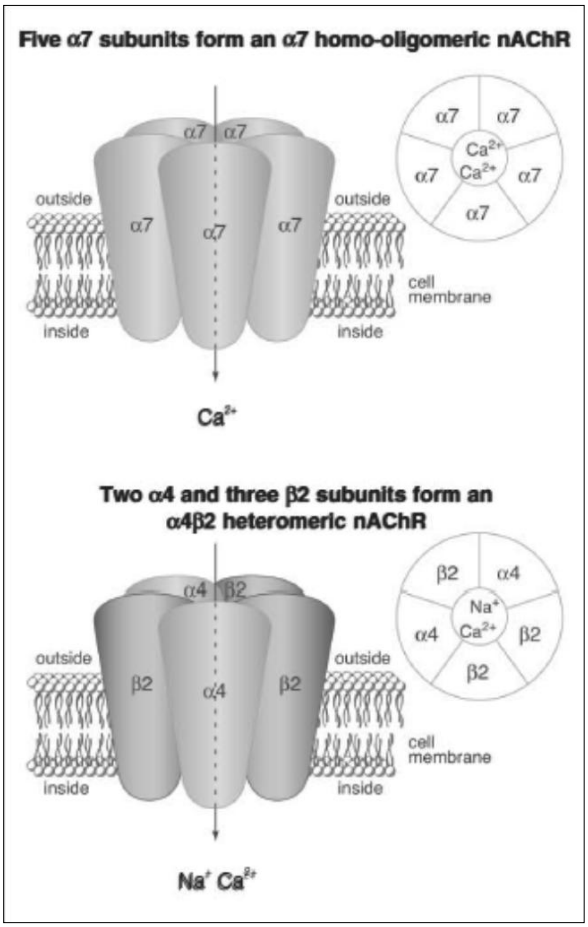

Figure 9: Structure and permeability of the α_7 and $\alpha_4\beta_2$nAChR subtypes. The former is permeable to only calcium, while the latter is permeable to both sodium and calcium. From Davis and de Fiebre, 2006.

Alcohol's effect on nAChRs is, predictably, inconsistent and varies by subtype. Current knowledge is mostly based on studies of cloned *Xenopus* frog eggs which artificially express normally absent nAChRs. The eggs are genetically modified so that they contain only one type of nAChR, thereby eliminating additive effects from multiple receptor subtypes. Ethanol changes transmission in the two previously mentioned common subtypes and in $\alpha_4\beta_4$, $\alpha_2\beta_2$, and $\alpha_2\beta_4$ receptors. Alcohol postsynaptically activates all of these receptors except for the α_7 subtype, which it inhibits.

The mechanism of action of alcohol on acetylcholine receptors is unknown, but is likely quite similar to that seen with $GABA_A$ receptors. Important to note, however, is that ethanol does not bind to the same area on nAChRs and $GABA_A$ receptors. Mutations in two amino acids essential for ethanol/$GABA_A$ receptor binding in the TM2 domain of $GABA_A$ receptors did not produce the same effect when mutated in nAChRs, implying that there is a different binding site (Borghese et al., 2002).

ii. Locations of Modified Acetylcholine Transmission in the CNS

nAChRs are not as ubiquitous as GABA receptors, but are still found in many regions of the CNS. The concentration of acetylcholine in the brain is estimated to be ten times that of norepinephrine and serotonin, and unlike monoamine systems it forms a substantial part of the basal forebrain. nAChRs are sequestered by subtype, with some subtypes being especially prevalent in certain parts of the brain. $\alpha_4\beta_2$ receptors are the most common and are not as localized as the other types. $\alpha_2\beta_2$ receptors are found in the hippocampus and interpeduncular nucleus, $\alpha_4\beta_4$ and $\alpha_2\beta_4$ are located in the dorsal interpeduncular nucleus and medial habenula, and α_7-containing receptors are found in the cortex and limbic system (Cardoso et al., 1999). $\alpha_4\beta_2$

receptors have been shown to stimulate GABA release from interneurons in the hippocampus and the medial septum. This stands as an example of the complexity of neurotransmission; an excitatory neurotransmitter may activate an inhibitory neuron, or vice-versa if the inhibitory neuron de-activates an interneuron that inhibits an excitatory neuron. There is no simple direct link from changes in neurotransmission to changes in whole brain regions, much less in behavioral manifestations.

iii. Physiological/Behavioral Changes

Nicotinic acetylcholine receptors are activated by nicotine and most studies examining behavior have focused on ethanol's interaction with nicotine. Others have shown the influence of nAChRs on ethanol consumption. The α_7 receptor subtype seems to mediate a number of alcohol's effects.

Smoking and alcohol dependence are highly correlated. Alcoholics are 60% more likely to smoke than the general population (Corrigall et al., 2000), and nicotine dependence is three times more prevalent among alcoholics (Kamens, Andersen, and Picciotto, 2010). Genetics explain 68% of the covariance between alcohol and cigarette use among those being treated for addiction (Kamens, Andersen, and Picciotto, 2010). The nAChR is linked to the comorbidity of these disorders. Alcohol has been shown to slow desensitization of the common $\alpha_4\beta_2$ receptor subtype, presumably potentiating the effects of nicotine. Conversely, pretreatment with nicotine increases the self-administration of alcohol. Alcohol consumption was no different among groups when the group treated with nicotine also received mecamylamine, a nAChR antagonist. Nicotine may sensitize nAChRs, with subsequent alcohol producing a stronger effect (Corrigall

et al., 2000). In a study using rats bred for high or low alcohol sensitivity, the high sensitivity rats were also more sensitive to nicotine.

Deletion of the allele for the α_7 subunit significantly reduces ethanol consumption. Mice lacking this subunit are more susceptible to the locomotor effects of alcohol, but show less memory impairment. They consume less alcohol than genetically "normal" mice. The β_2 subunit mediates alcohol withdrawal and sedation. Deletion of this subunit reduces withdrawal symptoms (Kamens, Andersen, and Picciotto, 2010).

D. Dopamine Transmission

i. Pharmacology

Dopamine is an important neurotransmitter in areas of the brain that control pleasure and reward. It has been well-studied due to its effects on behavior, particularly its relation to addiction. It has important roles in the CNS and is also a hormone (Niznik, 1987). It is a catecholamine and is structurally related to epinephrine and norepinephrine (Figure 10).

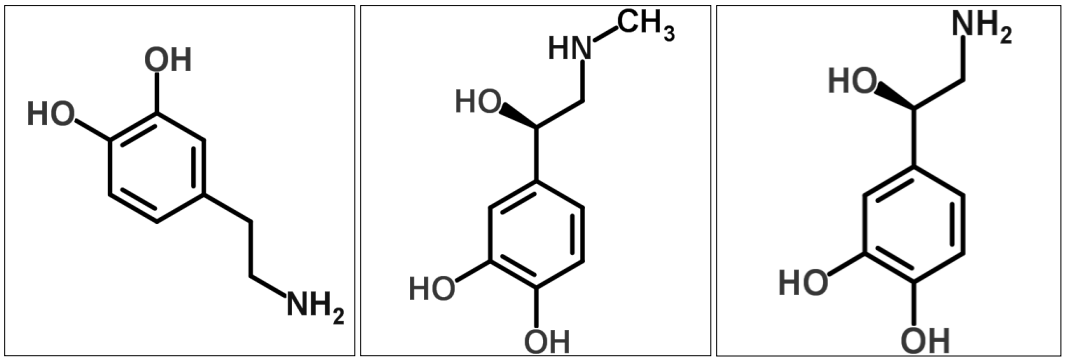

Figure 10: From left to right: Dopamine, epinephrine, norepinephrine

Dopamine receptors are universally G-protein coupled receptors. They are Class A GPCRs, structurally distinct from Class C $GABA_B$ receptors. Dopamine receptors belong to two main classes: D_1-like and D_2-like receptors. D_1-like receptors include D_1 and D_5; D_2-like includes D_2, D_3, and D_4 (Neve, 2010). In this section, the term D_1 receptor will refer to that specific subtype rather than all D_1-like receptors; the same applies to D_2 receptors.

The type of dopamine receptor determines its sensitivity to dopamine agonists and antagonists, with differences most significant among classes, i.e. D_1-like and D_2-like. However, there is overlap with some substances and the final effect of one receptor may be dependent on or modulated by the function of others, as is the case in reinforcing behavior (Missale et al., 1998).

Dopamine receptors do not have an ion channel and are unable to directly influence the permeability of ions and the voltage potential of the cell. Dopamine is often considered a "neuromodulater" rather than a true neurotransmitter because of this, although it certainly has net effects on neurotransmission (Chiara, 1997). Instead of opening or closing an ion channel, dopamine receptors act on second messenger systems. A major aspect distinguishing the two classes of dopamine receptors are their effects on adenyl cyclase. D_1 receptors activate a coupled adenyl cyclase while D_2-like receptors are not coupled to it and instead inactivate it by indirect mechanisms (Missale et al., 1998).

Dopamine receptors consist of seven transmembrane domains (Figure 11). The D_1-like receptors and D_2-like receptors are distinguished in part by amino acid differences in TM domains. Some dopamine receptor types have multiple genetic variants, but in functional terms they are fairly indistinguishable (Missale et al., 1998).

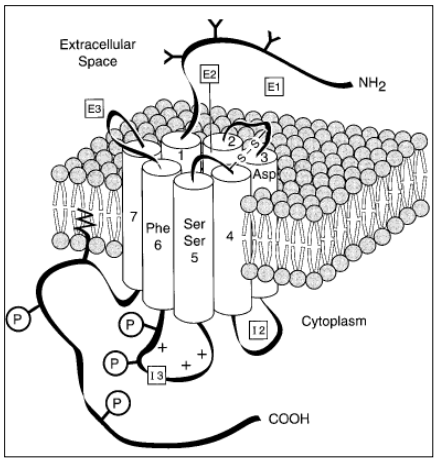

Figure 11: Representation of a dopamine receptor. Class C GPCRs have seven transmembrane domains. From Missale et al., 1998.

The indirect action of dopamine receptors manifests itself in two main ways: By modifying the activity of a dopaminergic neuron's other ion channels, or by influencing neurotransmitter release via extrasynaptic receptors. In the first case, the postsynaptic binding of dopamine changes the activity of ion channels, particularly glutamate. The nature of the change is based on the type of dopamine receptor activated. D_1-like receptors potentiate the effects of NMDARs, a type of excitatory glutamate receptor. AMPARs, another type of glutamate receptor, are inhibited by D_2-like receptor activation (Chiara, 1997).

Dopamine that does not bind to the postsynaptic cell or get degraded at the synapse diffuses into the extracellular fluid and may bind to extrasynaptic receptors. The activation of these receptors is a presynaptic effect, and serves to enhance or inhibit neurotransmitter release from the presynaptic neuron. In general, activation of extrasynaptic D_1 receptors increases neurotransmitter release, with D_2-like receptors producing the opposite effect. The specific neurotransmitter released is dependent on the individual characteristics of the neuron (Chiara, 1997).

Dopamine receptors alter neurotransmission through multiple indirect second messenger intracellular mechanisms. The primary mechanisms involve adenyl cyclase, the sodium-potassium pump, calcium channels, potassium channels, and the release of arachidonic acid. As mentioned previously, D_1-like receptors are coupled to adenyl cyclase and stimulate its activity. D_2-like receptors are not coupled to adenyl cyclase but may inactivate it by other means. D_1 receptors inhibit the activity of the sodium-potassium pump, which actively transports potassium into the cell in exchange for sodium (Missale et al., 1998). This pump maintains the electrochemical gradient of all cells, and is particularly important in the case of neurons.

Both D_1 and D_2-like receptors can affect intracellular calcium levels. D_1 receptors most likely increase Ca^{2+} levels by the activation of protein kinase A (PKA). PKA causes the release of intracellular Ca^{2+} by increasing cAMP, and also increases the permeability of Ca^{2+} ion channels by phosphorylation. D_2-like receptors can increase or decrease calcium levels depending on the specific receptor type and its location. D_2-like receptors also cause an efflux of potassium, resulting in hyperpolarization. This is thought to inhibit further dopamine release and causes the secretion of prolactin in the pituitary. Lastly, D_2 receptors cause the release of arachidonic acid, which is involved in its own second messenger pathway (Missale et al., 1998).

The current understanding is that ethanol does not bind to dopamine receptors. Ethanol increases dopamine release by acting upstream of dopaminergic neurons which innervate the mesolimbic system, particularly the nucleus accumbens (Chiara, 1997). This process seems to be dependent on acetaldehyde and opioid peptides (Chiara, 1997; Melis, et al., 2007). Spontaneous drinking behavior is suppressed by chelation of acetaldehyde by $_D$-penicillamine, and dopamine release associated with ethanol in some neurons of the brain stem is blocked by

inactivating alcohol dehydrogenase (Melis et al., 2007). Dopamine release in the nucleus accumbens is blocked by opioid peptide antagonists (Chiara, 1997).

ii. **Locations of Modified Dopamine Transmission in the CNS**

The location of dopaminergic neurons is relatively specific and well-mapped. Dopaminergic neurons originate in three basal areas of the brain: The substantia nigra, the ventral tegmental area, and the hypothalamus (Missale et al., 1998). These areas give rise to three pathways; the one most activated by alcohol is the mesolimbic system, stemming from the substantia nigra and ventral tegmental area. The mesolimbic system includes the ventral striatum, olfactory tubercle, and nucleus accumbens. The other two pathways are commonly known as the mesocortical and nigrostriatal systems (Chiara, 1997).

The D_1 receptor is the most prevalent in the central nervous system, and is found in all three mesolimbic system regions. The D_2 receptor is not as widespread but is also distributed across the mesolimbic system; D_3, D_4, and D_5 receptors are rare. D_1 and D_2 receptors work in tandem to produce the reinforcing effect of alcohol.

iii. Physiological/Behavioral Changes

Dopaminergic pathways are the brain's reward system. The release of dopamine is associated with pleasurable stimuli and behavioral reinforcement. In simple terms, dopamine release motivates an individual to seek out and engage in certain activities; for example, the acquisition and consumption of food. Motivational and participatory stimuli are known as "appetitive" and "consummatory," respectively. Certain areas of the reward system are involved

in each aspect of behavior. In the case of food, the mesolimbic system is activated only by consummatory stimuli while the mesocortical system is activated by both (Chiara, 1997).

The reward system is also involved in learning. Individuals begin to associate neutral stimuli, such as the smell of alcohol, with the actual reward brought about by drinking it. The behavior is reinforced, making it more likely to be repeated. In the case of alcohol, this can lead to habit-forming and dependence.

Dopamine has been implicated as a crucial neurotransmitter for the development of alcoholism and addiction in general. Substances that cause the release of large amounts of dopamine, such as cocaine and amphetamines, are uniformly addictive. Withdrawal from these drugs is associated with sub-threshold dopaminergic transmission due to down-regulation of dopamine receptors in the reward system of the brain. Anhedonia and depression are common manifestations of dopamine-specific withdrawal.

Dopamine release in the nucleus accumbens is correlated with feelings of reward and pleasure; not surprisingly, alcohol increases this effect. While normal reward stimuli, like the taste of food, cease to be pleasurable with repeated exposure, ethanol has an unlimited reinforcing effect via its chemical activation of the reward pathway (Chiara, 1997).

D_1 and D_2 receptors mediate different aspects of reinforcement. The activation of D_2 receptors actually reinforces the behavior, but requires the activation of D_1 receptors by endogenous dopamine (Missale et al., 1998). Polymorphism in the D_2 receptor is correlated with risk of alcoholism and quantity of alcohol consumed per week. Individuals considered $A1^+$, i.e. having an A1A1 or A1A2 genotype, were more likely to be dependent on alcohol and consumed more of it. The same trend was seen in nicotine consumption but not dependence (Table 5) (Connor et al., 2007

Measure	$A1^+$ Allele		$A1^-$ Allele		Effect Size	
Smoking Indices	Mean	S.D.	Mean	S.D.	F	P
Cigarettes per day	36.2	16.8	27.5	14.3	6.43	0.014
Nicotine Content (mg)	1.36	0.41	1.17	0.47	3.55	0.063
Fagerstrom Tolerance Questionnaire	7.07	2.61	6.24	2.83	1.74	0.191
Drinking Indices						
Drinking Frequency (days per week)	6.20	1.58	6.06	1.37	0.19	0.662
Drinking quantity per day (g)	217	106	167	84	5.70	0.019
Alcohol Dependence Scale (ADS)	33.76	9.34	29.94	8.89	3.44	0.067
Composite Variables						
Nicotine Dose per week (mg)	364	203	235	168	9.81	0.002
Ethanol Dose per week (g)	1366	768	1031	587	5.00	0.028

Table 5: Nicotine and alcohol consumption and dependence based on dopamine receptor allele. From Connor et al., 2007.

E. Glutamate Transmission

i. Pharmacology

Glutamate is the major excitatory neurotransmitter of the CNS (Figure 12). It plays a part in synaptic plasticity, learning, and development (VanDongen, 2008). Glutamate-mediated transmission is modified by alcohol.

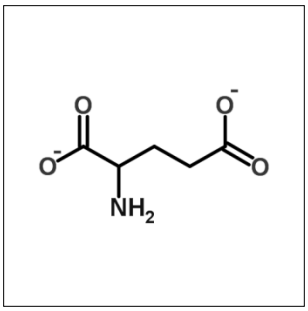

Figure 12: Glutamate

Glutamate receptors come in ionotropic and metabotropic varieties. Most important in mediating the effects of alcohol are the ionotropic receptors known as AMPA and NMDA. Their names are derived from their respective synthetic ligands, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-Methyl-$_D$-aspartate (NMDA). Both are ligand-gated ion channels in the same class as $GABA_A$, nACh, and serotonin receptors.

NMDA receptors are responsible for long-term changes following glutamate release. NMDARs are associated with neural plasticity and the modulation of synaptic signal strength through their regulation of intracellular calcium. NMDAR antagonists include ketamine, nitrous oxide, and PCP, well-known for their anesthetic and dissociative effects.

NMDA receptors have seven possible subunits: NR1, NR2A, NR2B, NR2C, NR2D, NR3A, and NR3B (Petralia and Wenthold, 2008). They always contain one NR1 along with one or two subunits of the others, typically NR2. The most common configuration in adult rat brains is NR1/NR2A/NR2B (Dodd et al., 2000). To be activated, NMDARs require glutamate, glycine, and depolarization. Mg^{2+} blocks the ion channel in a voltage-dependent manner; at resting potential, the channel is blocked. When glutamate is released, it most likely first activates faster AMPA receptors, which depolarize the neuron and remove the Mg^{2+} (Dodd et al., 2000). When

activated, NMDARs open a calcium ion channel. Ca^{2+} is a second messenger and can have a variety of effects, including neurotransmitter release.

Ethanol inhibits the function of NMDARs non-competitively, most likely binding to its own unique area on the NR1 subunit (Krystal et al., 2003[2]). An early study demonstrated ethanol's ability to reduce NMDA-activated currents in mouse hippocampal neurons (Lovinger, White, and Weight 1989). The receptor site may be located on a transmembrane component of the NR1 subunit. However, ethanol more readily inhibits NMDARs containing NR2A and NR2B subunits than those with NR2C or NR2D, so the other subunits are also important (Algaier, 2002). Receptor subunit composition varies by brain area, and accordingly alcohol does not affect all NMDA neurons equally.

Long-term exposure to the inhibitory effect of alcohol causes neurons to up-regulate NMDAR expression. Upon cessation of ethanol consumption, the CNS experiences hyper-excitability that manifests itself as muscle tremors and seizures (Hoffman et al., 1990). It is also thought that this excessive NMDAR activity can result in neuronal death (Hoffman, 1995). This glutamate-mediated toxicity is the result of an unusually large influx of Ca^{2+} ions that overwhelms and kills the cell (Dodd et al., 2000).

AMPA transmission is also modified by ethanol. These receptors are the most prevalent excitatory neurotransmitter receptor in the nervous system, rapidly facilitating neurotransmission in essentially all brain areas. They are composed of GluR1, 2, 3, and 4 subunits. AMPAR activation quickly opens an ion channel permeable to Na^{+} and K^{+} ions. This results in a net inward flux of positive ions and depolarization of the neuron. The entire process is over within milliseconds (Petralia and Wenthold, 2008).

Ethanol inhibits AMPARs, but this effect is not as significant or well-studied as its inhibition of NMDARs. AMPARs appear to be involved in relapse as well as some other behavioral effects of ethanol. Mice bred to lack the Glur3 subunit were less likely to relapse with alcohol (Sanchis-Segura, et al., 2006). GluR1-deficient mice showed no behavioral differences when compared to wild-type, but were resistant to the hypothermic effect of alcohol (Cowen, et al., 2003). AMPARs are also up-regulated with chronic ethanol exposure, an important factor in alcohol withdrawal.

ii. Locations of Modified Glutamate Transmission in the CNS

NMDARs and AMPARs are found throughout the CNS, but some brain regions are more sensitive to alcohol-induced inhibition than others. NMDARs in the hippocampus and parts of the cortex are particularly vulnerable. Ethanol also inhibits NMDARs in the cerebellum and inferior colliculus (VanDongen, 2008). NMDARs are up-regulated in the hippocampus and to a lesser extent in the cortex, thalamus, and striatum (Dodd et al., 2000). The role of AMPARs is less clear.

iii. Physiological/Behavioral Changes

NMDAR inhibition is one of the primary reasons for the behavioral changes seen in alcohol intoxication. It is suspected that GABA tends to play a greater role at low doses and NMDARs are responsible for higher dose effects. NMDAR antagonists like ketamine and PCP produce effects subjectively similar to high doses of ethanol, and cross-tolerance has been observed (Krystal et al., 2003[1]).

NMDARs are involved in forming long-term memories through a process termed long-term potentiation, or LTP. In LTP, stimulation of a neuron induces Ca^{2+} influx and a subsequent long-term change in the activity of the cell. Upon receipt of familiar stimuli, the neuron's response will be greater than it was in the past. Alcohol's inhibition of NMDARs in hippocampal CA1 pyramidal cells prevents LTP and thus the formation of memories, at least in part. Numerous brain regions and neurotransmitter systems are involved in memory formation (White, 2003).

In regards to the development of alcoholism, it is theorized that NMDAR inactivation acts as a negative feedback system that encourages an individual to stop drinking. Former alcoholics and non-alcoholics who are genetically predisposed to develop alcoholism show a blunted response to NMDA antagonists, suggesting that a lack of NMDAR-mediated negative feedback may promote excessive drinking and the eventual development of alcoholism (Krystal et al., 2003[1]). Mutations in rats that decrease the sensitivity of NMDARs to NMDA antagonists or increase overall NMDA sensitivity are correlated with a higher preference for alcohol (Krystal et al., 2003[2]).

Increased glutamatergic transmission by activation of both NMDARs and AMPARs during withdrawal increases the likelihood of relapse after drinking cessation. Some withdrawal symptoms such as depression and insomnia may persist for months and seem to be mediated by NMDA dysfunction that will return to "normal" with the consumption of alcohol (Krystal et al., 2003[2]). As previously mentioned, mice with a knockout of the Glur3 subunit of AMPARs are not as likely to relapse and have decreased withdrawal, suggesting that AMPA plays an important role in alcoholism and withdrawal (Sanchis-Segura et al., 2006).

F. Serotonin Transmission

i. Pharmacology

5-hydroxytryptamine, also known as 5-HT or serotonin (Figure 13), is a monoamine neurotransmitter that performs a variety of functions in the CNS in addition to functioning as a hormone in the rest of the body. It was first recognized as a regulator of blood pressure.

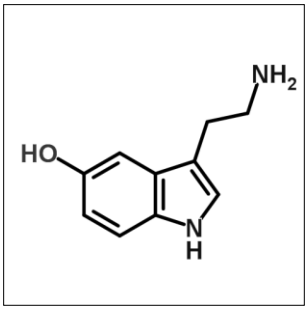

Figure 13: Serotonin

Serotonin has been remarkably conserved throughout evolution and is used in neurotransmission by organisms as simple as protists (Nichols and Nichols, 2008).

Serotonin receptors in mammals are very diverse; there are six classes of G-protein coupled receptors and one ligand-gated ion channel, 5-HT_3. Both types are implicated in the neurological effects of alcohol and in alcoholism (Wallis, Rezazadeh, and Lal, 1993). The GPCRs are further split into subclasses. 5-HT_1 has a, b, d, e, and f variants; 5-HT_2 is split into a, b, and c, and 5-HT_3 comes in a and b varieties. 5-HT GPCRs are in the same class as dopamine receptors and share a similar structure, consisting of seven transmembrane regions (Roth, 2006).

5-HT receptors produce different effects depending on their class, and may excite or inhibit the neuron. Ethanol has been shown to affect 5-HT_{1a}, 5-HT_{1b}, 5-HT_2, and 5-HT_3 receptors. 5-HT_{1a} receptors are one of the most thoroughly researched classes due to their clinical significance, having been implicated in disorders such as anxiety and depression. Upon activation by serotonin, these receptors inhibit adenyl cylase and in turn reduce the cell's

concentration of cAMP (Nichols and Nichols, 2008). This typically leads to hyperpolarization of the neuron through the activation of inwardly-rectifying K^+ channels (Roth, 2006). The rest of the 5-HT_1 receptor subtypes act through a similar mechanism, although their ultimate effects on cognition vary by virtue of their distribution in the CNS.

5-HT_2 receptors stimulate the hydrolysis of phosphoinositides which in turn leads to activation of protein kinase C and increased Ca^{2+} levels, a commonly used second messenger system. The increased Ca^{2+} results in neurotransmitter release (Nichols and Nichols, 2008). Ethanol's interaction with the 5-HT_2 receptor may stimulate dopamine release in the ventral tegmental area (Lovinger, 1999).

5-HT_3 receptors are ligand-gated ion channel similar to the nicotinic acetylcholine receptor in both structure and function (Nichols and Nichols, 2008). Ethanol activates these receptors (Lovinger and White, 1991).

5-HT_3 receptors mediate the activity of GABAergic and dompaminergic neurons in a way that may potentiate the effects of alcohol. Activation of 5-HT_3 receptors in the hippocampus increases GABA release and downstream inhibition of hippocampal neurons, possibly contributing to the cognitive changes in alcohol intoxication. Dopamine release in the nucleus accumbens is enhanced by 5-HT_3 activation (Lovinger, 1999).

ii. Locations of Modified Serotonin Transmission in the CNS

Serotonin itself is manufactured and released primarily by the raphe nuclei of the brain stem (Lovinger, 1999). The distribution of serotonin receptors the CNS as a whole is widespread but nevertheless well-documented. 5-HT_{1a} receptors are fairly ubiquitous and can be found in the raphe nucleus, cortex, limbic system including the hippocampus, and basal forebrain; they

are present as autoreceptors in the raphe nucleus and postsynaptic receptors elsewhere (Wallis, Rezazadeh, and Lal, 1993). 5-HT_{1b} receptors are located presynaptically on axon terminals in many areas of the brain including the raphe nucleus, CA1 pyrimidal cells of the hippocampus, the cerebellum, and the cortex (Nichols and Nichols, 2008). Notably, activation of 5-HT_{1b} receptors has been conclusively shown to modulate the depression of Purkinje cell response in the cerebellum to a greater extent than GABA alone (Wang et al., 1996).

The presence of 5-HT_2 receptors has been reported in the cortex and limbic areas. The distribution of these receptors is not exceptionally localized and is highly dependent on subtype. The 5-HT_{2a} and 5-HT_{2c} receptors are the most important for cognitive changes related to drug action. Antagonists of the 5-HT_{2a} receptor block the hallucinogenic effects of psilocybin and hallucinogenic stimulants (Nichols and Nichols, 2008).

5-HT_3 receptors are found in highest concentrations in the brainstem, where they seem to be involved in the induction of vomiting (Nichols and Nichols 2008). In regards to ethanol, however, their presence in the nucleus accumbens is considerably more important. Activation of 5-HT_3 receptors by ethanol increases dopamine release in the nucleus accumbens, indirectly contributing to the rewarding effects of alcohol (Wallis, Rezazadeh, and Lal, 1993).

iii. Physiological/Behavioral Changes

From a therapeutic perspective, alcohol's modulation of serotonin transmission may be the most important among all neurotransmitter systems. Mood, motivation, reward, and cravings for food and water are mediated by serotonin release (Nichols and Nichols, 2008; Kalant, 1988). These constructs tend to be altered in alcoholics as normal functions are overridden by a desire for alcohol. Serotonin abnormalities have been implicated in pathological aggression,

compulsive behavior, and bulimic binges, all disorders frequently co-morbid with alcoholism (LeMarquand, Pihl, and Benkelfat, 1994).

One contemporary theory is that some individuals have unusually low levels of serotonin or a lack of serotonin transporters, resulting in depression and anxiety. They may compensate for this "serotonin dysfunction" by self-medicating with alcohol (Heinz et al., 1998). Some studies have found reduced concentrations of serotonin metabolites in the cerebrospinal fluid and urine of alcoholics (LeMarquand, Pihl, and Benkelfat, 1994; Lovinger, 1999). A group of 22 male alcoholics were found to have 30% less serotonin transporters in their brainstem than healthy men; the number of serotonin transporters was further correlated with lifetime alcohol consumption and withdrawal-induced depression and anxiety. Serotonin transporters in the raphe nucleus are under-expressed in recently abstinent alcoholics; whether this is due to the use of alcohol or a genetic condition is unknown (Heinz et al., 1998). It is possible that metabolic changes brought about by alcohol intake may be the culprit for low total serotonin instead of a pre-existing condition, but pharmacological and genetic studies have supported the serotonin dysfun/ction theory.

Buspirone, a 5-HT_{1a} agonist, decreases alcohol cravings and increases length of time between heavy drinking episodes (LeMarquand, Pihl, and Benkelfat, 1994; Lovinger 1999). Odansetron and other 5-HT_3 antagonists reduced daily alcohol consumption in alcoholics presumably by blocking serotonin-facilitated dopamine release in the nucleus accumbens (LeMarquand, Pihl, and Benkelfat, 1994; Wallis, Rezazadeh, and Lal, 1993). Selective serotonin reuptake inhibitors, commonly prescribed for the treatment of anxiety and depression, potentiate the effects of alcohol by activating some of the same receptors (Sellers and Naranjo, 1988).

Genetic variations in serotonin receptors have been implicated in alcoholism, particularly among aggressive alcoholics. Mice lacking the gene for the 5-HT_{1b} receptor are more aggressive and show a higher preference for ethanol (Crabbe et al., 1996). These mice may be a good model for the subtype of alcoholics that exhibit violent and aggressive behavior along with their substance abuse, so-called "anti-social" alcoholics. Variations in the 5-HT_{1b}-encoding $HTR1_b$ gene have been linked to this subtype of alcoholism in a Finnish population (Lappalainen et al., 1998). A similar correlation has been established with the serotonin transporter gene, 5HTTLPR. This result was not restricted to anti-social alcoholics (McHugh et al., 2010). Rats with less 5-HT_2 receptors show a higher preference for alcohol (Wallis, Rezazadeh, and Lal, 1993).

Commonly prescribed antidepressants that increase serotonin concentrations in the brain have been successful in reducing drinking behavior and withdrawal symptoms in some individuals. Most of these drugs are selective serotonin reuptake inhibitors (SSRIs) and decrease the reuptake of serotonin at neural synapses, enabling serotonin to persist in the synaptic cleft for a longer period of time. SSRIs such as fluoxetine (Prozac) reduce the desire to drink and the pleasure derived from alcohol in some alcoholics but generally do not totally eliminate drinking (Lovinger, 1999). In male moderate problem drinkers, defined as those who have 7 drinks per day on average, SSRIs are effective in reducing drinking days, number of drinks, the desire to drink, and the behavioral reinforcement of alcohol (LeMarquand, Pihl, and Benkelfat, 1994). SSRIs should be particularly effective in treating alcoholics suffering from depression and/or anxiety, but ostensibly could help other alcoholics that do not have these conditions by inhibiting the rewarding effect of alcohol (Lovinger, 1999).

G. Effects on Opioid Peptide Transmission

i. Pharmacology

Opioid receptors and their endogenous ligands, opioid peptides, are an integral part of the CNS's natural painkilling system. The most discussed and researched opioid receptors are the μ (mu), δ (delta), and κ (kappa) receptors. Their endogenous ligands are β-endorphin, enkephalins, and dynorphins, respectively (Herz, 1997). However, it should be noted that these receptor-ligand relationships are not exclusive, for example some enkephalins may bind to mu opioid receptors.

These three receptors and their ligands are present in nearly all vertebrate species and are thought to have evolved into their modern form around half a billion years ago (Dreborg et al., 2008). Unlike other neurotransmitter systems, opioidergic transmission is relatively inactive under normal circumstances. Endogenous opioids are only released when required, like in instances of extreme stress or pain (Herz, 1997).

Other than their preference for different ligands, the three opioid receptor types are nearly indistinguishable in regards to function. All are class A G-protein coupled receptors with seven transmembrane regions, analogous to dopamine receptors and most serotonin receptors. Variation in opioid receptor activity is highly dependent on the specific bound G-protein rather than the receptor type. mu, delta, and kappa receptors are associated with the same G-protein subtypes at similar frequencies, although individual receptors may differ. Opioid receptors are usually bound to the G_i G-protein and their activation is inhibitory, with some exceptions (Conner and Christie, 1999).

Activation of opioid receptors inhibits neurotransmission in the postsynaptic neuron by reducing the activity of adenyl cyclase, closing voltage-gated calcium channels, and opening K^+ channels. Potassium follows the electrochemical gradient and flows outward, hyperpolarizing the cell. Atypical stimulatory opioid receptors of the rat olfactory bulb have been shown to increase Ca^{2+} conductance and cAMP production (Lambert, 1995).

Drugs that target opioid receptors are some of the most effective known analgesics but also number among the most frequently abused and addictive substances. The source of natural opiates is opium harvested from the seed pods of the opium poppy. Opium contains a variety of opioid alkaloids and has been used as a painkiller for thousands of years. One of the primary narcotic components of opium, morphine, was isolated in the early 1800's (Waldhoer, Bartlett, and Whistler, 2004). The acetylation of morphine produces the highly addictive diacetylmorphine, or heroin. Other synthetic opiates such as oxycodone and the potent fentanyl have also been developed for medical use.

The traditional opiate effects of analgesia, euphoria, respiratory depression, and addiction are due to activation of the μ opioid receptor. Morphine, its derivatives, and other opiates that produce similar effects are strong μ opioid agonists. μ receptors are further split into μ_1, μ_2, and μ_3 subtypes. μ_1 receptors bind morphine-like drugs and enkephalins. μ_2 receptors only have an affinity for morphine-like compounds. Analgesia and addiction have been attributed to the μ_1 and μ_2 receptor, respectively (Thompson et al., 1993). μ_3 receptors, which were only recently discovered, are sensitive to opioid alkaloids but not peptides (Cadet, Mantione, and Stefano, 2003). Mice lacking the gene for the μ receptor are virtually unaffected by morphine, exhibiting no analgesia, buildup of tolerance, or withdrawal symptoms (Matthes et al., 1994).

Opiate overdose can be fatal but is easily reversed as a consequence of the very specific neurochemical binding properties of these drugs. All opiate receptors, including μ receptors, are blocked by the non-selective opioid antagonists naloxone and naltrexone (Herz, 1997). Narcan, a brand name version of naloxone, is one of the most widely-used versions of naloxone. Heroin overdose is especially prevalent due to the unknown purity of the drug and rapid loss of tolerance with abstinence. Abstinent users are unable to estimate the proper dose and inject too much of the drug, resulting in fatal respiratory depression. Even extreme cases of overdose can be reversed if an opioid antagonist is rapidly administered.

Delta receptors are involved in olfaction, motor control, cognition, and behavioral reinforcement (Mendez and Morales-Mulia, 2008). They also mediate some of the effects of opiates. Delta receptor agonists produce analgesia while producing fewer side effects than μ agonists. Delta receptors are also involved in the acquisition of morphine tolerance (Waldhoer, Bartlett, and Whistler, 2004).

In contrast to the two aforementioned opioid receptors, kappa receptors produce a negative behavioral response when activated and seem to be the most complex opioid receptor in terms of drug action. Their activation produces dysphoria and limited analgesia. Mice lacking kappa receptors have more severe morphine withdrawal symptoms (Waldhoer, Bartlett, and Whistler, 2004). They are probably crucial in balancing the effects of the other two receptors on the dopaminergic reward system (Herz, 1997).

The activation of μ and δ receptors increases the release of dopamine in the nucleus accumbens and ventral tegmental area. Activation of kappa receptors has the opposite effect. Dopamine release is reduced during opiate withdrawal (Herz, 1997). Alcohol's modulation of opioidergic transmission centers around this system.

Alcohol ingestion increases the activation of μ and δ receptors and, accordingly, dopamine release in downstream neurons. The exact mechanism for this activation has not been elucidated but seems to involve modification of endogenous opioid production and release instead of direct receptor binding. Ethanol stimulates the production of enkephalins and the release of β-endorphin (Mendez and Morales-Mulia, 2008). The magnitude of β-endorphin release is increased in individuals with a family history of alcoholism (Sander, et al., 1998). Ethanol inhibits the binding of enkephalins but not exogenous opioid alkaloids (Hiller, Angel, and Simon, 1981). It does, however, produce cross-tolerance to opiates (Herz, 1997).

ii. Locations of Modified Opioid Peptide Transmission in the CNS

Opioid receptors are distributed throughout the CNS and are also found in the spinal cord. Areas of high concentration include the caudate-putamen, nucleus accumbens, amygdala, and hypothalamus (Mendez and Morales-Mulia, 2008).

μ receptors are localized in the thalamus, nucleus tractus solatarius, nucleus accumbens, and a few other areas (Thompson et al., 1993, Mendez and Morales-Mulia, 2008). Delta receptors are more highly concentrated in sensory areas regions like the amygdala and olfactory bulb, and additionally in the nucleus accumbens. Kappa receptors comprise only 10% of the total opioid receptors in rats and are found the same locations as the other receptors (Mendez and Morales-Mulia, 2008).

The opioid receptors located in the ventral tegmental area (VTA) and nucleus accumbens are the most relevant when discussing the effects of alcohol. Stimulation of μ and δ receptors in the VTA results in dopamine release in the nucleus accumbens. There is also evidence to

suggest that activation of opioid receptors in the hypothalamus and amygdala may have an influence on the rewarding effects of alcohol, independent of dopamine release (Herz, 1997).

iii. Physiological/Behavioral Changes

As evidenced by a wealth of behavioral and genetic studies, opioidergic transmission modulates the rewarding and addictive qualities of alcohol. Multiple theories exist on this topic. Alcoholics may experience greater endogenous opioid release in response to alcohol, or might be more sensitive to this release due to receptor variation (Herz, 1997). Genetic variation in the μ receptor specifically is correlated with alcoholism (Mendez and Morales-Mulia, 2008).

The use of specific and non-specific opioid agonists and antagonists has been crucial in understanding the role of endogenous opioids on drinking behavior. The μ receptor specific antagonists β-funaltrexamine and CTOP are effective at reducing drinking behavior in rats (Roberts et al., 2000; Hyytia, 1993), as did the δ receptor antagonists naltrindole and naltribene (Herz, 1997). DAMGO, a μ receptor agonist, prompts an increase in ethanol consumption (Roberts et al., 2000). Low doses of morphine appear to "prime" alcohol consumption, but high doses discourage further drinking. The rewarding effect of the low dose seems to facilitate drinking behavior that may compensate for low endogenous opioid activity, whereas the high dose alone is sufficient to make up for the deficiency (Herz, 1997). Enkephalins, δ agonists, also reduce ethanol consumption (Mendez and Morales-Mulia, 2008; Herz, 1997).

Predictably, the non-specific opioid antagonists naloxone and naltrexone reduce ethanol consumption. They have been effective in mitigating alcohol craving and reward when used to treat some alcoholics, but results have been inconsistent. This may be due to genetic variation among opioid receptors (Mendez and Morales-Mulia, 2008).

A relatively common mutation in the μ opioid receptor gene has been shown to increase β-endorphin binding three-fold. This mutation results from an Asp for Asn substitution at the 118 position of exon 1, and is referred to as 118G (Ray and Hutchinson, 2004). In a German population, the allele frequency was 7.8% (Sander et al., 1998). Individuals with this allele show a more profound response when treated with naltrexone, and report higher levels of euphoria from alcohol consumption. They also have a much higher incidence of alcohol problems among family members, 69% versus 25% in the A variants' families (Sander et al., 1998). The allele also modulates changes in dopaminergic transmission during withdrawal (Smolka et al., 1999). Taken together, this evidence may explain why naltrexone is an effective treatment in some but not all alcoholics. Naltrexone would hypothetically reduce the euphoric effect of alcohol to a greater extent in those with the G variant than A variant (Mendez and Morales-Mulia, 2008).

Further points to consider are variations in μ receptor prevalence and β-endorphin release. μ receptor knockout mice do not self-administer alcohol (Roberts et al., 2000). Ethanol-preferring rats have higher μ receptor densities in brain areas including the nucleus accumbens (Mendez and Morales-Mulia, 2008) and show a larger and longer increase in β-endorphin release following alcohol administration (Herz, 1997). A similar trend in β-endorphin release was observed in humans at a high genetic risk for alcoholism (Herz, 1997).

IV. Neurotoxic Effects of Alcoholism

A. Mechanisms of Neurotoxicity due to Prolonged Alcohol Use

i. Thiamine Deficiency and Wernicke-Korsakoff Syndrome

Chronic consumption of alcohol compromises the body's ability to absorb and use thiamine, a B vitamin necessary for energy metabolism in CNS neurons. Mammals do not have the ability to produce thiamine from other substances, so it must be obtained through dietary means (Todd, Hazel, and Butterworth, 1999). An Australian study found that 15% of alcoholics were thiamine deficient (Kril, 1995). Thiamine deficiency among alcoholics can occur through both indirect and direct mechanisms. Alcoholics often have poor diets which may indirectly lead to thiamine deficiency through malnutrition. Alcohol directly inhibits the absorption and utilization of thiamine through its effects on digestive organs and the enzymes of thiamine metabolism.

Alcoholics typically do not have healthy lifestyles and this extends to their diet. Alcohol itself is a source of calories and alcoholics may only rarely eat actual food; in one study, alcoholics on average obtained 60% of their calories from alcohol. Even when food is consumed, it tends to be mostly carbohydrates and is lacking in vitamins and minerals. The digestion of carbohydrates requires thiamine, reducing an already insufficient supply of the vitamin (Langlais, 1995). Alcoholics that have episodes of extreme binge drinking may be more vulnerable as food is rarely eaten during these episodes and thiamine is rapidly depleted (Sullivan and Pfefferbaum, 2009).

Even if enough thiamine is consumed, alcohol may inhibit its absorption to the extent that malnutrition will still occur. Most thiamine is absorbed in the small intestine by both passive and active transport. Active transport is the primary method of thiamine absorption when it is present at normal physiological concentrations, i.e., from food rather than a vitamin supplement. Alcohol directly inhibits this active transport of thiamine, possibly by reducing the activity of the sodium-potassium pump (Hoyumpa, 1983).

Folate, B_6, and B_{12} deficiencies are also common among alcoholics and limit thiamine absorption. Thiamine facilitates its own absorption, so its depletion is partially self-perpetuating (Hoyumpa, 1983). Alcohol can damage the lining of the intestine, further restricting nutrient availability (Langlais, 1995).

Thiamine reserves are stored in the liver, but this supply will become depleted in alcoholics. Alcohol-induced damage to the liver further compounds the problem by interfering with the organ's ability to store thiamine. Alcoholics with liver cirrhosis are more likely to be thiamine deficient and have greater neurodegeneration (Kril, 1995).

Alcohol further inhibits the uptake of thiamine at the blood-brain barrier. Since the amount of thiamine absorbed by the brain seems to be just marginally more than what is required, this alone could account for a deficiency (Langlais, 1995; Todd, Hazel, and Butterworth, 1999).

The combination of thiamine deficiency and the direct actions of alcohol interferes with the thiamine phosphorylation pathway and three thiamine-requiring enzymes in the brain: Alpha-ketoglutarate dehydrogenase (αKGDH), pyruvate dehydrogenase (PDHC), and transketolase (TK) (Langlais, 1995). The phosphorylation and dephosphorylation of thiamine is essential to its proper use once it enters the cell. Phosphorylation is catalyzed by thiamine

pyrophosphokinase, which converts thiamine to thiamine diphosphate (TDP). It is reversed by thiamine diphosphatase. αKGDH, PDHC, and TK require TDP. These enzymes are involved in basic energy production in addition to neurotransmitter synthesis and their interruption will lead to devastating effects on the neuron. Long-term deactivation of αKGDH in particular induces the accumulation of lactate and alanine while simultaneously depleting acetylcholine, GABA, and glutamate. Lactic acid may cause neural damage by lowering cell pH (Todd, Hazel, and Butterworth, 1999). TK catalyzes the synthesis of lipids that form the components of myelin sheaths (Langlais, 1995).

Alcohol directly inhibits thiamine pyrophosphokinase and increases the activity of TDAase, limiting the available supply of TDP. In addition, it directly reduces the activity of TK (Todd, Hazel, and Butterworth, 1999). The end result of this insufficient intake, absorption, and enzymatic incorporation of thiamine is widespread cell damage and the profound interruption of neural function.

The most noticeable change in the brain of alcoholics is the loss of total brain volume due to white matter degradation. The white matter consists of the myelin-coated axons that form connections between neurons. There is evidence to support loss of axonal integrity, or Wallerian degeneration, in addition to demyelination. The latter seems to be at least partially reversible especially when aided by thiamine supplementation (He et al., 2007).

In a study by He et al., the significance of thiamine deficiency in alcoholism-induced brain damage was examined. The brain damage itself was also described. Acute thiamine deficiency was induced in rats by pyrithiamine, a thiamine antagonist. Rats were split into four groups: Alcohol-exposed rats treated with pyrithiamine, rats never exposed to alcohol treated with pyrithiamine, alcohol-exposed rats treated with thiamine, and rats never exposed to alcohol

treated with thiamine. All rats were fed a thiamine-deficient diet independent of their treatment protocol for two weeks, after which it was changed to a normal thiamine-containing diet. Two weeks after that, the rats treated with pyrithiamine received thiamine injections.

The pyrithiamine-treated groups had the most extensive neural degradation. By order of damage severity, the groups were: alcohol/pyrithiamine, no alcohol/pyrithiamine, alcohol alone, and no alcohol. Significant deficits were observed in myelin thickness, axon diameter, and corpus callosum thickness in the two groups treated with pyrithiamine; alcohol alone did not produce this damage but did increase the severity. Based on other studies, brain damage related to thiamine deficiency is accelerated by alcohol consumption but not dependent on it (Sullivan and Pfefferbaum, 2009). Thiamine deficiency alone, however, can cause extensive degradation (He et al., 2007).

Pyrithiamine-treated groups also had greater small fiber densities. There are two proposed explanations for this phenomenon: First, that the smaller fibers are a sign of neural regrowth. Alternatively, that thiamine deficiency selectively destroys large fibers. There is a lack of evidence for axonal regrowth in the CNS, calling into question the validity of the first hypothesis. Recovery is more appropriately attributed to myelin regeneration (He et al., 2007).

Long-term thiamine deficiency can result in extensive damage which will commonly manifest itself as Wernicke-Korsakoff's syndrome (WKS), a disorder characterized by major cognitive deficits. WKS is named Wernicke's encephalopathy and Korsakoff's syndrome, of which it shows a combined symptomotalogy. It is a surprisingly common disorder; studies have found post-mortem incidence rates of 12.5% among alcoholics and 2% among the general population (Langlais, 1995).

Wernicke's encephalopathy is caused by the formation of lesions and edema in the thalamus, mammillary bodies, periaqueductal gray matter, and colliculi, among other regions (Sullivan and Pfefferbaum, 2009; Todd, Hazel, and Butterworth, 1999). Korsakoff's syndrome is secondary to untreated Wernicke's encephalopathy and is associated with profound amnesia as a result of brain shrinkage that is most severe in the limbic system, pons, thalamus and cerebellum (Sullivan and Pfefferbaum, 2009). Enlarged ventricles and dramatic changes in the gyri and sulci can be observed in MRI images (Figure 15).

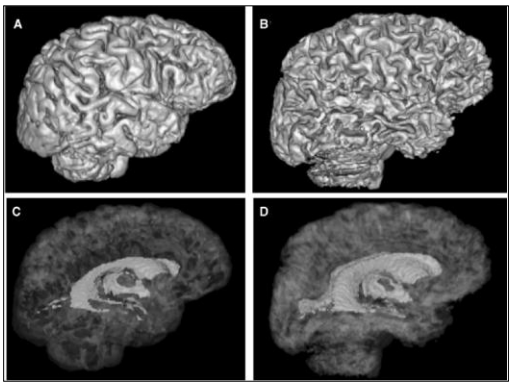

Figure 15: Shrinking of gyri (B) and enlargement of sulci (B) and ventricles (D) in a WKS brain (B, D) compared to a healthy brain (A, C). From Sullivan and Pfefferbaum, 2009.

The physical damage of Wernicke's encephalopathy leads to noticeable behavioral changes. The main symptoms are ataxia, confusion, opthalmoloplegia, and uncontrollable eye movements (Sullivan and Pfefferbaum, 2009; Todd, Hazel, and Butterworth, 1999). With no clinical intervention, alcoholics may develop the more severe ataxia and extreme memory impairment of Korsakoff's syndrome (Sullivan and Pfefferbaum, 2009). Korsakoff's syndrome results in both anterograde and retrograde amnesia for episodic memories. Affected individuals may completely lose their ability to form new memories. It is thought that damage to connections between the hippocampus, thalamus, cortex, and other structures results in significant impairments in attention, perception, and information processing. In a facial

recognition task, WKS patients had abnormally low activation of the hippocampal-anterior thalamic axis, which is necessary in the encoding of episodic memories (Caulo et al., 2005). Some WKS patients have a reduced ability to concentrate, distinguish between similar stimuli, and process visual information. Additionally, dementia-like symptoms are comorbid in 60% of WKS patients. These individuals exhibit an overall decrease in intelligence and show blunted emotional responses (Langlais, 1995).

The mechanism by which thiamine deficiency leads to Wernicke's encephalopathy has not been thoroughly researched. Neural damage has been proposed to occur through lactic acidosis, energy deficits, increased glutamate excitotoxicity, breakdown of the blood-brain barrier, and free radical formation. Nimodipine, which increases cell pH by inhibition of a Ca^{2+} channel, has a protective effect on brain structures commonly damaged in WKS. The glutamate antagonist MK801 protected the thalamus from damage (Todd, Hazel, and Butterworth, 1999). The development of WKS is genetically influenced. Genetic differences in TK, NMDA receptors, ADH, and apolipoprotein E are associated with the risk of developing the disease (Carota and Schnider, 2005; Todd, Hazel, and Butterworth, 1999). It is likely that many other genes are involved. Considerably more research will be necessary to fully understand the neurobiological basis of WKS. Thiamine is an essential cofactor in some of the most basic cellular processes and its deficiency has complex ramifications.

Fortunately, WKS can be prevented with thiamine supplementation. Fortifying alcoholic beverages with thiamine should reduce the incidence of the disease (Hoyumpa, 1983). Partial and even complete recovery from WKS has been reported as well, but is not the norm. Due to limited understanding of the disease, making a prognosis is difficult. Time since onset is the most reliable predictor. Up to 75% of WKS patients will have permanent memory impairments.

About a quarter show no improvement in ataxic symptoms. Even when thiamine is given early, a full recovery is rare (Carota and Schnider, 2005).

ii. NMDA/Glutamate Excitotoxicity

Another primary source of alcoholism-induced brain damage is glutamate excitotoxicity. Ethanol inhibits NMDA receptors. With chronic consumption, the CNS adapts by up-regulating NMDA receptor expression to preserve NMDAergic transmission. When ethanol consumption is ceased, CNS neurons do not immediately re-absorb these receptors and hyperactive NMDAergic transmission occurs leading to seizures and neurotoxicity. Damage is not restricted to the axons as in thiamine deficiency; whole cell death is common.

Chronic ethanol consumption increases the concentration of NMDA receptors and the sensitivity of CA^{2+} channels (Chandler et al., 1993; Witte et al., 2003). After three days of chronic ethanol exposure, there was a 40% increase in NMDA agonist binding in rat cerebellar granule cells (Hoffman, 1995). Ethanol also seems to increase the sensitivity of NMDA receptors to glutamate binding by changing their subunit composition (Nagy, Muller, and Laszlo, 2001; Nagy and Laszlo, 2002), and may facilitate the release of glutamate from presynaptic terminals (Hazell and Butterworth, 2009).

Cell death can occur as a result of changes in osmotic pressure and the activation of second messenger systems. *In vitro* studies have shown interruptions in Na^+ and Cl^- balance; whether this occurs *in vivo* has not been determined. NMDA receptor activation causes a large calcium influx and the over-activation of second messengers like protein kinase C as well as possible changes in neurotransmitter release, ultimately causing the death of neurons (Lovinger, 1993). This process may be partially mediated by nitric oxide (NO). Nitric oxide synthase is a

Ca-dependent enzyme and is found at a high concentration in the cerebellum. At excessive levels, nitric oxide is a neurotoxin. A large Ca^{2+} influx may result in the production of toxic amounts of NO (Lancaster, 1992; Hazell and Butterworth, 2009).

Ethanol consumption will prevent withdrawal symptoms and excitotoxicity. This serves as a strong motivator for alcoholics to continue their drinking (Chandler et al., 1993). Excitotoxicity can also be prevented with the use of NMDA antagonists or gangliosides. Ethanol preferentially up-regulates the NR2B subunit of NMDA receptors. NR2B antagonists have milder side effects than non-selective inhibitors like MK-801 so they may be appropriate for clinical treatment (Nagy et al., 2004). Gangliosides may help by blocking some of the long-term effects of calcium influx, such as the activation of protein kinase C. There is also evidence that they down-regulate NDMA receptors (Hoffman, 1995).

In humans, the cerebellum, hippocampus, and frontal cortex are most vulnerable to NMDA-mediated excitotoxicity (Chandler et al., 1993; Witte et al., 2003). Damage to these respective areas results in a loss of coordination, inability to form memories, and impaired executive functioning (Witte et al., 2003).

V. Conclusions

In humans, the vast majority of alcohol is metabolized in the liver by ADH and ALDH; the microsomal ethanol oxidizing system and catalase become more active at high ethanol concentrations. The microsomal ethanol oxidizing system can be up-regulated and adapts to chronic consumption. More efficient forms of ADH and less efficient forms of ALDH are correlated with a lower risk of alcoholism. In both cases it is due to the rapid accumulation of toxic acetaldehyde and an accompanying unpleasant flush reaction. More research needs to be conducted on first-pass metabolism to determine its significance and the role of the stomach.

Alcohol is an extremely non-selective drug and as such its effects on neurotransmission are still poorly understood. Alcohol's depressant properties seem to be mediated primarily by GABA at low doses and NMDA at higher doses; these two neurotransmitter systems are also responsible for withdrawal hyperexcitability. Alcohol's stimulation of the dopaminergic reward system through the release of serotonin, dopamine, and opioid peptides can produce behavioral reinforcement and addiction, particularly among genetically high-risk individuals. Drugs targeting serotonin and opioid receptors have been successful in reducing drinking behavior in clinical settings. While there is a wealth of information on how neurotransmitter release is affected by alcohol, knowledge of the chemical basis for this is lacking. Future studies should examine which receptors and ion channels are directly activated by ethanol.

Chronic consumption of ethanol leads to significant metabolic and neurologic disruptions. Two frequent consequences of alcoholism are thiamine deficiency and glutamate receptor over-activation, both of which are toxic to neurons. Thiamine deficiency disrupts energy production in neurons, resulting in white matter destruction and an overall decrease in brain volume. Severe damage may manifest itself as Wernicke-Korsakoff syndrome. The brain

adapts to chronic ethanol exposure by up-regulating glutamate receptors; upon cessation of alcohol consumption, glutamate transmission becomes pathologically over-active and can kill whole neurons. Numerous mechanisms have been proposed for this phenomenon, but there is little experimental evidence for any of them. Recovery from Wernick-Korsakoff syndrome has been observed, but is highly variable. It remains unclear why some alcoholics are able to make a virtually full recovery while others show no improvement in symptoms after years of abstinence and thiamine supplementation.

VI. References

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