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A Control System Hypothesis of the *N*-methyl-D-aspartate Glutamate Receptor's Role in Alcoholism and Alcohol Withdrawal

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Abstract: Alcoholism and alcohol withdrawal, complicated physiological conditions with significant consequences for physical and emotional health, have been the focus of extensive studies in various scientific disciplines for centuries. In America alone, alcoholism is known to affect 14 million people; it accounts for an estimated annual cost of $100 billion in healthcare and related productivity losses, and according to the Centers for Disease Control, there were 34,833 chronic alcohol-related deaths in 2001.

Various experimental studies have strongly suggested that many of the physiological consequences of alcoholism and alcohol withdrawal may be associated with alcohol-induced inhibition of *N*-methyl-D-aspartate (NMDA) glutamate receptors, but the mechanisms are not completely known. (Glutamate is an important excitatory neurotransmitter and its NMDA receptors are expressed widely throughout the brain.) In this paper, we postulate the following hypothesis: *that the level of unblocked NMDA receptors is controlled in alcoholism to maintain at a constant level, the amount of glutamate release and extent of glutamatergic excitation; that ethanol blocks these receptors, and additional receptors are generated to compensate for the loss;* we then develop and analyze a control system model predicated on this hypothesis. Our model shows, among other things, that upon cessation of alcohol consumption, no additional generation of NMDA receptors occurs, but the number of unblocked NMDA receptors increases dramatically, leading to the excitotoxicity observed clinically. This first attempt at a control system representation of the NMDA receptor's role is thus able to capture the essence of some key organism-wide responses observed in alcoholism and alcohol withdrawal. Future work will involve incorporating additional molecular details to obtain a more accurate model which can then be potentially useful in postulating treatment regimens for alcohol withdrawal.

1. INTRODUCTION

1.1 Background

Evidence of alcohol use and abuse dates back to the Stone Age (Patrick, 1952), and its prevalence in modern history is well-documented. In the United States alone, there were approximately 424,000 hospital discharges in 2003 that listed alcohol-related morbidity as the principal diagnosis; an additional 1.6 million discharges were documented to be alcohol-induced conditions, illnesses, or injuries (Chen et al., 2005). Furthermore, the Centers for Disease Control reported 34,833 chronic alcohol-related mortalities in the United States in 2001 (Stahre, 2004). These alcohol-related morbidities and mortalities constitute a significant healthcare epidemic. It is estimated that alcohol abuse affects 14 million Americans and accounts for an annual cost of $100 billion in healthcare and related productivity losses (Rich, 1998). However, despite centuries of alcohol research, the neurological basis, epidemiology, optimal treatment regimen, and disease progression of chronic alcoholism have not been elucidated completely.

Typically, chronic alcoholism develops in individuals who consume excessive quantities of alcohol at regular intervals over an extended period of time. These individuals adapt to the effects of alcohol, and in some (but curiously not all), this leads to a physical dependence upon alcohol. (It is important to note that not all individuals progress to alcohol dependence in this manner, nor do all excessive drinkers develop alcohol dependence.) Alcohol dependent individuals both depend upon alcohol for normal function and function normally with high circulating levels of alcohol in the bloodstream. Upon the cessation of alcohol intake, these individuals experience physical and emotional symptoms characteristic of alcohol withdrawal which may include anxiety, excessive sweating, agitation, altered consciousness, increased blood pressure, increased heart rate, seizures, hallucinations, delirium tremens, cardiac arrhythmias, or sudden death; the collection of these symptoms is known as alcohol withdrawal syndrome (AWS) (Alcohol, 2007; Kähkönen, 2004). The symptoms of AWS imply dysregulation of the body's internal equilibrium, or homeostasis, in response to a sudden decrease in blood alcohol content. Homeostasis is regulated by the brain, and our research interests lie in understanding the neurobiological maintenance of both homeostatic and emotional control in response to chronic alcohol intake and acute alcohol withdrawal.

Ethanol, the primary alcohol molecule in consumable alcoholic beverages, is absorbed into the bloodstream during digestion and then travels throughout the body via the bloodstream. The majority of absorbed ethanol is metabolized in the liver to form carbon dioxide and water, but ethanol molecules can also escape the bloodstream into other organs and tissues. A small portion of alcohol metabolism is performed in these tissues, but ethanol molecules can also interact with and affect the molecular machinery subtending normal function. Previous studies have examined and modeled alcohol metabolism (e.g. Umilis et al., 2005), alcoholism susceptibility and familial connections (e.g. Chester et al., 1998; Cowley et al., 1994), alcohol withdrawal treatment (e.g. Crabbe et al., 1994; Dahchour & de Witte, 1999; Danysz et al., 2000; Krupitsky et al., 2007), and the molecular changes that underpin the behaviours associated with alcohol dependence (e.g. Krystal et al., 2003; Roberto et al., 2004). These studies have identified many molecular changes in the brain resulting from chronic alcohol intake and withdrawal that may explain alcohol's affect on the neurobiological maintenance of homeostatic and emotional control.

In general, molecular studies of alcoholism and alcohol withdrawal in the brain have focused on the two primary neurotransmitter systems in the central nervous system: excitatory neurotransmission by glutamate and inhibitory neurotransmission by gamma-aminobutyric acid (GABA). Alcohol acts to enhance inhibition in the central nervous system by synergistically increasing GABA activity and decreasing glutamate activity (Krystal et al., 2003 & 2006). The temporal and spatial dynamic balance of these neurotransmitters within specific brain regions leads to profound effects on the behavior, mood, memory, and function of the individual. We have chosen to restrict this initial study to alcohol's effects on glutamate transmission via a specific glutamate receptor, the *N*-methyl-D-aspartate (NMDA) receptor.

1.2 N-methyl-D-aspartate (NMDA) Receptors

NMDA receptors are heteromeric ionotropic glutamate receptors found on neurons throughout the brain. Each NMDA receptor is composed of two NR1 subunits (of which there are 8 slice variants) and two NR2 subunits (of which there are four subtypes), and the specific subtype compositions differ in functionality and brain region localization allowing for brain region variability and glutamate neurotransmission specificity (Krystal et al., 2003). NMDA receptors form calcium channels that are blocked by Mg^{2+} under resting conditions. Membrane depolarization (a decrease in the electrical potential across the neuron's outer membrane caused by a shift in the electrochemical gradient across the membrane) leads to the displacement of the Mg^{2+} blockade. The NMDA receptor is then activated by the binding of both glutamate (to the NR2 subunit) and the amino acid glycine (to the NR1 subunit). The activated NMDA receptor then allows an influx of calcium, the release of glutamate, and the release of other neurotransmitters such as dopamine and norepinephrine. Tsai et al. hypothesize that it may be via the dysregulation of these other neurotransmitters that NMDA receptors contribute to the dysregulation of the homeostatic and emotional control observed in alcohol withdrawal (1995).

1.3 NMDA Receptors and Ethanol

NMDA receptors have been shown to be high-affinity targets for ethanol in the brain (Grant and Lovinger, 1995; Krystal and Tabakoff, 2002). Like known NMDA receptor antagonists, ethanol molecules attenuate NMDA receptor function (e.g. Grant and Lovinger, 1995). Several studies have shown that to compensate for the chronic blockade of NMDA receptors by ethanol, chronic ethanol treatment induces some brain regions to generate new NMDA receptor subunits (e.g. Roberto et al., 2004, 2006; Rossetti et al., 1999). Ethanol dissociates from the NMDA receptors during withdrawal, leading to an increased number of unblocked NMDA receptors. Unblocked NMDA receptors promote the release of additional glutamate via a positive feedback mechanism of glutamate-induced glutamate release (Rossetti et al., 1999). The excess glutamate from the additional unblocked NMDA receptors could then lead to excessive excitation known as excitotoxicity, which can be harmful to the cells. Increased glutamate release is observed experimentally in chronic alcohol treated animals withdrawing from alcohol (Rossetti et al, 1999). Many of the symptoms of alcohol withdrawal are associated with excess excitation, such as that resulting from glutamate-induced excitotoxicity. NMDA receptor antagonists have been shown to reduce withdrawal symptoms and provide dopamine-independent euphoria comparable to that provided by ethanol consumption (Krupitsky et al., 2007; Danysz et al., 2000). Conversely, NMDA receptor agonists increase the magnitude of dysphoria and the symptoms of withdrawal (Krystal et al., 2003).

We hypothesize that, in some brain region(s) critical to homeostatic and emotional control, the number of NMDA receptors, under feedback control, increases with chronic ethanol intake such that the number of unblocked, viable NMDA receptors remains approximately constant in order to maintain, at a desired level, the glutamatergic inhibition and the amount of glutamate release. Between drinking sessions and upon the initiation of withdrawal, ethanol dissociates from the blocked NMDA receptors leading to an increase in the NMDA receptor response to extracellular gluatamate and increased physical and emotional discomfort. This drives the alcohol adapted individual to consume more alcohol to raise the blood alcohol level and repress the withdrawal symptoms. In the case of a more prolonged withdrawal, the increase of unblocked, viable NMDA receptors leads to increased glutamate via the positive feedback mechanism mentioned earlier and increased response to glutamate by virtue of the increased number of NMDA receptors—thereby contributing to the more severe withdrawal symptoms and dysphoria observed during such an episode of withdrawal. It is important to note that in some brain regions, kinases and phosphotases act at modulatory sites on the NMDA receptor (e.g. Wang et al., 2007), but the ultimate result is a sensitization of NMDA receptors to ethanol, and the present

analysis of increased receptor number is analogous to any such increase in sensitization.

2. CONTROL SYSTEM ABSTRACTION

2.1 Alcohol Intake and Response

Alcohol intake inhibits dysphoria by modulating the concentrations and activities of neurotransmitters and neurotransmitter systems. Figure 1 shows our abstraction of the concepts of euphoria maintenance by alcohol intake in a control system block diagram. The behavioral processes and consequences associated with the intent and action of alcohol consumption control the individual's desire for and consumption of alcoholic beverages based upon the deviation between actual (perceived) and expected states of euphoria. As the perceived level of euphoria decreases below expectations, this composite controller acts to adjust neurotransmitter concentrations and encourage additional intake of ethanol. The alteration of neurotransmitter concentration or blood alcohol level then affects the neurotransmitter response. Increased ethanol concentration in the brain leads to augmentation of GABA activity and inhibition of NMDA activity. The activity of both neurotransmitters are integrated into the neuronal processes of generating mood and euphoria.

2.2 NMDA Receptor Dynamics

We are currently focusing on the NMDA receptor subsystem highlighted by the red dotted box in Figure 1, and we hypothesize that the activity of the NMDA receptor system is itself under control with the objective of maintaining a reasonably constant level of NMDA receptors for normal function. We assume that the glutamate load of these neurons from other processes is constant such that the differential input corresponding to the alcohol effect can be represented by a single alcohol intake function.

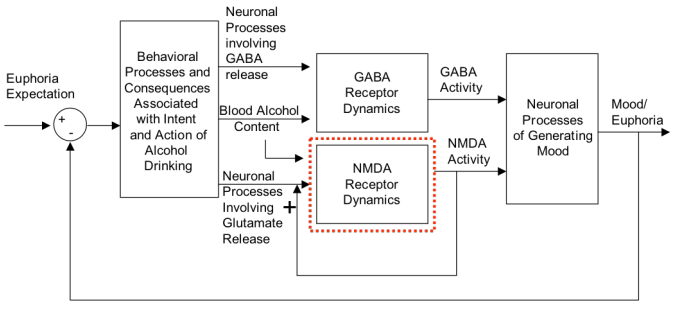

Figure 1: Block diagram representation of euphoria maintenance by alcohol intake. Consumption of alcohol is accompanied by an expectation of euphoria. The brain controls the behavioral and molecular processes associated with obtaining the expected euphoria in response to alcohol intake via two major systems of neurotransmission: GABAergic and glutamatergic (primarily via NMDA receptors). Each system is independently controlled, and it is the principal portion of the NMDA receptor system (highlighted by the dotted box) that we have chosen to study further.

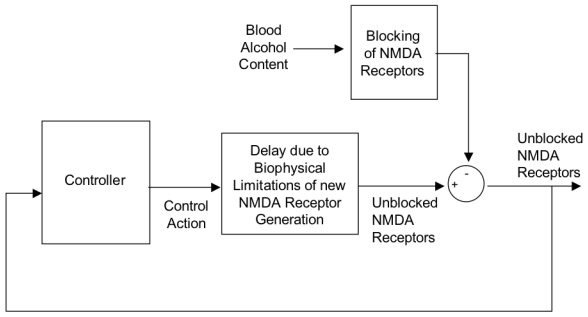

Figure 2: Control system block diagram for the control of unblocked NMDA receptors. The controller has an implicit set point and generates new unblocked NMDA receptors with a delay due to the biophysical limitations of the cell's transcription, translation, and post-translational machinery. Blockage of NMDA receptors by alcohol is represented as a disturbance.

As illustrated in the block diagrammatic representation in Figure 2, a feedback controller controls new NMDA receptor generation in order to maintain a certain number of unblocked NMDA receptors (U), or equivalently, to regulate the total amount of glutamate release. Alcohol intake is a disturbance to the process, decreasing U via ethanol blockage of NMDA receptors. From this control system perspective, the decrease in U causes an increase in controller action, which leads to an increase in NMDA receptor generation, consistent with experimental observations in certain brain regions (e.g. Roberto et al., 2004, 2006; Rossetti et al., 1999). The control system in Figure 2 thus represents our hypothesis *that the level of unblocked NMDA receptors is controlled in alcoholism to maintain at a constant level, the glutamatergic inhibition and the amount of glutamate release; that ethanol blocks these receptors, and additional receptors are generated to compensate for the loss.*

3. MODELING AND ANALYSIS

The model consists of two ordinary differential equations describing the number of unblocked (U) and ethanol-blocked (B) NMDA receptors in a generic neuronal population. The total number of NMDA receptors can be determined at any time as the sum of the blocked and unblocked species.

As illustrated in Figure 2, the number of unblocked NMDA receptors is influenced by both the controller activity and the blockage of NMDA receptors by ethanol molecules. We assume that implicit within the process of new NMDA receptor generation are terms accounting for the natural degradation of both species. If we include these terms and assume elementary mass action kinetics for the blocking reactions:

$$U + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B, \qquad (1)$$

where A represents a dimensionless blood alcohol content, the equations governing the number of unblocked NMDA receptors (U) and blocked NMDA receptors (B) are:

$$\frac{dU(t)}{dt} = -k_1 A(t)U(t) + k_2 B(t) + C(t-\alpha) - k_{du}U(t) \quad (2)$$

$$\frac{dB(t)}{dt} = -k_2 B(t) + k_1 A(t)U(t) - k_{dB}B(t) \quad (3)$$

where k_{du} and k_{dB} represent the rates of natural degradation of unblocked and of blocked NMDA receptors respectively; C(t-α) represents the controller action indicating the creation of new unblocked NMDA receptors via transcription and translation; and the indicated delay, α, accounts for the time required for transcription, translation, and post-translational processing. We estimate α to be 3 hours.

The control law that we hypothesize for this system is shown in Equation 4. Various previous studies of biological control systems have shown similar relationships and/or utilized similar mathematical representations of steady state controller action (e.g. alveolar ventilation rate: Guyton and Hall, 1996; Glass and Mackey, 1988; pupillary reflex: Clarke et al. 2003). We chose this formuation to mimic the biophysical limitations of a cell's transcription and translation machinery.

$$C(t) = y_{\max}\left(\frac{a^n}{[U(t)]^n + a^n}\right) \quad (4)$$

In Equation 4, y_{max} represents the maximal rate of production of new NMDA receptors; *a* and *n* are location and shape parameters, respectively. In this model, we chose y_{max}=4 receptors/hour, *a*=100 receptors, and *n*=4. The steady state characteristics of the controller with these parameters are shown in Figure 3.

We chose to represent the blood alcohol level as a gradually increasing function with regular periodicity, as shown in Equation 5:

$$A(t) = \begin{cases} 0 & \\ 0 & if \begin{cases} \sin(pt) < 0 \\ t > t_w \end{cases} \\ Z\sin(pt)\exp(gt) & otherwise \end{cases} \quad (5)$$

The periodicity parameter, *p*, was chosen to be 0.75hr^{-1} such that the period of the waveform was approximately 8 hours. This corresponds to three daily alcohol intakes. The growth parameter, g, was chosen to be 0.001hr^{-1} so that the intake increased gradually over time. In order to generate a dimensionless representation of blood alcohol content and to scale the blood alcohol content to different levels for different populations (such as different genders or organisms), the scalar Z is incorporated in the definition of A(t). Z represents the ratio of blood alcohol level after consumption of a specific volume of ethanol in the population of interest to the blood alcohol level after consumption of the same volume of ethanol in the reference sample. In this study, we model the reference sample, and therefore Z was set to unity. The parameter, t_w, is the time at which the withdrawal period begins; it was set to 48 hours prior to the end of the simulation to mimic a 48 hour withdrawal. Figure 4 shows the alcohol input for the first 24 hours of simulation with three periods of alcohol intake. The alcohol input for all 1000 hours of simulation is shown in Figure 5B.

We implemented the model with the following parameter set (k_1=0.05hr^{-1}, k_2=0.0005hr^{-1}, k_{du}=0.02hr^{-1}, k_{dB}=0.02hr^{-1}, U(0)=100 receptors, B(0)=0 receptors, t_w=48hr), and were able to generate the following response characteristics: an increase in the number of blocked and total NMDA receptors with repetitive alcohol intake, a sharp decrease in the number of blocked NMDA receptors and a corresponding increase in unblocked NMDA receptors upon withdrawal, and dynamic changes in unblocked receptors with alcohol intake. Figure 5 shows the resulting number of unblocked, blocked, and total NMDA receptors, over a 1000 hour period, along with the

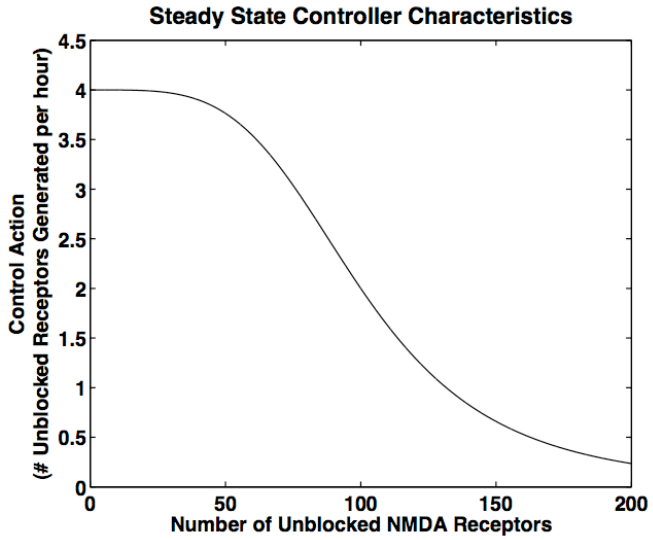

Figure 3: Steady state characteristics of the controller action as a function of the number of unblocked NMDA receptors as described in Equation 4 (ymax=4 receptors/hr, a=100 receptors, n=4).

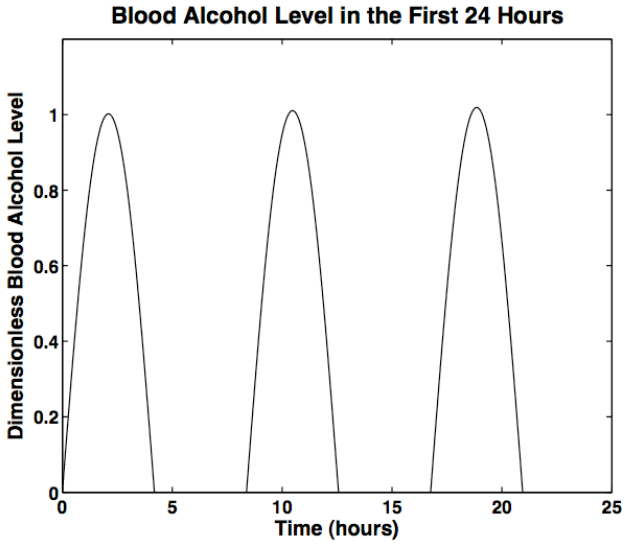

Figure 4: Dimensionless blood alcohol level disturbance function for the first 24 hours of simulation. The three intakes are approximately eight hours apart.

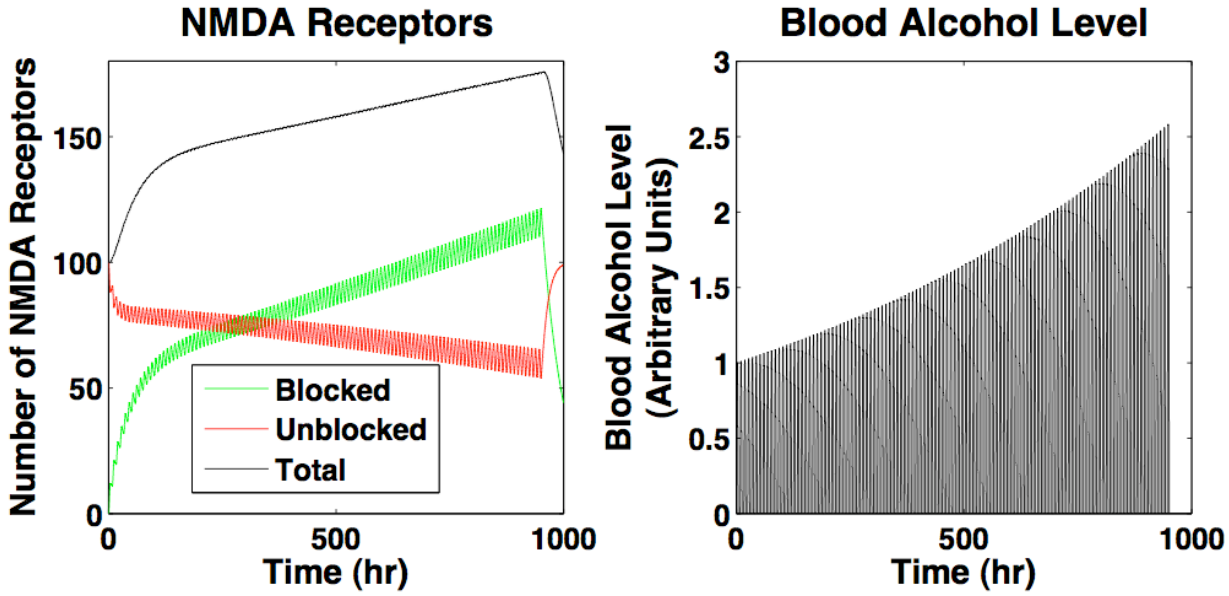

Figure 5: A) The dynamics of blocked, unblocked, and total NMDA receptors in response to a growing periodic alcohol input function (B). The total number of NMDA receptors increases with each dose of alcohol as well as throughout the simulation, and also drops abruptly during withdrawal, as expected. Similarly, the number of blocked receptors increases until the alcohol input is removed, when it drops rapidly. Conversely, the number of unblocked receptors decreases until the point when the alcohol input is removed, when it increases, perhaps leading to the excitotoxicity observed clinically.

input blood alcohol level function. These are all consistent with previous experimental results of increased NMDA receptors in response to chronic alcoholism. Furthermore, the sharp increase in the number of unblocked NMDA receptors during the final withdrawal period agrees with the experimental observation of increased glutamate response.

4. DISCUSSION

Complex physiological disorders such as alcoholism and alcohol withdrawal involve the conglomeration of many different interacting molecular systems. While we do not intend to diminish the importance of the other systems, we were able to generate results with our reduced model that match the experimentally observed organism-wide responses to chronic alcohol and withdrawal. Our model captures an increase in the total number of NMDA receptors with prolonged alcohol intake. Upon the cessation of alcohol intake (t=952 hours, ~40 days, in this simulation), the total number of NMDA receptors decreases, but the number of unblocked NMDA receptors increases dramatically. This leads to an increased *response* to glutamate as well as an increased *release* of glutamate via the positive feedback loop indicated in Figure 1. Increased glutamate release leads to the excitotoxicity and seizures, and these are also the symptoms observed clinically during alcohol withdrawal.

The experimental evidence of increased NMDA receptor concentration during chronic alcoholism is conflicting, however. Some groups found statistically significant NMDA subunit gene expression differences between control and chronic alcohol-treated animals (e.g. Roberto et al., 2004), while others found no significant changes (e.g. Läck et al., 2005). These studies were conducted in various brain regions, but although different brain regions may exhibit different gene expression patterns, our model can be adapted to any method of sensitization to alcohol (e.g. inhibition of NMDA receptors by phosphorylation of NMDA receptor subunits) to achieve a similar increase in activity and glutamate release upon alcohol withdrawal.

We have described here a relatively simple first attempt at a control system representation of a hypothesis regarding alcoholism and alcohol withdrawal. Future work will involve expanding the model to introduce more molecular details including the activity of and interactions with the other predominant neurotransmitter, GABA. Clinical evidence shows that the co-administration of NMDA receptor antagonists *and* GABA receptor agonists yields more ethanol-like effects than either alone (Hodge and Cox, 1998). Our ultimate goal is to create a model that includes both of these systems and would allow *in silico* experiments with different treatment regimen hypotheses in order to assess their effectiveness and potential consequences. We also aim to use the model to generate hypotheses for the molecular differences between excessive drinkers who develop physical dependence and those who do not so as to motivate the development of drugs to prevent physical dependence and the adverse effects of chronic alcoholism. Finally, we hope to identify experimentally if these molecular changes occur in the brain regions controlling physical and emotional homeostasis.

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