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A mathematical model of a control systems hypothesis of N-methyl-D-aspartate-receptor-mediated ethanol dependence and withdrawal dynamics

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A MATHEMATICAL MODEL OF A CONTROL SYSTEMS HYPOTHESIS OF N-METHYL-D-ASPARTATE RECEPTOR-MEDIATED ETHANOL DEPENDENCE AND WITHDRAWAL DYNAMICS

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Abstract

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The biochemical effects of ethanol on the human brain are manifested through many neurological pathways. Chronic exposure to the depressant has been shown to result in physical dependence. Subsequent cessation results in withdrawal symptoms such as seizures and both short- and long-term changes in neurological activity. One of the primary conduits implicated in the pathways of ethanol dependence and withdrawal is the detection of glutamate via N-methyl-D-aspartate (NMDA) receptors (NMDARs). Ethanol molecules inhibit these receptors, and consequent NMDA-induced glutamatergic changes can result in dependence on ethanol in order to sustain normal brain function. This study considers the relocation control of NMDARs in response to chronic alcoholism and withdrawal as a dynamic control system. Specifically, the system is modeled as a negative feedback control system with a dual-action relocation controller and an explicit set point. The model is used to investigate the effects of ethanol consumption frequency, duration, and magnitude as well as various withdrawal profiles on both the NMDAR population and withdrawal symptoms. The model results are consistent with published trends in NMDAR populations in response to ethanol. Simulated results suggest that withdrawal severity is independent of dependence dynamics, and that regulating the blood alcohol level throughout the progression of withdrawal can minimize withdrawal symptoms. Furthermore, the model suggests that the development of dependence is a function of the frequency of exposure, while the degree of dependence is related to the combination of duration and magnitude of intoxication. Finally, the model enables the possibility of capturing individualized patient neuroexcitatory states by adjusting controller parameters. The mathematical model of NMDAR dynamics provides a platform for analyzing alcohol dependence, predicting withdrawal severity, and designing treatments to minimize excitotoxic insult during alcohol withdrawal.

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Chapter 1

Introduction

1.1 Motivation

In the new millennium, alcohol abuse has represented a large healthcare risk that has steadily grown with time. In the United States alone, hospital discharges for incidents related to drug abuse grew from 2.5 million in 2004 to 4.6 million in 2009 – an 81% increase[1,2]. The frequency of discharges which listed alcohol-related morbidity as the primary diagnosis has similarly increased from 424,000 discharges in 2004[3] to 658,000 in 2009 – a 55% increase[1,2]. In 2012 it was estimated that 17 million American adults suffered from an alcohol use disorder, 1.4 million of which were treated at specialized facilities that year[4]. This leads to significant healthcare costs, which were estimated to total $223.5 billion in the United States in 2006[5]. The epidemic of alcohol abuse in the United States has sparked renewed interest in the study of alcohol dependence. Despite this, much is still unknown about the neurological and biological mechanisms of alcohol dependence or its epidemiology, disease progression, and optimal treatment.

Chronic alcoholism develops when individuals regularly consume pharmacologically significant quantities of ethanol over an extended period of time. The constant presence of ethanol leads to homeostatic adaptations in the brain and neurotransmitter systems to compensate for neurological effects of ethanol; given enough time, this can lead to a state where the brain is physically dependent upon ethanol to function[6]. That is to say that the brain does not function appropriately without a high concentration of ethanol in the bloodstream.

Once dependence has developed, if the individual ceases to consume alcohol they may experience symptoms of withdrawal. These symptoms can manifest physically or emotionally and include anxiety and agitation, sweating, increased blood pressure and heart rate, altered consciousness, seizures, hallucinations, delirium tremens, cardiac arrhythmias, or sudden death. Collectively, these symptoms are termed alcohol withdrawal syndrome and they imply a dysregulation of the body's internal equilibrium or homeostasis[7, 8]. The focus of this work is on the mechanism by which homeostasis is maintained in response to ethanol and the dynamics of that system through chronic ethanol ingestion and subsequent withdrawal.

Many studies have implicated neuroexcitatory transmitter systems as part of the neurological response to ethanol[9, 10], the homeostatic development of dependence[11-13], and withdrawal symptoms[14-16] and behavior[17]. In particular, these studies have identified molecular changes in the excitatory and inhibitory neurotransmission systems which are modulated by glutamate and gamma-aminobutyric acid (GABA) respectively. It has been shown that ethanol acts to inhibit the central nervous system by synergistically increasing GABA activity and decreasing glutamate activity[18-20]. The dynamic balance of the excitatory and inhibitory systems and disturbances which affect them are of fundamental importance to understanding the mechanisms behind the development and withdrawal from ethanol dependence. This study focuses on the inhibition of glutamate transmission via glutamate receptors. There are two types of glutamate receptors. Ionotropic or ligand-gated receptors are comprised of an extracellular binding site and an ion channel pore which spans the cell membrane and allows transduction signals. Metabotropic receptors or G-protein coupled receptors rely on intermediary G-proteins to act on an ion channel

rather than operating via direct action[21]. Of the former type, the ionotropic N-methyl-D-aspartate (NMDA) receptor is of particular interest, as it has been most directly related to withdrawal symptoms such as seizures[22] and, consequently, is one of the most widely studied neuroexcitatory pathways for ethanol. Therefore, while the complete response to alcohol is likely to involve a multitude of pathways, this work will focus on the role of NMDA receptors.

1.2 Purpose of Experiment

In this study, the neuroexcitatory system was considered from a dynamic control systems perspective. The primary goal of this study was to isolate individual system parameters to gain fundamental insight into the variables that affect the development of ethanol dependence and withdrawal and the resultant severity of symptoms. To that end, a model was developed utilizing control system dynamics as a basis. This model was found to be capable of converging for a broad range of controller parameters and disturbance profiles and the resultant behaviors were consistent with characteristic behaviors of the NMDA neuroexcitatory complex in response to ethanol. What follows is a brief summarization of the process by which this study was conducted.

A two-component controller model with explicit set point was constructed, as well as a governing system of differential equations describing the dynamic response of the NMDA population. The control system hypothesis was developed and coded in both Visual Basic Applications and MATLAB®. A baseline sensitivity study was performed to

better understand parameter functions and to select a set of parameters which prominently displayed characteristic behaviors for further study.

In order to study the effects of system disturbances and sensitivity on ethanol withdrawal, it is necessary to have some measure of the severity and extent of withdrawal. Two methods for measuring this are proposed based on analogy to observed symptoms. The peak value of NMDARs is considered a measure of maximum synaptic activity and, consequently, the likelihood for the development of seizures. The area under the NMDAR curve above the original set point is proposed as a measure of adaptive changes in the neuroexcitatory system.

A variety of ethanol dependence and withdrawal profiles were proposed and tested to observe the effects that these disturbances had on measures of withdrawal severity. The frequency and magnitude of consumption were varied to order to study the development of dependence. Pairs of parameters were studied to determine how results change based on patient-specific changes in neuroexcitatory dynamics and kinetics. The final goal is to provide a more methodical method for understanding the conditions which underlie the development of dependence and subsequent withdrawal in order to provide a basis for the development of proactive, patient-specific treatment rather than a reactive, symptomatic regimen.

Chapter 2

Literature Review

2.1 NMDA and GABA as a Two-Component Neuroexcitatory Control System

Recent molecular studies of neuroexcitatory activity in mammals have identified glutamate as the principle neurotransmitter by which excitatory signals are propagated across a synaptic junction[12, 21]. A synapse is a junction between neurons that allows signals to be sent from the presynaptic region (axon) across the synaptic junction to the postsynaptic density (dendrite) of the next neuron. Studies describe a bimodal compensatory system that controls neuron activity through mechanisms involving N-methyl-D-aspartate (NMDA) and gamma-aminobutyric acid (GABA). Glutamate is the excitatory neurotransmission agent while gamma-aminobutyric acid (GABA) is the inhibitory neurotransmission agent. The effect that these chemicals have on brain function is dependent on their activation of NMDA and GABA receptors, both of which come from families of hetero-oligomeric, ligand-gated ion channels[23, 24] and are cotranslationally assembled from a multitude of subunit types in the endoplasmic reticulum[25]. The subunit composition of these receptors has been shown to have significant effects on resultant physiological and pharmacological properties[24, 25].

NMDA and GABA receptors modulate synaptic activity in regions of the brain such as the hippocampus, nucleus accumbens, cerebral cortex, and striatum[12] by fluxing ions such as Ca^{2+}, Na^{2+}, and Cl^{-} in a voltage- and activity-dependent way[19, 23, 24]. The synaptic transmissions are regulated by glutamate availability and lead to substantial effects on subjects' behaviors such as impulse control[26], memory[27], and mood, and can

have long-lasting impacts on neuronal development[21]. Consequently, it has been proposed that the number, composition, activity, and types of receptors are not constant[25]. Rather, activity and composition can change in a cell- and synapse-specific manner in response to factors such as age[23], brain region[23], long-term neuronal potentiation[25, 28], and sensory stimuli[25, 28].

The dynamic, homeostatic adjustments to the receptor populations trigger neurochemical cascades which affect the concentrations and activity of calpain, fyn tyrosine, and Mg^{2+} among other neurologically active agents, as well as their interactions with NMDA, GABA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, and Kainate receptors[29]. These changes in glutamatergic receptor population and the resultant cascade effects are the underlying mechanism of synaptic plasticity[21, 28] and provide protective functions that stabilize long-term synaptic efficacy and strength[30] as well as preventing synaptic degeneration and apoptosis[31].

It is important to note that the activity of GABA receptors (GABARs) and NMDA receptors (NMDARs) can be substantially modulated by agonists and antagonists[32, 33]. This is because the subunits composing NMDARs and GABARs are encoded by gene families which confer sensitivity to drugs as well as regional and functional sensitivity and specificity[25,34]. Consequently, these receptors are the target of action for psychotropic drugs such as ketamine, phencyclidine, cocaine, heroin, and ethanol. NMDA and GABA receptors are implicated in the pharmacological effects of these drugs[32], as well as the development of dependence and negative symptoms of withdrawal, such as seizures[12, 32, 35].

There are many theories as to the exact mechanism by which ethanol modulates the glutamatergic neuroexcitatory system, but it is generally accepted that the result is a synergistic increase in inihibitory GABA activity and a decrease in excitatory glutamatergic activity[19, 20]. This is because alcohol and other psychotropic drugs engage homeostatic processes which attempt to stabilize the neuronal network by affecting changes to the number, density, structure, and subunit composition of GABARs and NMDARs. This study focuses in particular on the response of the NMDAR population to ethanol and investigates the dynamics of ethanol dependence and withdrawal.

2.2 Neurological Function of NMDA Receptors

Before beginning to construct mechanisms that control neuronal activity in response to the depressant effects of ethanol, it is of critical importance to understand synaptic structure and function as well as the role of NMDARs therein. Much of synaptic function and activity is regulated by the availability of the neurotransmitter glutamate. This is because glutamate exerts control over both metabotropic glutamate receptors, which are implicated in signaling cascades that result in the production of secondary neuronal messengers, as well as ionotropic glutamate receptors, which flux ions such as sodium, potassium, and calcium when they are activated[6, 21]. NMDARs are ionotropic receptors whose activation results in the flux of Ca^{2+}, thereby triggering numerous intracellular signaling cascades[21]. Consequently, the intracellular concentrations of calcium and glutamate are co-dependent, as calcium is effectively released in a glutamate-dependent manner mediated by NMDARs[36]. The balance between glutamate concentration and synaptic activity is critical to normal brain function, as glutamate-gated

currents in the hippocampus have cascading symptomatic effects on long-term potentiation, mood, behavior, and seizure emergence[12, 26].

However, not all glutamate affects all glutamate-gated receptors in the same way. There are two glutamate concentrations which are of interest: synaptic and ambient concentrations. The synaptic concentration of glutamate is subject to short, high concentration bursts in response to synaptic signaling[21]. On the other hand, the ambient or extracellular glutamate concentration remains relatively constant[21]. The ambient concentration of glutamate in brain and neuronal tissue ranges from 5-15 mmol/kg in human neurons, with the concentration in axon terminals being two to three times higher due to the synthesis of glutamate occurring locally in the mitochondria[21].
Synaptic and ambient glutamate concentrations are mediated by separate populations of receptors with analogous nomenclature. Synaptic glutamate receptors, which include NMDARs, are inserted and clustered in the dendritic regions localized in the synapse[21]. Approximately half the NMDAR population is synaptic, with the remaining receptors being located in the extrasynaptic region[21].

According to Featherstone, the process of glutamate signaling occurs in several steps as shown in Figure 1[21]. The first step involves mitochondrial glutamate synthesis and transport through the cytoplasm of glutamatergic neurons. Second, glutamate is pumped into a secretory vesicle near the axon that is called a synaptic vesicle. The synaptic vesicle fuses with the plasma membrane and releases the glutamate into the synaptic cleft. This creates a burst of increased glutamate concentration that is detected

and transduced by NMDARs and other glutamate receptors. Excess glutamate is removed from the synaptic cleft by excitatory amino acid transporters.

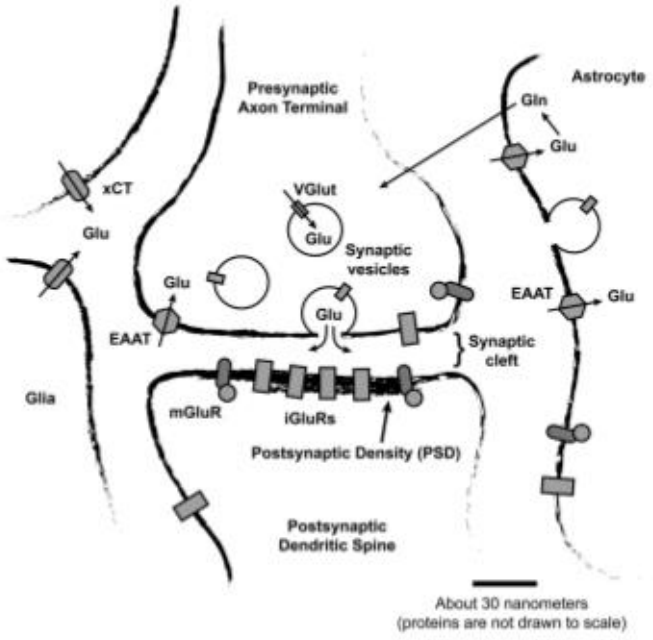


Figure 1. Glutamate signaling process. Glutamate is synthesized in the mitochondria of the presynaptic axon terminal and transported to the synaptic vesicle. The vesicle fuses with the extracellular membrane and releases glutamate into the synaptic cleft. The burst of high glutamate concentration is detected by ionotropic receptors (iGluR) as well as metabotropic receptors (mGluR) in the dendritic postsynaptic density. Any excess glutamate is collected by excitatory amino acid transportors (EAAT) and returned to the axon. Figure reproduced from Featherstone[21].

To summarize, NMDARs are glutamate-gated excitatory neurotransmitters. When glutamate is released from the presynaptic region into the synaptic cleft, NMDARs in the postsynaptic density detect the synaptic glutamate concentrations, and respond by fluxing Ca^{2+}, allowing the excitatory signal to propagate along the neuronal network. Normal neuronal function is maintained by achieving an equilibrium between the excitatory effects of NMDARs and the inhibitory effects of GABA.

2.3 Structure and Function of Ionotropic NMDA Receptors

NMDARs are ligand-gated ion channels with a high permeability to Ca^{2+} ions[19] that are activated by the excitatory amino-acid neurotransmitters glutamate and glycine[23, 37, 38]. Particularly, NMDARs are hetero-oligomeric assemblies of NR1, NR2, and NR3 subunit complexes which cotranslationally self-assemble in the endoplasmic reticulum of the dendritic terminal of the synapse[25]. Each receptor has at least two active binding sites for glutamate[19]. The activity of the receptors is modulated by the allosteric binding of small compounds to the amino-terminal domain[37].

Each subunit type has unique functions and variations. The purpose of the NR1 subunit is to provide the backbone for the receptor complex. It forms the ion channel[27] and maintains normal receptor function[12]. The NR2 subunit builds on the functionality of the NR1 by mediating the effects of pharmacological agents such as ethanol and determining the channel kinetics and activity[27]. In general, the NR2 subunit potentiates the electrophysical response of the NMDAR. Finally, the role of NR3 subunits is to regulate the glutamate-gated flux of calcium[39].

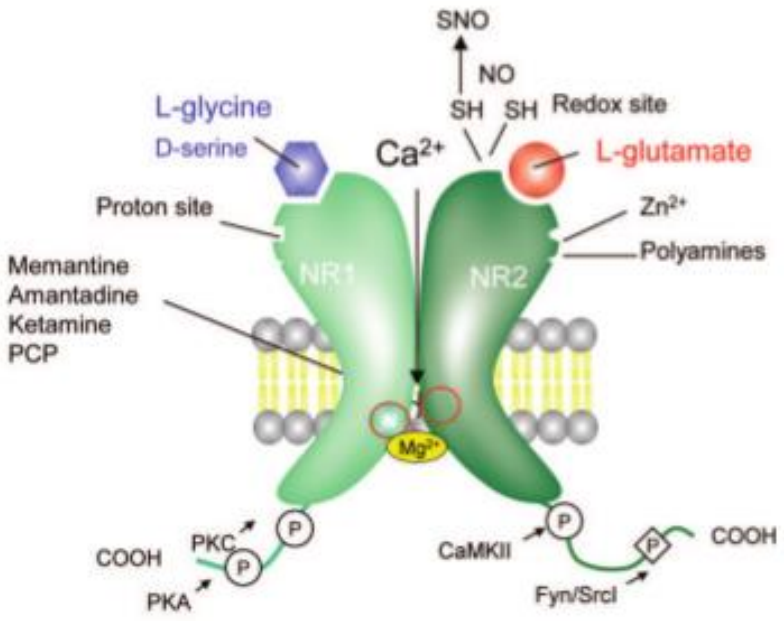


Figure 2. Structure and Binding Sites of NMDARs. NMDARs are heteromeric ion channels composed of NR1, NR2, and NR3 subunits. The NR1 and NR2 subunits are the primary active sites, and will always contain an extracellular amino-terminal, intracellular carboxyl-terminal domain with phosphorylation sites, and binding sites for glutamate and glycine. NMDARs also contain a number of allosteric binding sites for polyamines, Zn^{2+}, and protons. These binding sites have a direct effect on the activity of the receptor[40]. Figure reproduced from Benarroch[40].

This study focuses on the NR1 and NR2 subunit complexes. NR1 subunits are required for baseline receptor function, and are consequently a reasonable measure of the density of receptors. NR2 subunits describe the kinetics and activity and provide a means of describing changes in receptor functionality as a response to stimuli and disturbances such as ethanol.

There are four types of NR2 subunits, designated as NR2A, NR2B, NR2C, and NR2D. Adult neurons are primarily composed of NR1/NR2A and NR1/NR2B complexes[41] with NR2A receptors being primarily synaptic while NR2B receptors are primarily extrasynaptic[38]. The reason for this is because these two subunits provide different properties, specificities, and sensitivities. NR2A subunits provide faster kinetics as well as greater channel open probability and more prominent desensitization in response to Ca^{2+} ions[25]. NR2B subunits are more sensitive to glutamate[10].

NR2 subunits are encoded by a family of genes which determine functional specificity and sensitivity[34], resulting in subunit combinations displaying different kinetics and divalent action sensitivities[32] as well as unique numbers and types of binding sites and affinities for agonists and antagonists[42]. As a consequences of these varied sensitivities, the physical and chemical properties and responses of NMDARs are highly dependent on receptor composition.

2.4 Pathways for the Development of NMDA-Mediated Ethanol Dependence

The effects of ethanol on the central nervous system are manifested as selective and non-competitive inhibition of ligand-gated ion channels, particularly NMDARs[18] at behaviorally relevant levels of ethanol exposure. In general, these levels range from 10-100 mM, with 50 mM being accepted as well within the relevant range of intoxication[23, 30]. Treatment of NMDARs with agonists such as ethanol results in desensitization of the receptors, which means a decreased ability for glutamate to generate the typical large, post-synaptic Ca^{2+} currents. This behavior can be observed in Figure 3[43].

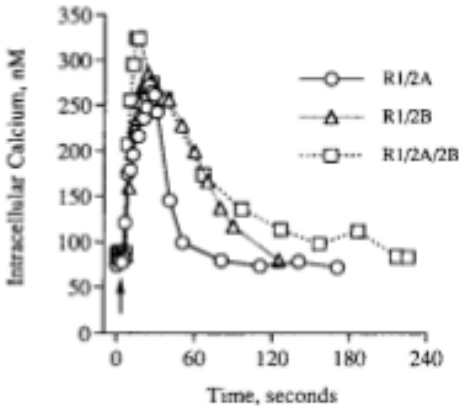


Figure 3. Typical synaptic firing behavior. Complexes of NR1, NR2A and NR2B. The characteristic have fast-onset, burst responses which occur as a result of NMDAR activation by glutamate. Figure reproduced from Blevins et al[41].

The desensitization of NMDARs by ethanol effectively removes receptors from the functional pool, thereby affecting activity, trafficking, and localization[21]. Consequently, the channel open probability of the NMDARs is reduced in the presence of ethanol, which inhibits the Ca^{2+} current generated upon activation[22]. As a result, the extrasynaptic concentration of Ca^{2+} builds up and the neurons adapt to slow-onset, small changes in Ca^{2+} rather than the typical behaviors observed above[43]. This neuronal tolerance can be developed in as little as minutes[23], and long-term exposure can generate additional negative effects.

There are two theories for explaining the inhibitory effects of ethanol on NMDARs. The first is that ethanol interacts directly with the receptors, either through an allosteric or competitive binding site. Studies in the response of NMDARs to antagonists have shown that the non-competitive blockade of NMDARs produces results which correspond to chronic ethanol treatment[44]. This means that ethanol likely binds to allosteric sites in the ion channel which produces a non-competitive inhibition of glutamate-mediated responses[18]. A second theory is that ethanol modulates the activity of receptors through the addition of PO_4^{3-} groups (phosphorylation) or by some other mechanism[27]. It is likely that the observed inhibitory effects of ethanol cannot be wholly attributed to one mechanism or the other, but rather that the effects are achieved by a combination of these and other neurochemical processes.

It has also been observed that varying the duration, frequency, and time-dependent profile of ethanol consumption and withdrawal can induce unique patterns of gene expression[9, 42]. Because of this dependence, it has been proposed that the adaptive

responses occur by multiple, independent post-translational mechanisms[45]. This is supported by the fact that chronic ethanol treatment does not significantly alter the short-term sensitivity of the NMDAR population to acute ethanol exposure[46].

This gives rise to the hypothesis that the short- and long-term effects of ethanol are realized by different pathways. It is currently hypothesized that short-term exposure to alcohol alters the functionality and sensitivity of existing receptors by altering subunit and cytoskeleton interactions as well as phosphorylation[47]. This results in short-term, subunit specific suppression of receptor function, manifested as an inhibition of excitatory glutamatergic transmission as observed in Figure 4[6, 10].

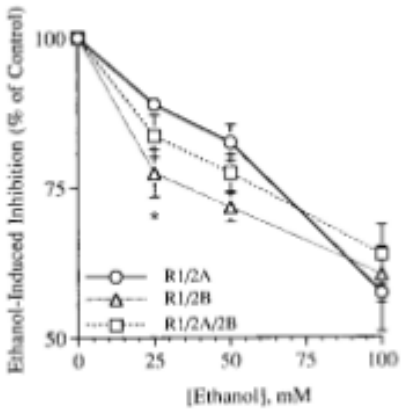


Figure 4. Subunit-specific inhibition of NMDAR activity. NR2 splice variants respond to ethanol in a subunit-specific manner. Here the inhibition is represented as a percent of normal function (control). This demonstrates the difference in subunit activities and sensitivity to ethanol. Of particular note is the decreased sensitivity of NR2B in the presence of ethanol, represented as a percent of the activity of the control sample. Figure reproduced from Blevins et al[41].

Conversely, long-term exposure to ethanol results in increased function and number of NMDARs in response to chronic inhibition[6, 11]. This is a result of homeostatic adaptations in response to decreased receptor activity due to ethanol-mediated blockage[11], the purpose of which is to return stability and normal function to the neuronal network[30]. Consequently, it is believed that during chronic ethanol exposure, additional NMDARs are recruited to the synapse to compensate for the blocked receptor population and to maintain baseline neuronal activity[9, 14, 30, 33]. It has also been observed that blocking excitatory neuronal activity accelerates the trafficking of receptors to the

synapse[25] and that chronic exposure leads to an increase in the clustering of dendritic binding sites[30, 44], particularly in the hippocampus[34].

These long-term adaptations in receptor population in response to inhibition are implicated in the pathways of physical dependence[48]. The natural adaptation to long-term inhibition is to upregulate the number and density of binding sites by recruiting additional receptors[33]. However, decreased receptor function results in an increase in ambient and synaptic glutamate concentrations[11, 36]. As the concentration of ethanol declines, excitatory activity moves beyond normal function and into a region of hyperexcitability due to the increased receptor population[14]. Indeed, patients with chronic ethanol dependence express higher concentrations of excitatory neurotransmitters[33]. These maladaptive changes in receptor population occur as a direct response to chronic inhibition of NMDARs by antagonists[49] and create an environment where normal function is only possible in the presence of ethanol[15, 48], as that reduces the excitatory load on the neuron to normal levels.

As a consequence of these maladaptive changes, alterations may be observed in a patient's control over alcohol intake in order to manage their neuroexcitatory state[26]. This type of physical dependence is developed in order to maintain normal brain function in response to the depressant effects of ethanol[6] and the resultant changes in NMDAR population and density. The relationship between these responses demonstrates that upregulation of NMDARs is an important substrate for modulating long-term synaptic efficacy, but is also directly implicated in the development of ethanol tolerance and dependence[12, 19].

This section describes two mechanisms by which the NMDAR population can be affected by the presence of ethanol. Short-term exposure results in shifts in NMDAR activity via phosphorylation and other modulations via allosteric binding sites. These short-term changes are entirely reversible. Chronic inhibition of NMDARs by ethanol results in the compensatory recruitment of additional NMDARs from extrasynaptic regions in order to maintain normal brain function.

2.5 Mechanisms for the Manifestation of the Biochemical Effects of Ethanol on NMDARs

As outlined in Section 2.4, the dynamics of NMDAR activity can be described by two primary mechanisms, distinguished by the speed of their responses. Changes in activity occur quickly, on the order of minutes[21, 23], and are believed be the primary response to acute exposure to ethanol. Chronic exposure, on the other hand, engages homeostatic upregulation of NMDARs in order to restore normal brain function[14, 27, 30]. This two-mechanism system is supported by observations that changes in NMDAR population activity, composition, and density are time- and dose-dependent[9, 30, 42]. Potential pathways by which the activity, composition, and density of the population are modulated will be discussed in more detail in this section.

There are two primary mechanisms by which the activity of NMDARs can change in response to various stimuli. First, it must be considered that the composition of the NMDAR population changes in response to system disturbances. This is supported by studies that have shown that the polypeptide[48] and mRNA levels of NR2B subunits[10, 50] are upregulated during chronic ethanol dependence. These behaviors are shown in

Figures 5 and 6 respectively. Because NR2B subunits are more sensitive than NR2A[10], this represents a homeostatic response which upregulates activity in response to an antagonist. By analogy with processes observed during synaptogenesis, it is believed that the scaffolding protein PSD 95 modulates changes the surface expression of NR2A and NR2B subunits[51].

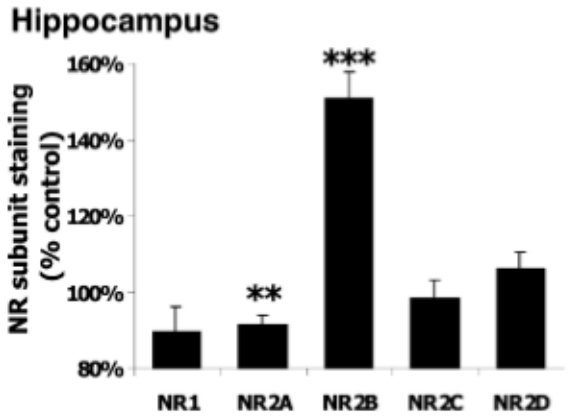


Figure 5. Expression of NMDAR subunit polypeptides at the synapse changes in response to chronic ethanol treatment. NR1 and NR2A polypeptides are downregulated, potentially due to inactivation due to inhibition. NR2B polypeptides are upregulated. This is likely a homeostatic response to stabilize neuronal activity by increasing the sensitivity of NMDARs. Figure reproduced from Nagy et al[48].

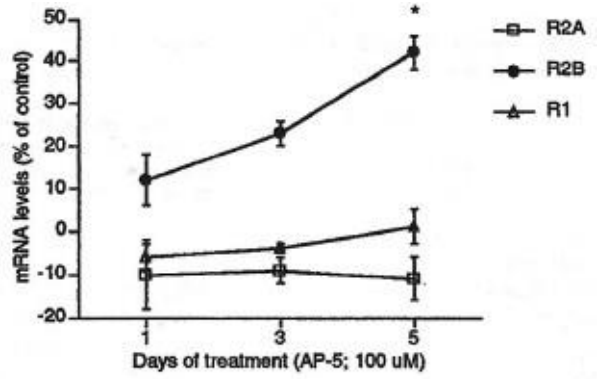


Figure 6. NMDAR subunit mRNA expression changes in response to chronic ethanol treatment. mRNA for NR1 and NR2B subunits are upregulated. This represents the potential for substantial changes in population composition and the insertion of newly synthesized receptors. Figure reproduced from Follessa et al[50].

It is also possible that a multitude of proteins and other neuroexcitatory transmitters can engage cascades to affect changes in the activity of receptors. There is no shortage of neurochemical agents that have been implicated in mechanisms which may attenuate the activity of NMDARs in response to ethanol.

One proposed mechanism is that NMDAR activity is regulated by phosphorylation of tyrosine phosphatases, as has been shown in the hippocampus[52]. Striatal-enriched protein tyrosine phosphatases are brain-specific protein highly expressed in the striatum, hippocampus, and cortex that form a complex with NR2B subunits to regulate NMDAR activity[27]. Research has shown that there is a correlation between the desphosphorylation via tyrosine phosphatases and the inhibition of receptor

function[27]. Fewer tyrosine phosphatases have been shown to reduce NMDAR sensitivity to ethanol, and inhibitory effects are recovered as the proteins are reintroduced[27].

Tyrosine kinase can also be implicated in changes to NMDAR potentiation[52]. Insulin receptor activation has been shown to initiate a neurotransmitter cascade that results in the activation of cystolic tyrosine kinases that may affect NMDAR activity via phosphorylation[52].

It has also been suggested that localization of fyn kinases, particular fyn-rack1 account for NMDAR sensitivity to ethanol in brain regions such as the hippocampus and dorsal striatum[23, 53]. NR2B subunits are targeted by fyn-rack1 complexes, and as rack1 dissociates, fyn phosphorylates the NR2B unit and consequently increases receptor activity. The dissociation is activated by ethanol and occurs via protein kinase A mediated nuclear translocation[54]. This hypothesis is further supported by evidence that fyn kinases and rack1 are only co-localized in brain regions affected by ethanol[53, 54]. Anders et al. have proposed that ethanol activates calmodulin, a protein which binds via the c-terminal domain of the NR1 subunit to enhance calcium-dependent inactivation[23]. Ethanol sensitivity has been positively correlated with affinity for calmodulin[23]. Littleton et al. have proposed that NMDAR function can be altered by cascades involving polyamines. NMDARs, particularly those with NR2B subunits have been shown to have their activity enhanced in the presence of polyamines produced during ethanol exposure and withdrawal[55].

Finally, nitrous oxides are involved in the long-term maintenance of NMDAR potentiation by acting as a retrograde messenger[45]. It is hypothesized that ethanol stimulates the production of nitrous oxides through neuronal NO synthase isoforms and that these nitrous oxides regulate several of the membrane-dependent pathways for realizing NMDAR sensitivity to ethanol[47]. This is supported by the observation that administration of NO synthase inhibitors decreases the severity of withdrawal symptoms, while treatment with nitrous oxide donors results in increased severity[45].

However, these short-term mechanisms for controlling synaptic activity do not account for the adaptive changes in NMDAR population observed in response to chronic ethanol treatment. In response to consistent inhibition, an increase in the density of active binding sites for antagonists, such as MK-801, is observed in mammalian hippocampus[33, 44]. This increase in available binding sites is accompanied by maladaptive upregulation of mRNA and protein levels of NMDAR subunits[44, 49]. Interestingly, this upregulation of NMDAR population does not alter the sensitivity of receptors to acute ethanol exposure[46]. This implies that the mechanisms for short- and long-term response are independent, and that increased activity is not associated exclusively with alterations in ligand-binding properties or composition[11], but rather that receptor population and density make contributions as well. This means that while bulk increases in population density of NMDARs at the synapse occur in response to ethanol, other processes for controlling receptor activity are still engaged[30].

Upregulation of NMDARs is achieved by selective, activity-dependent recruitment of NMDARs to the synapse. These changes in receptor population occur via

three mechanisms: insertion, lateral movement, or internalization[30]. This means that during prolonged periods of inhibition, one would expect to observe the synthesis and insertion of new receptors at the synapse as well as the relocation of additional receptors from extrasynaptic region to compensate for reduced sensitivity. The exact mechanism by which insertion and relocation occur is unknown, but it is hypothesized that the process involves synapse-associated protein 102 (SAP 102), which trafficks NR2A and NR2B during synaptogenesis or PDZ domain proteins, which are implicated in intracellular trafficking and synaptic delivery[25, 51]. The synthesis hypothesis is consistent with the observed increase in NR1 and NR2 mRNA in response to ethanol[50] (Figure 6), while the relocation hypothesis is supported by observed increases in NR1 polypeptide expression unaccompanied by an increase in mRNA expression[10, 44].

2.6 Withdrawal Dynamics

Chronic ethanol treatment results in maladaptive regulatory changes in activity, density, and composition of the synaptic NMDAR population[33, 48]. As ethanol is purged from the system, an increase in cerebral activity that enters the regime of hyperactivity is observed. It is believed that this hyperexcitability is a result of overstimulation of NMDARs[11] in the wake of maladaptive changes at the neuronal level to prolonged inhibition[45]. As ethanol is processed and removed from the system, the blockade of NMDARs is disrupted and neuroexcitatory activity begins to increase. The combination of increased ambient glutamate and NMDARs as a result of the prolonged blockage results in a state of hyperexcitability. This state can be observed in Figure 7[14, 17, 33].

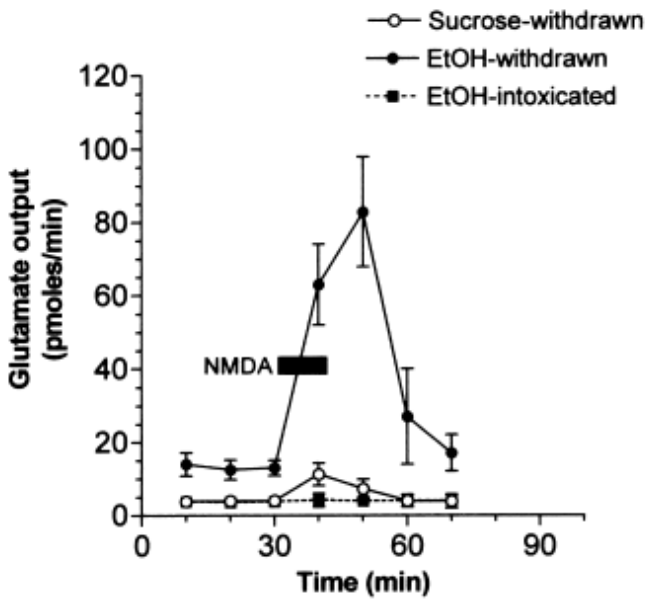


Figure 7. Prolonged inhibition due to chronic ethanol exposure results in a state of hyperexcitability upon cessation of treatment. Rats underdoing ethanol withdrawal show increased synaptic activity in response to direct stimulation of striatal NMDARS via focal application of NMDA (indicated by the black square) relative to rats that are intoxicated. Figure reproduced from Rosetti et al[36].

Excessive glutamatergic stimulation of NMDARs in the withdrawal state is believed to enhance the flux of Ca^{2+}, resulting in excessive neuronal activity. This type of neuronal hyperexcitability is termed excitotoxic and is related to seizures observed in ethanol withdrawal[11, 19, 36, 38]. NMDA is the neurotransmitter which has been most directly related to the development of seizures in response to ethanol withdrawal, despite the fact that NMDA and GABA are both known initiators of seizures[35]. This is because hyperexcitability has been shown be contingent on NMDA independently of other neurotransmitters[14]. This is supported by a myriad of studies which show that NMDA and other NMDAR agonists exacerbate withdrawal symptoms and increase the frequency and

intensity of seizures[33] while NMDAR antagonists such as ethanol and MK-801 ameliorate symptoms[15, 32, 33]. The electrical activity of neurons corresponding to this hyperactive excitotoxic state is shown in *Figure 8*. Additionally, studies have shown that cells pretreated with ethanol show increased sensitivity to NMDA and other agonists, requiring ethanol to maintain normal levels of excitation[15, 48]. This is further supported by the observation that the primary difference between seizure-prone and seizure-resitant mice is the density and number of binding sites for NMDA[34].

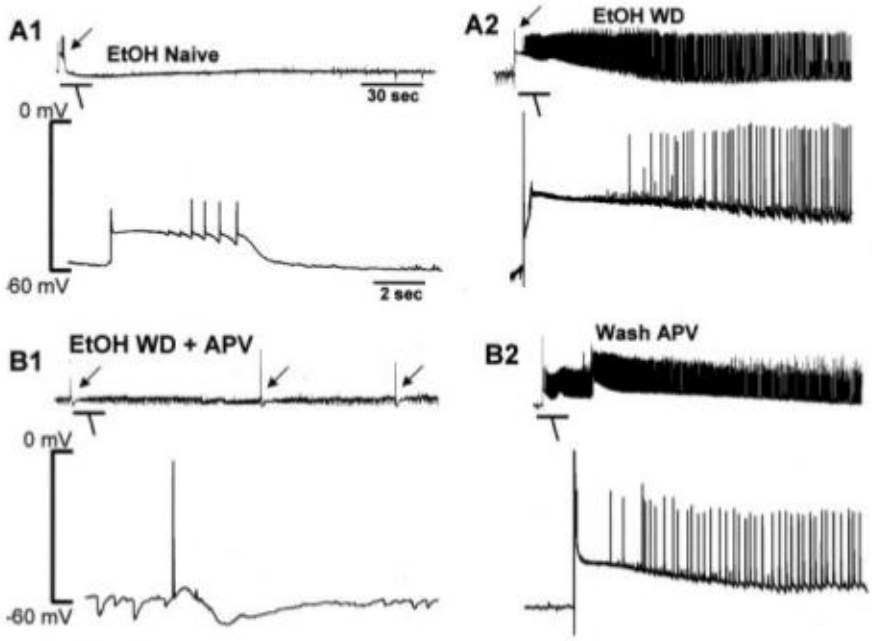


Figure 8. Neuronal activity varies in response to disturbances to the dependence or withdrawal state. A1 shows normal response in an ethanol naïve neuron. A2 shows a state of hyperexcitability after the neuron is exposed to a chronic ethanol treatment and washed of ethanol to induce withdrawal. B1 shows the response of an ethanol dependent neuron that has been exposed to (2R)-amino-5-phosphonovaleric acid (APV), an NMDA antagonist. B2 shows the return of withdrawal seizures once the APV has been washed from the system. Figure reproduced from Hendricson et al[14].

This ethanol-mediated state of hyperexcitability is implicated in the development of a number of short- and long-term withdrawal symptoms[16]. The sharp decline in NMDAR function during the period shortly following withdrawal is believed to be a general response to excitotoxic damage, wherein damaged cells undergo apoptosis in order to prevent the proliferation of damage[38]. There are two proposed mechanisms that contribute to this effect. First, the current population is downregulated by the calcium-dependent protein calpain-I. In response to the Ca^{2+} overload in postsynaptic neurons, calpain-I cleaves the c-terminal regions from NR2A and NR2B subunits[38]. NMDAR complexes are stable even when these interactive sequences have been removed from the NR2 subunits[38]. The second pathway is to downregulate the synthesis and insertion of new receptors, rather than the current population. It is believed that Ca^{2+} overload blocks the transcription of NR1 subunits, which are necessary for the formation of a functional ion channel[38]. It is also proposed that the stable, non-functional receptors impose geometric limitations on the density of active receptors at the synapse. These pathways represent long-term mechanisms for downregulation of synaptic activity[38]. The downregulation due to inactivation and excitotoxic apoptosis can be observed in Figure 9.

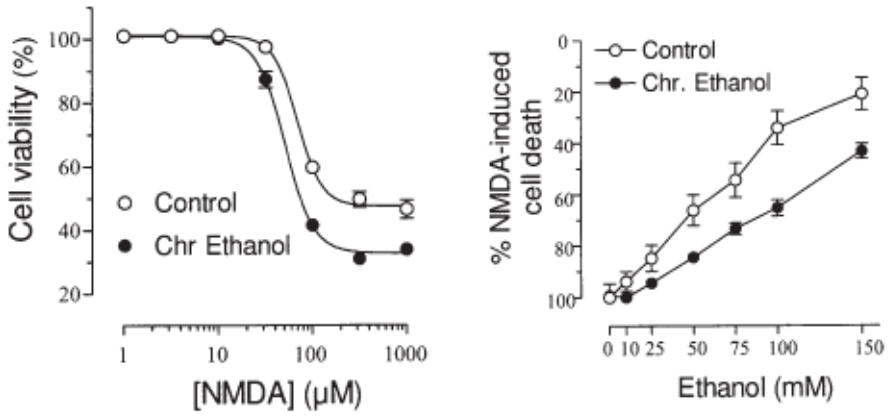


Figure 9. Chronic ethanol treatment induces NMDAR-mediated neuronal apoptosis. The left panel shows the effect of chronic ethanol on cell viability. Exposure to ethanol increases the likelihood of excitotoxic cell death, which decreases viability. The right panel demonstrates the effect of reintroduction of ethanol into a system in a state of hyperexcitability. Reintroduction reduces the induction of NMDA-mediated excitotoxic apoptosis, which implies a dependence on ethanol to maintain normal function. Figure reproduced from Cebere[11].

These changes in neuronal function leading up to, during, and post-withdrawal persist for approximately 36 hours post-withdrawal, which is consistent with the duration of withdrawal symptoms[22]. However, the excitotoxic stimulation of NMDARs in response to ethanol withdrawal triggers a series of events that result in delayed neuronal death[45] and excitatory downregulation as described previously. The resultant changes in ambient glutamate and synaptic activity are associated with permanent developmental and behavioral effects[21] and are associated with the gradual development of a seizure susceptibility[56]. In fact, NMDAR-mediated neurotoxicity is believed to play a role in the

development of conditions such as ischemia and epilepsy in which seizures are common and may be the trigger for degenerative diseases of the brainstem and thalamus, such as Wernicke-Korsakoff syndrome[22,32]. It is also observed that increased expression of NR2B subunits is related to the tendency of patients to relapse[13].

This section explores the neuroexcitatory role of NMDARs as a glutamate-gated ion channel. NMDARs are composed of three types of subunits, each with their own variations that confer specificity and sensitivity to different pharmacological agents such as ethanol. The effects of these subunits on ethanol are manifested by two types of mechanisms. Short-term changes in response to acute ethanol exposure are achieved by changes in NMDAR activity via allosteric inhibition and phosphorylation cascades. Long-term changes are achieved by the insertion of new receptors, recruitment of extrasynaptic receptors to the synaptic region, or internalization of excess receptors. Extended periods of inhibition result in the compensatory upregulation of the NMDAR population in order to maintain a normal excitatory state. As ethanol is removed from the system, this overpopulation causes excessive glutamatergic stimulation which is associated with the emergence of excitotoxic withdrawal symptoms and subsequent neuronal damage.

Chapter 3

Materials and Methods[1]

3.1 A Control System Description of the Ethanol-NMDAR System

In the hopes of better understanding biological responses to alcohol dependence and withdrawal at a systemic level, a mathematical model to describe the dynamics of NMDARs in response to ethanol was developed where the excitatory neurotransmission process was considered as a negative feedback control system wherein alcohol-induced blockage of NMDARs functions as a disturbance to normal maintenance of synaptic activity as described in Figure 10.

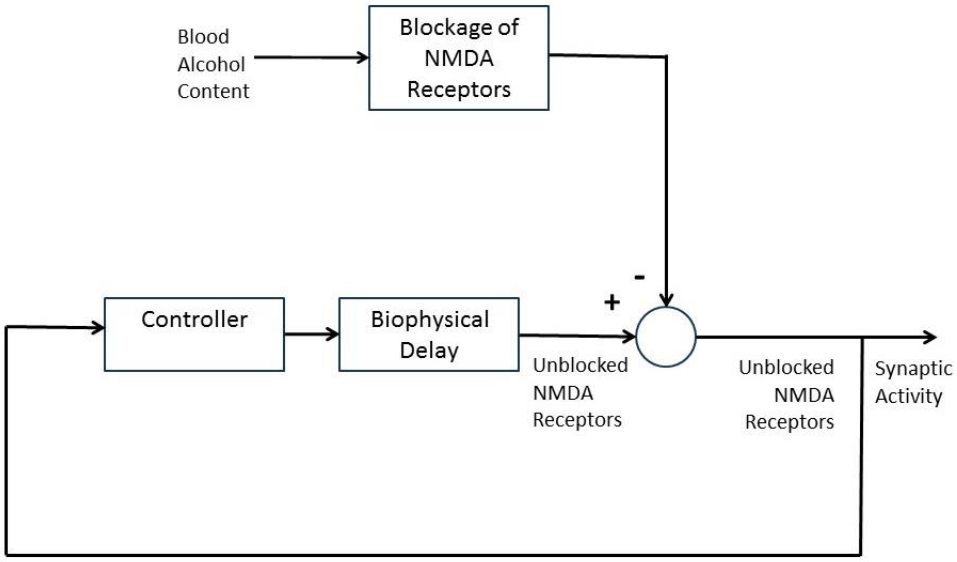


Figure 10. Block diagram showing a simplistic description of general system dynamics. The controller detects unblocked NMDARs as a measure of synaptic activity and takes appropriate action to restore the system to the desired set point. Alcohol-induced inhibition of NMDARs functions as a disturbance to the system.

[1] Substantial portions of this chapter were adapted from Gutierrez and Staehle[57].

Previous work by Staehle[58, 59], investigated descriptions of the NMDAR-ethanol system utilizing only synthesis and insertion as mechanisms of maintaining synaptic activity. However, a synthesis-only description is not sufficient to recreate the clinically relevant results. In order to create the sharp spike in neuronal activity upon cessation as well as a tendency to return to normal levels of synaptic activity, it is important for the controller to be able to move NMDARs both to and from the synapse.

This study proposes a composite controller with two active components: an activity controller that maintains synaptic activity by sending additional receptors to the synapse, and a density controller that moderates the population of NMDARs at the synapse by removing active, unblocked receptors from the synapse. Together, these controllers function to maintain a constant number of active synaptic receptors in the face of disturbances, such as inhibition of receptors by ethanol, as observed in Figure 11.

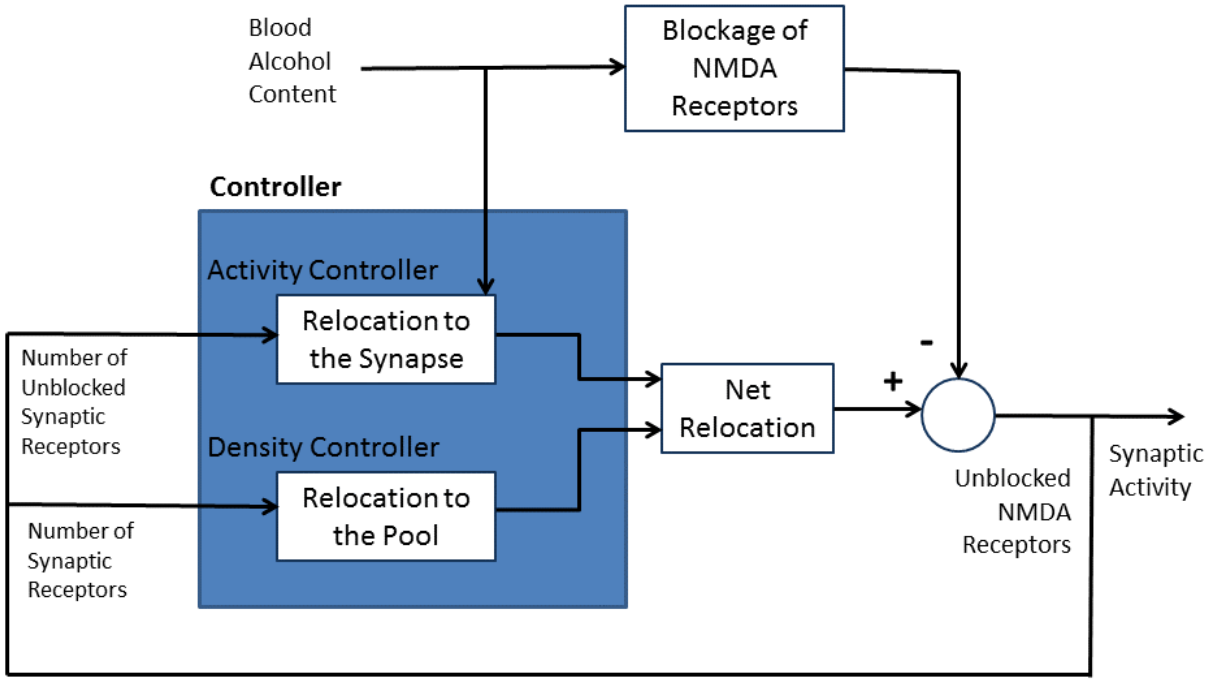


Figure 11. Block diagram of the relocation-only description of the NMDAR-ethanol system. The net action taken to modify the synaptic activity is the linear combination of desired actions of the activity and density controllers. Ethanol functions as a disturbance variable which modulates both the inhibition of NMDARs as well as the strength of the activity controller, allowing for the balance between the controllers to drift with chronic intoxication.

3.2 Developing Governing Equations from Elementary Kinetics

In this study, the NMDAR-ethanol system is considered as a compensatory negative feedback control mechanism, as described in the control system block diagram in Figure 11. The overarching control objective here is to maintain normalized brain function in the presence of ethanol by maintaining a constant level of unblocked NMDARs (*U*) at the synapse.

As ethanol is introduced to the system, unblocked receptors (*U*) become blocked (*B*) by alcohol (*A*) according to a reversible reaction with elementary kinetics, as shown in Equation 1:

$$U + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B \tag{1}$$

where k_1 = 0.05 hr^{-1} and k_2 = 0.03 hr^{-1}. The number of NMDARs is somehow sensed or measured by the cell in a process which is assumed to have a perfect gain and negligible dynamics, akin to most biological sensors. This information is then processed by a two-part composite controller, whose combined action, $C_T(t)$, changes the number of unblocked receptors (*U*) at the synapse. The overall changes in *U* and *B* are therefore governed by Equations 2 and 3:

$$\frac{dU(t)}{dt} = -k_1 A(t) U(t) + k_2 B(t) + C_T(t) \tag{2}$$

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

3.3 Developing a Bimodal Composite Controller

In order to achieve bi-directional action, the composite controller must be able to move receptors to and from the synapse. Previous work by Staehle et al. considered a unidirectional controller with sigmoidal-shaped steady state characteristic activity to insert newly-synthesized, unblocked NMDARs at the synapse[58, 59]. That controller was taken to be of the following form:

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

The form of control law selected for this system (Equation 4) is based on similar mathematical studies of steady state controller action in biological systems[60-62], where it is hypothesized that this sigmoidal formulation captures the physical limitations of biological processes. In these descriptions, y_{max} represents the maximum controller action, while *a* and *n* are position and shape parameters that shift steady state controller activity plots and change the curvature, respectively.

The problem with this formulation is that controller action is only positive. Even in the case that *U(t)* is taken as a deviation variable, negative action is possible only if *n* is odd. Without a mechanism for reducing the number of receptors at the synapse, this controller was unable to capture expected behavior during withdrawal. Therefore, for this study, a dual-mode, bi-directional composite controller for modulation of synaptic unblocked NMDARs was developed. The first subcontroller, termed the activity controller, inserts new unblocked NMDARs from an extrasynaptic "pool" based on current levels of synaptic unblocked NMDARs in an effort to maintain a defined population of unblocked receptors. The second subcontroller, termed the density

controller, removes unblocked NMDARs from the synapse in an effort to maintain a fixed number of NMDARs at the synapse. This controller does not discriminate whether the synaptic receptor is blocked or unblocked in its assessment of synaptic density, but only removes active, unblocked NMDARs from the synapse. This behavior was selected because NMDAR receptor trafficking is activity dependent[30] and consequently the trafficking and localization of receptors blocked by ethanol is inhibited[9, 21]. It is therefore assumed that inhibited receptors are inaccessible for the molecular mechanisms responsible for relocation.

The dual construction yields two subcontrollers of the following forms, where C_1 controls relocation to the synapse by the activity controller, C_2 controls relocation from the synapse by the density controller, and C_T represents the net control action:

$$T(t) = U(t) + B(t) \tag{5}$$

$$C_1(t) = y_{max1}\left(\frac{U(t)^{n_1}}{U(t)^{n_1} + a_1(A)^{n_1}}\right) \tag{6}$$

$$C_2(t) = -y_{max2}\left(\frac{T(t)^{n_2}}{T(t)^{n_2} + a_2{}^{n_2}}\right) \tag{7}$$

$$C_T(t) = C_1(t) + C_2(t) \tag{8}$$

The controller activity formulation of Equations 6-8 is complicated by the fact that the two subcontrollers cause significant deviation in the implicit set point. Changes to the parameters of either controller shifts the number of receptors at which the controller actions are balanced, which is the effective set point for the system. Manipulating parameters to achieve the desired set point is feasible when only one controller is involved, but with the additional complexity of a second subcontroller, an

explicit set point is required. The formulation utilizing an explicit set point is provided in Equations 9-12.

$$\Delta U(t) = U_{Desired} - U(t) \tag{9}$$

$$\Delta T(t) = U_{Desired} - T(t) = U_{Desired} - U(t) - B(t) \tag{10}$$

$$C_1(t) = y_{max1}\left(\frac{\Delta U(t)^{n_1}}{a_1(A)^{n_1} + \Delta U(t)^{n_1}}\right) \tag{11}$$

$$C_2(t) = -y_{max2}\left(\frac{\Delta T(t)^{n_2}}{a_2{}^{n_2} + \Delta T(t)^{n_2}}\right) \tag{12}$$

In this formulation, the controller activity is based upon the deviation of the measured value from the explicit set point. For this study the explicit set point, $U_{Desired}$, is defined as 100 receptors. This value is arbitrary and can be scaled according to biochemical data. It is also assumed that the population of NMDARs in the "pool" is never limiting and thus the calculated C_1 controller activity is always realizable. This is assumption is valid as long as both controllers are active, and would need to be revisited for scenarios in which the activity of one controller dominates (e.g. approximations of co-morbid disease states). Furthermore, both controllers are constrained in line with biophysical limitations on their control actions: C_1 has no activity if $U(t) > U_{Desired}$ and C_2 has no activity if $T(t) < U_{Desired}$.

Finally, it is necessary to develop a mechanism by which the balance between the subcontrollers, and consequently the desired set point for NMDAR population, shifts in response to alcohol intake. Thus, a position parameter of the activity subcontroller, a_1, was defined to be a function of blood alcohol content. In general, with smaller values of a_1, small changes in U create large changes in controller output. As a_1 increases, larger deviations in U are required to obtain the same controller action. To capture the alcohol

dependency, a_1 has been defined as an Arrhenius function deviation from an initial value, as shown in Equation 13.

$$a_1(A) = a_x + a_z e^{-k_a A(t)} \tag{13}$$

The position parameter is defined in this manner so that the controller response is quick when ethanol content is low but requires larger deviations when alcohol level increases.

In the development of this control scheme, a number of assumptions about the glutamatergic neurotransmission system were necessary. First, it is assumed that the overall glutamate load of these neurons is reasonably constant such that ethanol is the only stimulus modulating the number of NMDARs required at the synapse. This allows the disturbances to the system to be described as a single function representing alcohol intake. Second, it is assumed that synaptic activity is primarily a function of receptor population and density; the receptor population herein can therefore be considered as a homogenous population with characteristics of the average composition and activity of synaptic NMDARs.

3.4 Mathematical Simulation

Two methods were used to simulate this model and determine solutions to the system of differential equations (Equations 2 and 3). First, a stepwise integration using Euler's Method was performed in Visual Basic Applications utilizing a step size of 0.1 hours. This method is necessary to handle cases with any discontinuities.

In order to validate the results obtained in Visual Basic Applications, this system was also studied in MATLAB® by differentiating Equations 11 and 12 to obtain a system of equations as shown below. First, Equation 11 is differentiated yielding the following result. Recall that a_1 is dependent on alcohol, and consequently is a function of time:

$$\frac{dC_1}{dt} = \frac{y_{max1}}{({a_1}^{n_1} + \Delta U(t)^{n_1})^2}\left[\left(n_1 \Delta U(t)^{n_1-1}\frac{d\Delta U}{dt}\right)(a_1(t)^{n_1} + \Delta U(t)^{n_1}) - \left(n_1 a_1(t)^{n_1-1}\frac{da_1}{dt} + n_1 \Delta U(t)^{n_1-1}\frac{d\Delta U}{dt}\right)(\Delta U(t)^{n_1})\right] \quad (14)$$

A factor of n_1 can be pulled out of the differential terms, and the negative can be distributed.

$$\frac{dC_1}{dt} = \frac{y_{max1} n_1}{({a_1}^{n_1} + \Delta U(t)^{n_1})^2}\left[\left(a_1(t)^{n_1}\Delta U(t)^{n_1-1}\frac{d\Delta U}{dt} + \Delta U(t)^{2n_1-1}\frac{d\Delta U}{dt}\right) + \left(-a_1(t)^{n_1-1}\Delta U(t)^{n_1}\frac{da_1}{dt} - \Delta U(t)^{2n_1-1}\frac{d\Delta U}{dt}\right)\right] \quad (15)$$

The second term of each of the differential pieces are identical, allowing for the following simplification:

$$\frac{dC_1}{dt} = \frac{y_{max1} n_1}{({a_1}^{n_1} + \Delta U(t)^{n_1})^2}\left[a_1(t)^{n_1}\Delta U(t)^{n_1-1}\frac{d\Delta U}{dt} - a_1(t)^{n_1-1}\Delta U(t)^{n_1}\frac{da_1}{dt}\right] \quad (16)$$

Now consider the differential terms as described below to yield the final result, Equation 19.

$$\frac{d\Delta U}{dt} = -\frac{dU}{dt} \quad (17)$$

$$\frac{da_1}{dt} = -k_a a_z e^{-k_a A(t)} \quad (18)$$

$$\frac{dC_1}{dt} = \frac{-y_{max1} n_1}{({a_1}^{n_1} + \Delta U(t)^{n_1})^2}\left({a_1}^{n_1}\Delta U(t)^{n_1-1}\frac{dU}{dt} + k_a a_z a_1(t)^{n_1-1}\Delta U(t)^{n_1} e^{-k_a A(t)}\frac{dA(t)}{dt}\right) \quad (19)$$

A similar treatment is applied to Equation 12, wherein it is first differentiated:

$$\frac{dC_2}{dt} = \frac{-y_{max2}}{(\Delta T(t)^{n_2} + {a_2}^{n_2})^2}\left[\left(n_2 \Delta T(t)^{n_2-1}\frac{d\Delta T}{dt}\right)({a_2}^{n_2} + \Delta T(t)^{n_2}) - \left(n_2 \Delta T(t)^{n_2-1}\frac{d\Delta T}{dt}\right)(\Delta T(t)^{n_2})\right] \quad (20)$$

A factor of n_2 can be pulled out and the products distributed to show that the last two terms are equal and opposite, consequently dropping out:

$$\frac{dC_2}{dt} = \frac{-y_{max2} n_2}{(\Delta T(t)^{n_2} + {a_2}^{n_2})^2}\frac{d\Delta T}{dt}[{a_2}^{n_2}\Delta T(t)^{n_2-1} + \Delta T(t)^{2n_2-1} - \Delta T(t)^{2n_2-1}] \quad (21)$$

$$\frac{dC_2}{dt} = \frac{-y_{max2} n_2}{(\Delta T(t)^{n_2} + {a_2}^{n_2})^2}\left({a_2}^{n_2}\Delta T(t)^{n_2-1}\frac{d\Delta T}{dt}\right) \quad (22)$$

Now consider the derivative of the total number of receptors at the synapse, and pull out the resultant negative to obtain the final result, Equation 25.

$$\frac{d\Delta T}{dt} = -\frac{dU}{dt} - \frac{dB}{dt} \quad (23)$$

$$\frac{dC_2}{dt} = \frac{-y_{max2} n_2}{(\Delta T(t)^{n_2} + {a_2}^{n_2})^2}\left[{a_2}^{n_2}\Delta T(t)^{n_2-1}\left(-\frac{dU}{dt} - \frac{dB}{dt}\right)\right] \quad (24)$$

$$\frac{dC_2}{dt} = \frac{y_{max2} n_2}{(\Delta T(t)^{n_2} + {a_2}^{n_2})^2}\left[{a_2}^{n_2}\Delta T(t)^{n_2-1}\left(\frac{dU}{dt} + \frac{dB}{dt}\right)\right] \quad (25)$$

The system of differential equations described by Equations 2, 3, 19, and 25 were solved using an ordinary differential equation solver (ode15s) in MATLAB®.

For the purposes of validation, it is important to note that both the absolute controller action and its derivative are consistent with the form used in previous work as y_{max2} and a_z approach zero. When controller action is described in terms of a deviation variable, it takes the following form:

$$C(t) = y_{max}\left(1 - \frac{a^n}{U(t)^n + a^n}\right) \quad (26)$$

$$C(t) = y_{max}\left(\frac{U(t)^n + a^n}{U(t)^n + a^n} - \frac{a^n}{U(t)^n + a^n}\right) = y_{max}\left(\frac{U(t)^n}{U(t)^n + a^n}\right) \quad (27)$$

In the limit that y_{max2} goes to zero, the composite controller action takes the following form:

$$\lim_{y_{max2}\to 0} C_T(t) = y_{max1}\left(\frac{\Delta U(t)^{n_1}}{a_1(A)^{n_1}+\Delta U(t)^{n_1}}\right) - y_{max2}\left(\frac{\Delta T(t)^{n_2}}{{a_2}^{n_2}+\Delta T(t)^{n_2}}\right) \quad (28)$$

$$C_T(t) = y_{max1}\left(\frac{\Delta U(t)^{n_1}}{a_1(A)^{n_1}+\Delta U(t)^{n_1}}\right) \quad (29)$$

A similar proof can be performed for the derivative of the respective control actions. Using the quotient rule:

$$\frac{dC(t)}{dt} = \frac{y_{max}}{(U(t)^n+a^n)^2}\left(nU(t)^{n-1}\frac{dU}{dt}(U^n + a^n - U^n)\right) \quad (30)$$

$$\frac{dC(t)}{dt} = \frac{y_{max}naU(t)^{n-1}\frac{dU}{dt}}{(a^n+U(t)^n)^2} \quad (31)$$

Similarly, when the composite controller action is considered and the limit as y_{max2} and a_z approach zero is taken:

$$\lim_{y_{max2}\to 0}\frac{dC_T}{dt}(t) = \frac{-y_{max1}n_1}{({a_1}^{n_1}+\Delta U(t)^{n_1})^2}\left({a_1}^{n_1}\Delta U(t)^{n_1-1}\frac{dU}{dt} + k_a a_z a_1(t)^{n_1-1}\Delta U(t)^{n_1}e^{-k_a A(t)}\frac{dA(t)}{dt}\right) + \frac{y_{max2}n_2}{(\Delta T(t)^{n_2}+{a_2}^{n_2})^2}\left[{a_2}^{n_2}\Delta T(t)^{n_2-1}\left(\frac{dU}{dt}+\frac{dB}{dt}\right)\right] \quad (32)$$

$$\frac{dC_T}{dt}(t) = \frac{-y_{max1}n_1}{({a_1}^{n_1}+\Delta U(t)^{n_1})^2}\left({a_1}^{n_1}\Delta U(t)^{n_1-1}\frac{dU}{dt} + k_a a_z a_1(t)^{n_1-1}\Delta U(t)^{n_1}e^{-k_a A(t)}\frac{dA(t)}{dt}\right) \quad (33)$$

$$\lim_{a_z\to 0}\frac{dC_T}{dt}(t) = \frac{y_{max1}n_1{a_1}^{n_1}\Delta U(t)^{n_1-1}\frac{d\Delta U}{dt}}{({a_1}^{n_1}+\Delta U(t)^{n_1})^2} \quad (34)$$

This shows that these two controller formulations are identical, allowing for the simplification of additional layers of complexity which are considered in this study.

3.5 Alcohol and Withdrawal Profiles

One of the goals of this study was to determine the effect of varying ethanol dependence and withdrawal profiles on the development of withdrawal symptoms. To that end, a variety of disturbance profiles were proposed and tested utilizing the model as derived previously.

The base case was previously developed by Staehle, wherein alcohol was represented by a chronically increasing sinusoid with fixed periodicity and abrupt withdrawal at a specified time t_w, as described in Equation 26:

$$A(t) = \begin{cases} 0 & \sin(pt) < 0 \\ 0 & for \quad t > t_w \\ Zsin(pt)\exp(gt) & otherwise \end{cases} \tag{26}$$

The periodicity parameter, p, describes the frequency of alcohol consumption, g describes the growth of ethanol consumption over time, t_w is the time at which the desired withdrawal profile is imposed, and Z provides a scaling factor for normalizing the dimensionless alcohol level. As in previous work[58, 59], the parameters used for the base case were p = 0.75 hr^{-1}, g = 5x10^{-4} hr^{-1}, t_w = 500 hr, and Z = 1. This case can be seen in Panel A of Figures 12 and 13.

Additional ethanol disturbance functions were developed to investigate the system response to varying dependence and withdrawal paradigms. Figure 12 and 13 show each of the unique dependence and withdrawal profiles respectively.

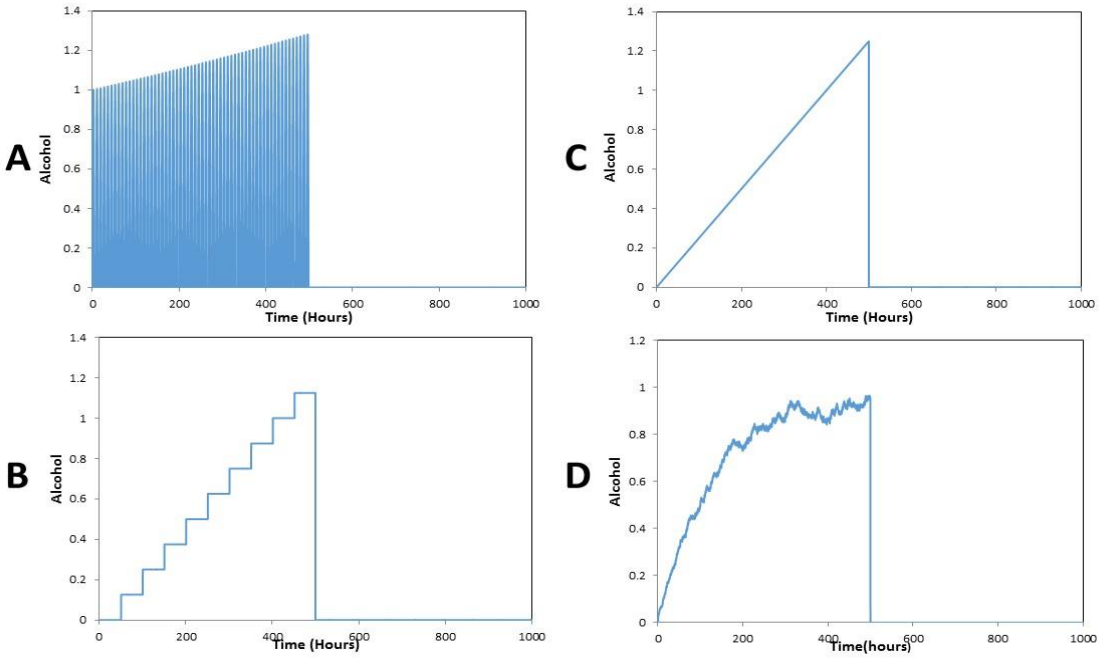


Figure 12. Four distinct ethanol dependence profiles are considered as disturbance functions. The four dependence profiles are: (A) sinusoidal; (B) linear; (C) step-up; (D) random consumption with exponential decay. All include abrupt withdrawal at t_w = 500 hours.

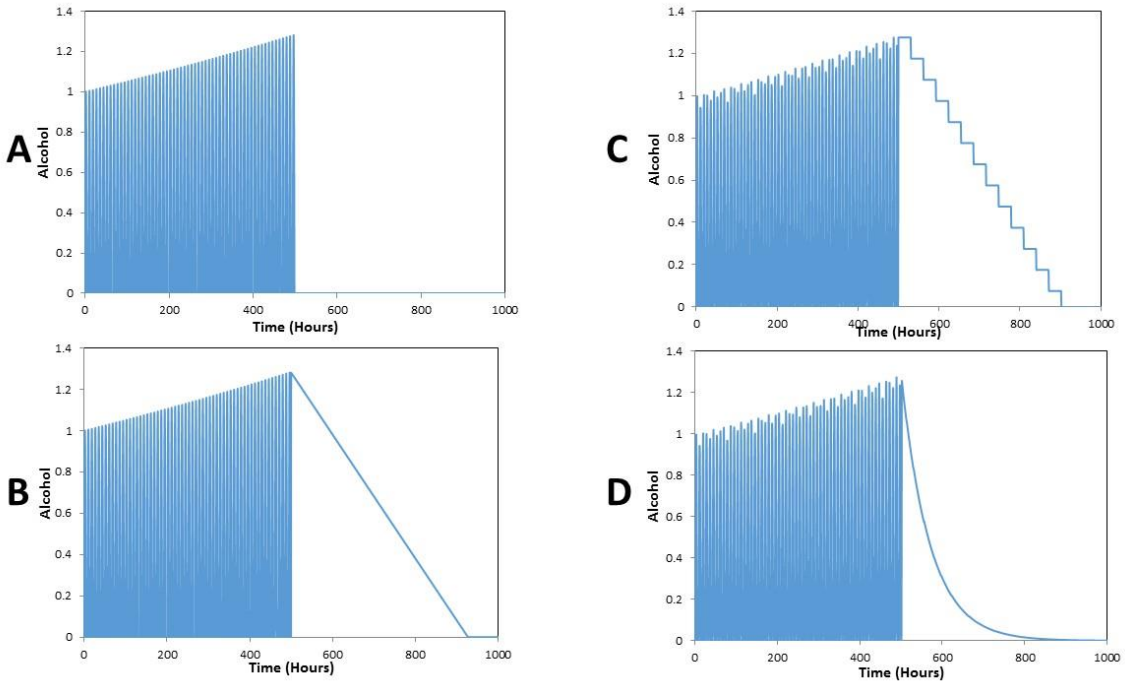


Figure 13. Four distinct withdrawal profiles are considered. The four withdrawal profiles are: (A) abrupt; (B) step-down; (C) linear; (D) exponential decay. All include increasing, sinusoidal consumption leading up to withdrawal at t_w = 500 hours.

3.6 Measures of Withdrawal

One of the primary purposes of this study was to determine the effects of varying patterns of consumption on the development of dependence and subsequent withdrawal. To that end, it is necessary to develop mathematical analogues to the development of both acute withdrawal symptoms as well as the more long-term consequences in order to enable the quantitative comparison of predicted severity.

Two measures have been proposed to measure the extent and severity of withdrawal: maximum synaptic NMDAR population and area under the NMDAR curve.

The first is the peak value of NMDARs at the synapse. One of the assumptions built into the relocation-only model is that activity is directly related to the density of receptors at the synapse. Consequently, the point of maximum hyperexcitability is hypothesized to a reasonable measure of the peak excitotoxic activity and resultant probability or severity of withdrawal-induced seizures.

The second measure of withdrawal is the area between the NMDAR curve and the explicit set point. The purpose of this measure is to determine the long-term deviation from normal brain function in the wake of ethanol withdrawal due to lasting changes to NMDAR population function, composition, and density. The area under the curve was calculated as an approximate integral between the explicit set point and the actual unblocked receptor curve following the initiation of withdrawal. This calculation was conducted using a midpoint approximation for all points after the initiation of withdrawal where the number of unblocked NMDARs was above the set point. The time step for this approximation was 0.1 hours.

Chapter 4

Results and Discussion[2]

4.1 A Control Systems Model of Dynamic NMDAR Populations

In the hopes of better understanding biological responses to alcohol dependence and withdrawal at a systemic level, a mathematical model was developed to describe the dynamics of NMDARs in response to ethanol where the excitatory neurotransmission process was considered as a negative feedback control system. As described in Chapter 3, a composite controller was considered with two active components: an activity controller that maintains synaptic activity by sending additional receptors to the synapse, and a density controller that moderates the population of NMDARs at the synapse by removing active, unblocked receptors from the synapse. Together, these controllers function to maintain a constant number of active synaptic receptors in the face of disturbances, such as inhibition of receptors by ethanol.

When simulated with the alcohol profile given in Equation 26 (Figure 12A), the model described in the Methods section with Parameter Set A (Table 1) produced results (Figure 14) that are qualitatively consistent with experimental data in four distinct ways: (1) the synaptic NMDAR population increases with alcohol (Figure 14C)[9, 10, 12, 14, 30, 32, 33, 44]; (2) alcohol consumption paradigms affect the severity of outcomes[9, 42]; (3) an excitotoxic withdrawal response is observed[6, 11, 14, 22]; and (4) NMDAR populations return to normal levels over time[33].

[2] Substantial portions of this chapter were adapted from Gutierrez and Staehle[57].

Here, the explicit set point was fixed at an arbitrary value of 100 NMDARs at the synapse, as indicated by the dashed line in Figure 14C. As alcohol molecules block the active, unblocked receptors, the composite controller attempts to maintain the set point value by receptor translocation to (activity controller, dark red) and from (density controller, navy blue) the extrasynaptic pool of receptors (Figure 14B).

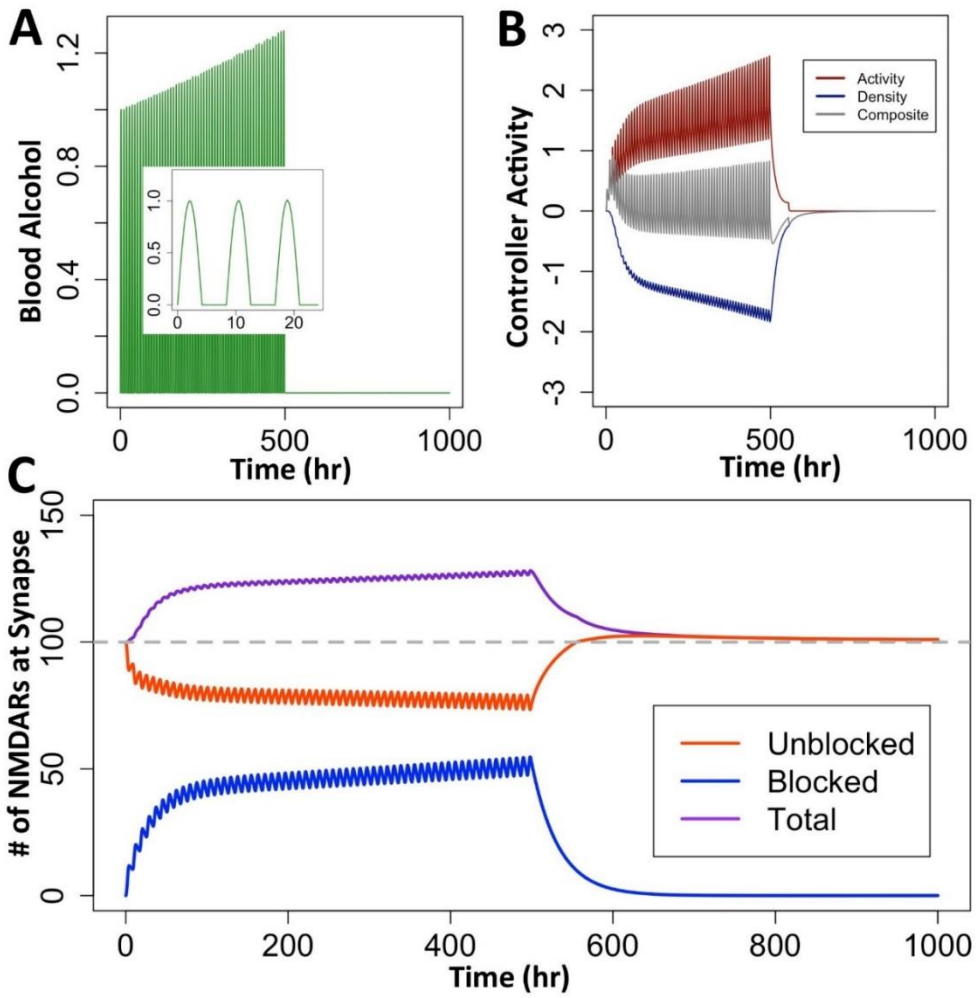


Figure 14. Simulated model response to alcohol consumption. (A) The model with Parameter Set A was simulated with a gradually growing dimensionless alcohol input that approximates three alcohol consumption peaks per day (as shown in the 24-hour inset) and an abrupt withdrawal after 500 hours (B) The resultant controller activity, expressed as number of NMDARs translocated to the synapse per hour. In response to the changing levels of active, unblocked NMDARs at the synapse, the activity controller (dark red) moves NMDARs from the extrasynaptic pool to the synapse, while the density controller (navy blue) removes NMDARs from the synapse. The overall, composite controller activity is shown in grey. (C) Dynamics of unblocked (red), blocked (blue), and total (purple) NMDARs at the synapse in response to the alcohol profile shown in Panel A.

4.2 A Robust, Tunable Composite Controller

The proposed composite controller consists of six primary parameters: y_{max1}, n_1, $a_1(A)$, y_{max2}, n_2, and a_2, where a_1 is a function of alcohol concentration involving three secondary parameters: a_x, a_z, and k_a (Equations 11-13). The steady state controller activity varies with changes in the primary parameters as shown in Figure 15. Increases in alcohol concentration enhance the actions of the activity controller through its effect on a_1 (Equation 13). This provides a mechanism for incorporating alterations in the apparent set point during long-term exposure to alcohol, which is one hypothesis for the development of alcohol dependence[6, 14, 15, 44].

Clinical reports suggest a wide variety among individuals' neuroexcitatory activity during alcohol dependence and withdrawal based on genetics[34], gender[9], and behavior[42]. The proposed composite controller is tunable to approximate a range of activity. Figure 16 shows the simulated results for four alternative controller configurations (Parameters Sets B-E, Table 1) responding to the same alcohol input (Equation 26). The magnitude and duration of the predicted excitotoxicity following withdrawal varies considerably among these alternative configurations.

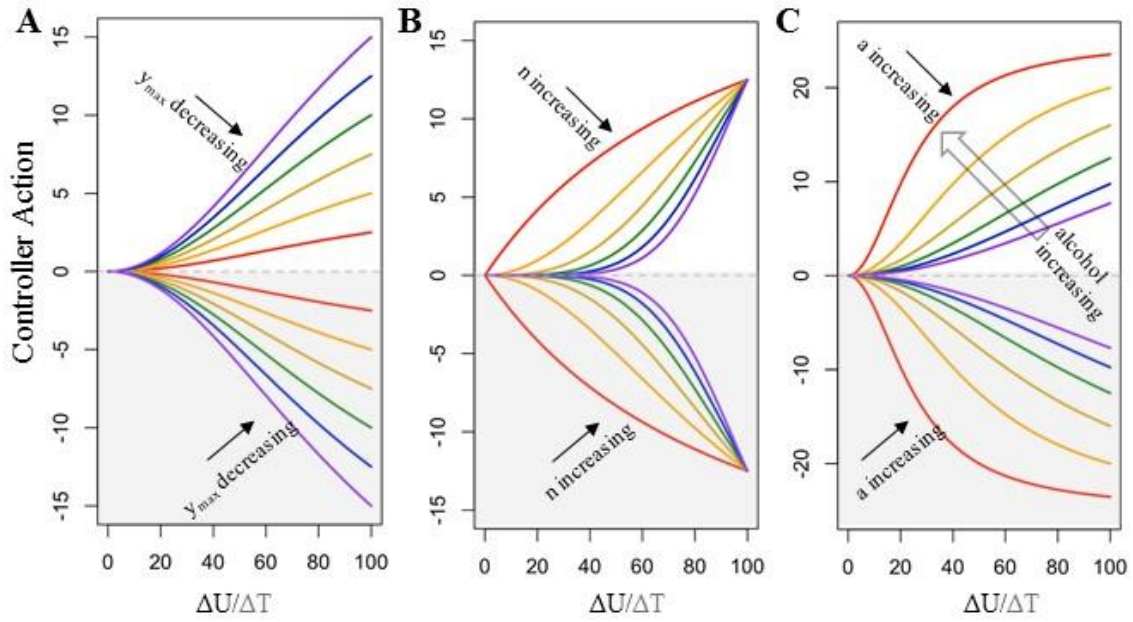


Figure 15. Steady state controller actions for various primary parameter alterations. The number of NMDARs translocated to the synapse per hour by each controller with various values of y_{max} (A), n (B), and a (C). In each panel, the action of the activity controller is positive, changing in response to ΔU, whereas the action of the density controller is negative and changes according to ΔT. Parameter values were altered in common intervals across both controllers. For (A), y_{max} = 5 (red), 10 (orange), 15 (yellow), 20 (green), 25 (blue), and 30 (purple) receptors/hour. For (B), n = 1 (red), 2 (orange), 3 (yellow), 4 (green), 5 (blue), and 6 (purple). For (C), a = 25 (red), 50 (orange), 75 (yellow), 100 (green), 125 (blue), and 150 (purple) receptors. Parameter values not explicitly changed are those of Parameter Set A. Increased alcohol concentration decreases the value of a_1, as shown by the open arrow in (C).

Table 1
Controller Parameter Sets.

Parameter	**Set A**	**Set B**	**Set C**	**Set D**	**Set E**
y_{max1} *(rec/hr)*	25	25	25	25	25
n_1	2	2	**4**	2	**4**
a_x *(rec)*	50	50	50	**25**	**25**
a_z *(rec)*	50	50	50	**25**	**25**
k_a	0.5	0.5	0.5	0.5	0.5
y_{max2} *(rec/hr)*	25	**12.5**	25	25	25
n_2	2	2	**4**	2	**4**
a_2 *(rec)*	100	100	100	**50**	**50**
Figure(s)	14,15*	16A	16B	16C	16D-23

Values of parameters used for various analyses and the figures associated with the corresponding analyses. Bolded numbers highlight differences from Parameter Set A. *: For each panel of Figure 15, the indicated parameter was altered from its nominal value in Parameter Set A.

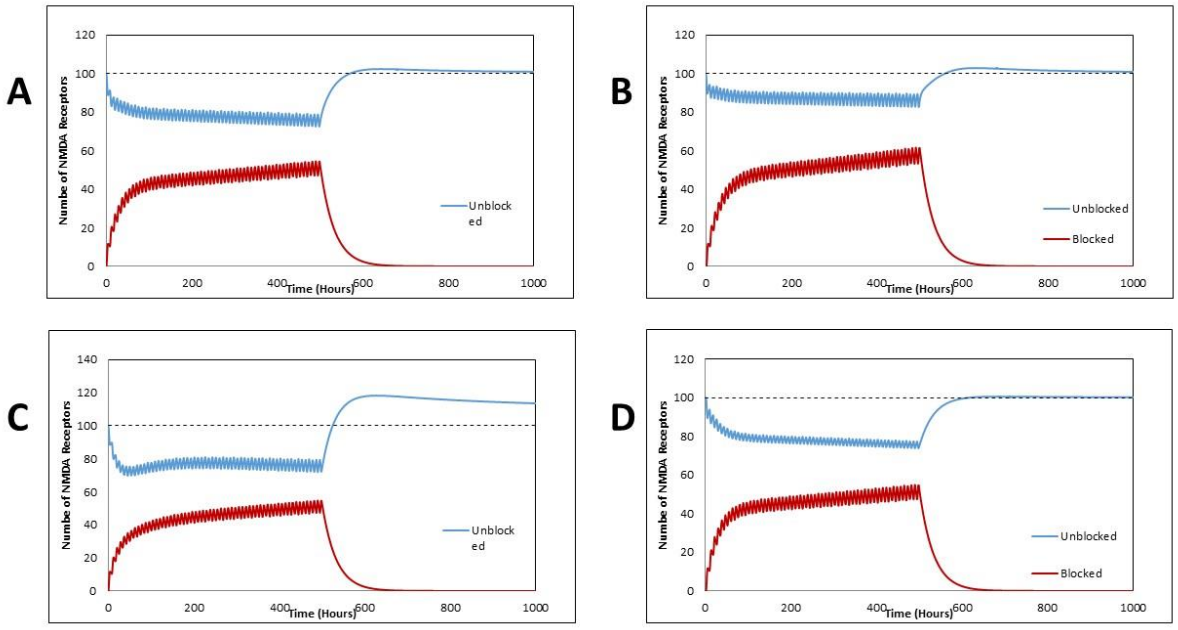


Figure 16. The magnitude and duration of predicted excitotoxicity varies with controller configuration. In response to the alcohol input of Equation 26 (shown in Figure 14A), the number of blocked (blue) and unblocked (red) NMDARs at the synapse varies with controller configuration. Controller parameters are listed in Table 1: (A) Parameter Set B, (B) Parameter Set C, (C) Parameter Set D, (D) Parameter Set E. Excitotoxicity is inferred when the number of unblocked receptors at the synapse is greater than 100 (the arbitrarily defined explicit set point).

4.3 Consumption Patterns Leading to Dependence Do Not Influence Predicted Withdrawal Severity

Clinicians have no control over the alcohol consumption pattern that leads to alcohol dependence, and frequently the pattern is unknown. In order to gauge the relative import of specific consumption patterns on predicted withdrawal severity, four alcohol consumption patterns were simulated. Parameter Set E was selected for these investigations, because, as shown in Figure 16, this configuration led to moderately severe predicted withdrawal upon cessation of alcohol input. As shown in Table 2 and the insets of Figure 17, all four proposed alcohol inputs involve a gradually increasing dimensionless alcohol level that ends abruptly after 500 hours. The profiles vary in consumption pattern from an idealized linear increase to a randomized pattern of intake.

The simulated results of NMDAR levels at the synapse are shown in Figure 17. Interestingly, the severity of alcohol withdrawal, as quantified by the area under the curve and the maximum number of unblocked receptors at the synapse, does not change appreciably (<10%, Table 2). In fact, as long as the consistency of exposure, peak ethanol concentration, and withdrawal profile are similar, the severity of withdrawal is largely the same. This suggests that although withdrawal severity differs considerably with controller parameters (Figure 16, akin to different activities in different individuals), the specific pattern of alcohol consumption with a given duration prior to withdrawal does not affect predicted withdrawal severity (Figure 17, Table 2).

It is important to note that this assumes that the controller parameters remain constant, and do not vary in response to chronic ethanol. It is possible that the values of these parameters are dependent on disturbances via ethanol, either directly or as a result

of neuroexcitatory cascades, which would add an additional layer of complexity to this issue.

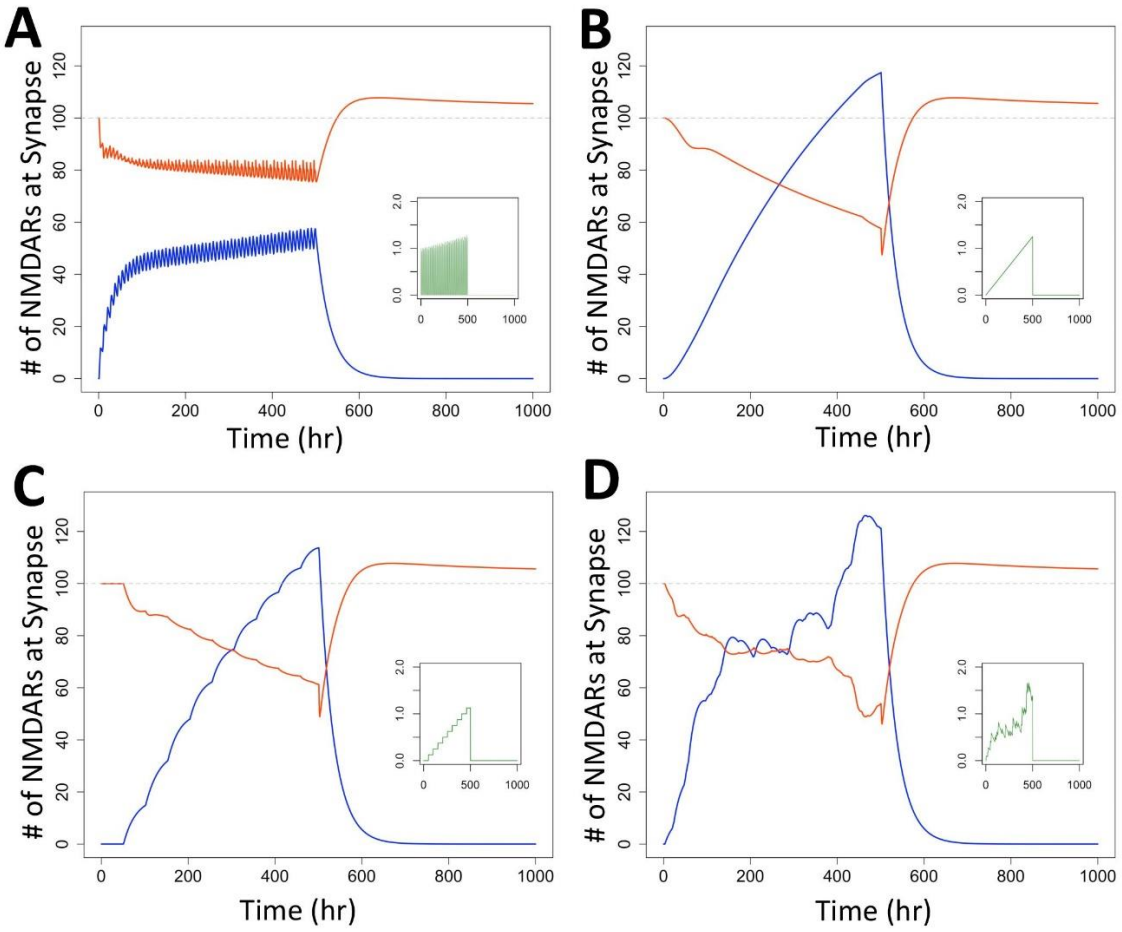


Figure 17. The specific pattern of alcohol consumption prior to withdrawal does not affect withdrawal severity. The number of unblocked (red) and blocked (blue) NMDARs at the synapse for various dimensionless alcohol consumption patterns (green insets). For periodic increases (A), constantly increasing levels (B), incremental increases (C), and randomly distributed dimensionless alcohol levels (D), the excitotoxicity after withdrawal is relatively uniform for the given parameter set (Parameter Set E, Table 1).

Table 2
Measures of withdrawal severity for the four alcohol dependence profiles tested.

Alcohol Profile	**Withdrawal Profile**	**Area Under Curve**	**Unblocked Max**
Equation 26	$\left\{\begin{matrix} t \geq 500 \\ A(t) = 0 \end{matrix}\right\}$	2893	107.77
$A(t) = 0.0025t$	$\left\{\begin{matrix} t \geq 500 \\ A(t) = 0 \end{matrix}\right\}$	2743	107.77
$\left\{\begin{matrix} A(t=0) = 0 \\ Incremental\ increase\ of \\ 0.125\ every\ 50\ hours \end{matrix}\right\}$	$\left\{\begin{matrix} t \geq 500 \\ A(t) = 0 \end{matrix}\right\}$	2743	107.77
$\left\{\begin{matrix} p \leq 0.1 \;\; A(t) = A(t-1) + 0.1 \\ p > 0.1 \;\; A(t) = A(t_{last})e^{-0.01(t_{last}-t} \end{matrix}\right\}$ Where p is a randomly generated probability and t_{last} is the time alcohol was last consumed.	$\left\{\begin{matrix} t \geq 500 \\ A(t) = 0 \end{matrix}\right\}$	2680	107.77

The corresponding dynamic responses are shown in Figure 17.

4.4 Alcohol Consumption During Withdrawal Affects Predicted Withdrawal Severity

During withdrawal, administration of ethanol and other NMDAR antagonists has been shown to decrease the severity of withdrawal symptoms in humans and rodents and decrease excitotoxicity in cultured neurons[32, 33]. Unfortunately, the frequency and dosage of NMDAR antagonist administration in in-patient settings is driven symptomatically and administered reactively. This model provides the opportunity to try any withdrawal pattern risk-free and evaluate the predicted withdrawal severity, even patterns that are not

easily implemented clinically. This could lead to proactive administration of antagonist thereby preventing symptoms and excitotoxic damage.

Whereas the alcohol pattern leading to dependence did not influence the quantified measures of withdrawal appreciably, the alcohol pattern during withdrawal has a large impact on these measures. For the pre-withdrawal alcohol input given in Equation 26 for $t < 500$hr, Figure 18 shows the predicted synaptic NMDAR populations during six withdrawal regimes. These regimes include complete cessation (Figure 18A), exponential decay (Figure 18B), step-wise decreases (Figure 18C), and linearly decreasing alcohol profiles with various initial amounts (Figures 18D-F). The quantified severity of withdrawal is shown in Table 3. As expected, additional alcohol present during the withdrawal period decreases the severity of withdrawal, primarily in terms of the area under the curve. The maximum number of unblocked receptors observed is fairly consistent during all withdrawal regimes tested. This is expected to be a function of the controller parameters, which were constant for all withdrawal regimes tested here.

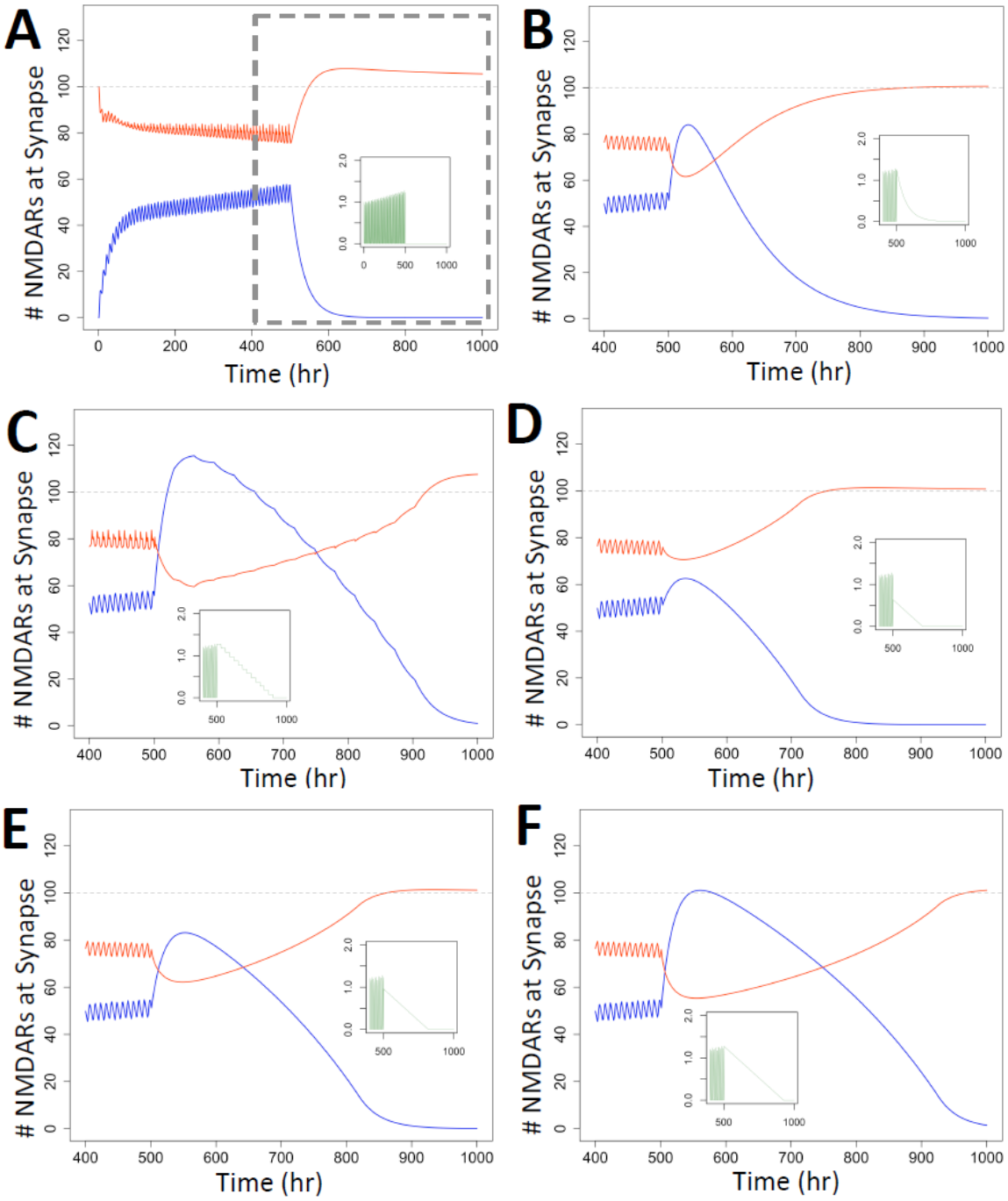


Figure 18. Alcohol levels during withdrawal affect the severity of withdrawal. The number of unblocked (red) and blocked (blue) NMDARs at the synapse in response to various withdrawal patterns (green insets). The full time course is shown in (A). The remaining panels show only t > 400hr, the region inside the dashed box in (A). The response is identical in all withdrawal schemes at t < 500hr. Withdrawal was initiated at t = 500hr in various patterns: (A) abrupt and complete cessation; (B) exponential decrease of alcohol; (C) gradual incremental decreases; (D) constant decrease from ½ maximum alcohol level; (E) constant decrease from ¾ maximum alcohol level; (F) constant decrease from maximum alcohol level.

Table 3
Measures of withdrawal severity for the six withdrawal profiles tested.

Alcohol Profile	**Withdrawal Profile**	**Area Under Curve**	**Unblocked Max**
Equation 15	$\begin{Bmatrix} t \geq 500 \\ A(t) = 0 \end{Bmatrix}$	2883	107.77
Equation 15	$\begin{Bmatrix} t \geq 500 \\ A(t) = A_{max} e^{-0.015(t - t_{withdraw})} \end{Bmatrix}$	54.1	100.64
Equation 15	$\begin{Bmatrix} t \geq 500 \\ A(t) = A_{max} \\ Step\ Down\ 0.1\ every\ 30\ hours \end{Bmatrix}$	2883	107.66
Equation 15	$\begin{Bmatrix} t \geq 500 \\ A(t) = 0.5A_{max} - 0.003(t - t_{withdraw}) \end{Bmatrix}$	260.5	101.38
Equation 15	$\begin{Bmatrix} t \geq 500 \\ A(t) = 0.75A_{max} - 0.003(t - t_{withdraw}) \end{Bmatrix}$	157.3	101.38
Equation 15	$\begin{Bmatrix} t \geq 500 \\ A(t) = A_{max} - 0.003(t - t_{withdraw}) \end{Bmatrix}$	19.3	101.11

The corresponding dynamic responses are shown in Figure 18.

The sudden drop in unblocked receptors and peak in blocked receptors observed in the ramp, step down, and exponential decay profiles may seem counterintuitive; a peak in unblocked receptors is expected to coincide with observed withdrawal symptoms. However, this behavior is due to the shift from periodic to sustained alcohol levels. When the alcohol level deviates between large values and zero (as it does at $t < 500$hr), the controller activity mimics these changes. Consistent controller response, however, leads to a large increase in the number of synaptic receptors, but the high ethanol level initiating this consistent response means that the receptors are quickly blocked, and become unblocked gradually as alcohol level diminishes. This suggests that even if it were possible to maintain a non-zero alcohol level during in-patient withdrawal, the effects on NMDAR-mediated neuroexcitatory processes would not be favorable.

4.5 Measures of Dependence

One of the goals of this study was to determine whether there is some quantitative measure of whether or not a patient has developed physical dependence on ethanol. Prior analysis has shown that the path by which dependence is achieved has little effect on subsequent measures of withdrawal symptoms. It is, however, still to be determined whether the consumption profile affects the development of dependence.

In examining this question, there are a number of parameters which much be considered. Foremost among these is the quantity, frequency, and duration of ethanol consumption. To study the effects of these parameters on the development of dependence, two ethanol consumption profiles were proposed. First, a step function is

considered to determine the combined effects of varying duration and amplitude of consumption. Second, a periodic function with fixed amplitude is considered to determine the effects of varying frequency and amplitude. These ethanol profiles are shown in Figure 19.

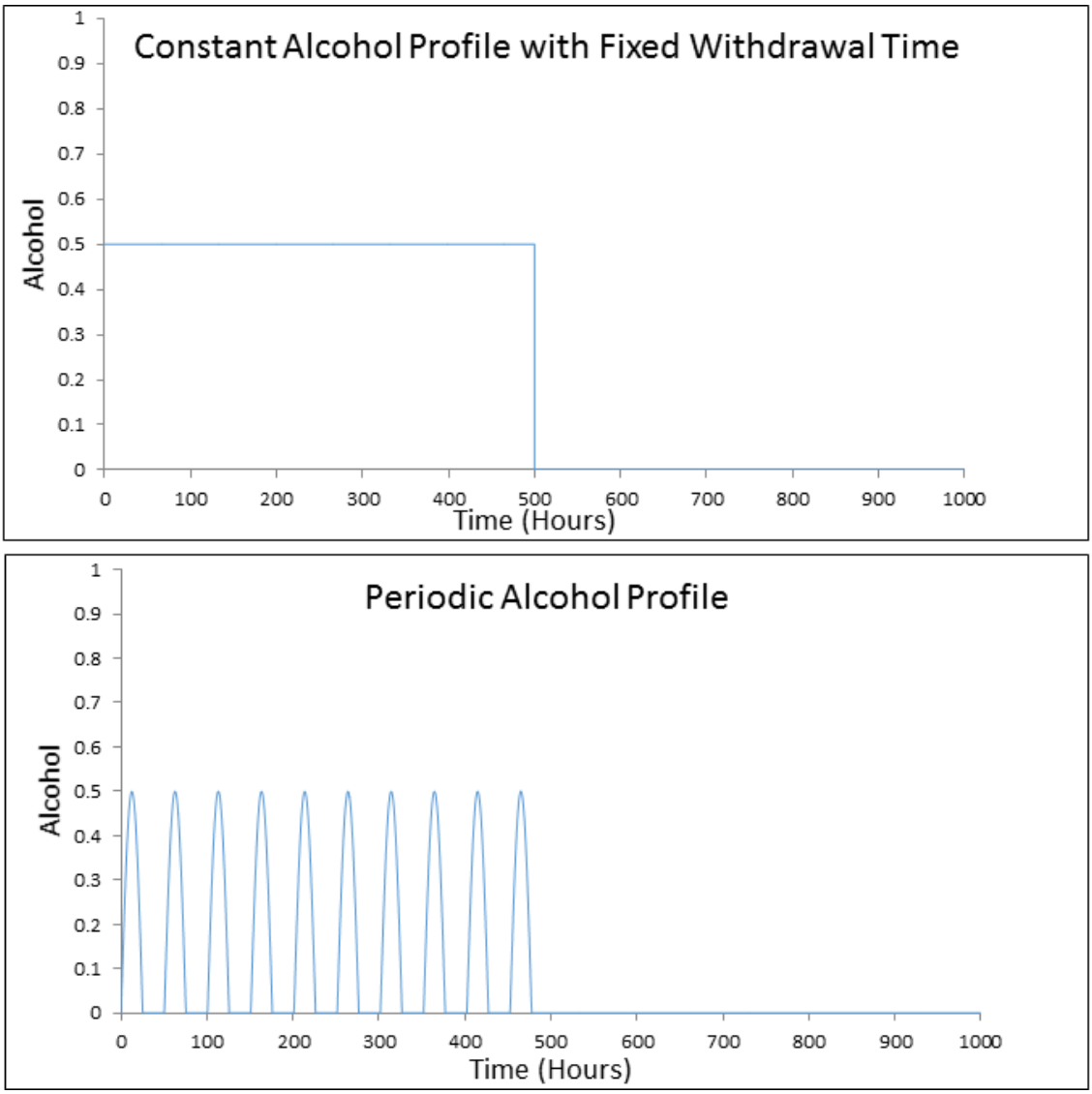
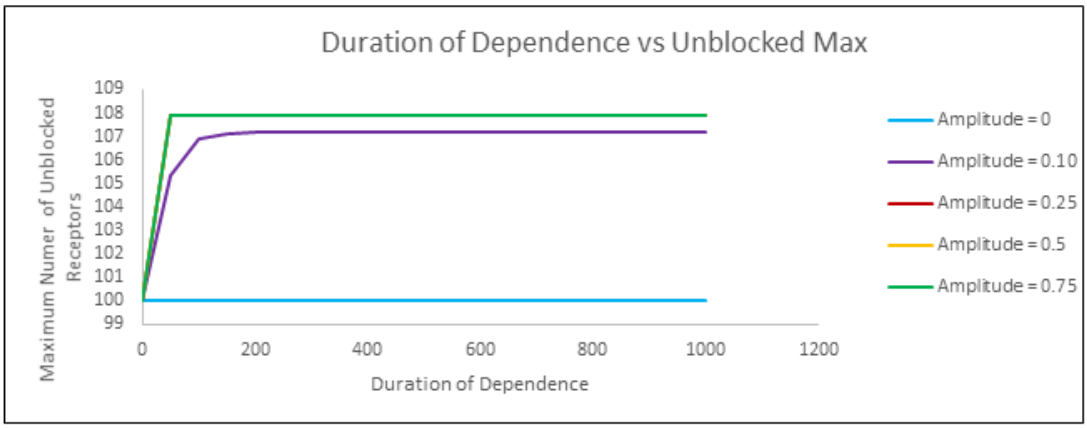


Figure 19. Patterns of ethanol consumption used in the study of dependence development. The Heaviside step function profile (top) involves a constant alcohol input for a predetermined amount of time. Both the duration and amplitude can be varied. The periodic function is the positive portion of a sine wave, where both the frequency and amplitude can be varied.

In order to test the effects of these parameters on the development of dependence, two trials were performed. First, the control system was studied using various constructions of the step function, where the alcohol input was varied from 0 to 1.00 by steps of 0.05 and the duration of consumptions was varied from 0 to 1000 hours by steps of 50 hours. The time allowed for the development of withdrawal symptoms was held constant at 500 additional hours beyond the time of withdrawal. Second, the control system was studied with the periodic consumption profile, where the amplitude of consumption was varied from 0 to 1.00 by steps of 0.05 and the frequency was varied from 0 to 5.00 by steps of 0.25. Controller parameter Set E was used for these trials (Table 1).

For each trial, both the maximum number of unblocked receptors and the area under the NMDA curve were recorded. The series of data were superimposed and plotted in order to identify possible patterns which could be used to indicate dependence. The results are shown in Figures 20 and 21.



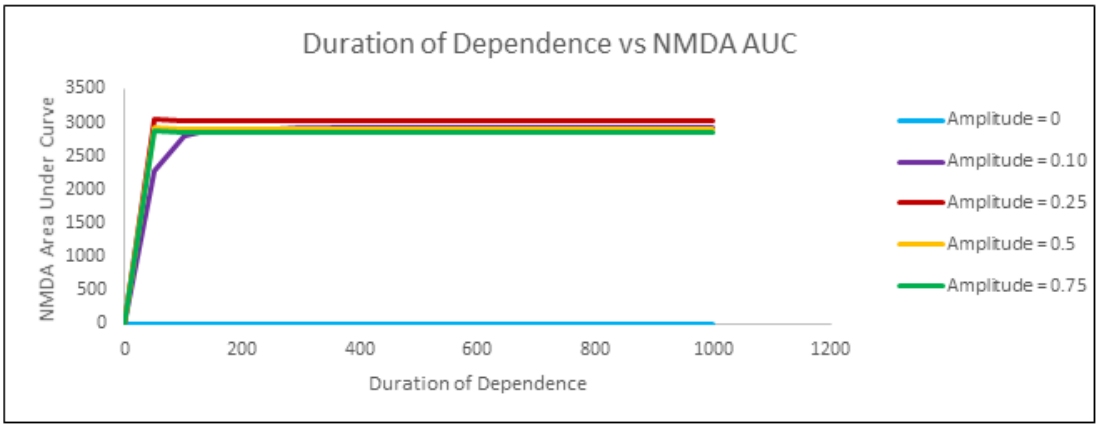


Figure 20. Measures of dependence vary with both duration and amplitude of consumption. The results show that both maximum number of receptors (top) and the area under the NMDA curve (bottom) vary with similar patterns in response to changes in the amplitude and duration of alcohol consumption. It is observed that as long as sufficient alcohol is in the system (amplitude ≥ 0.1) a similar pattern of withdrawal is observed.

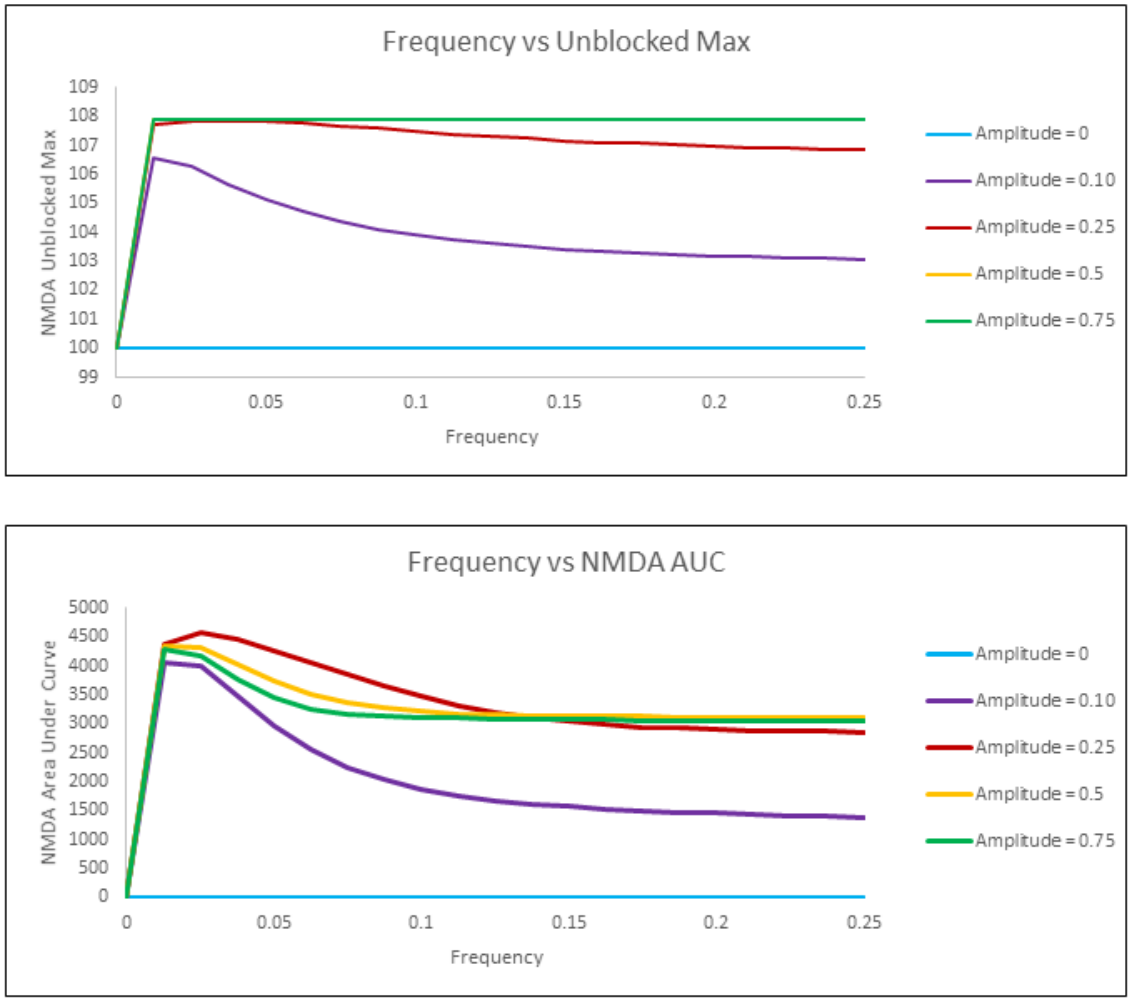


Figure 21. Measures of dependence vary with frequency and amplitude of consumption. Both the maximum number of unblocked receptors (top) and the area under the NMDA curve (bottom) vary with both frequency and amplitude as long as a minimum threshold of ethanol is met (amplitude ≥ 0.10). The peaks observed are similar to those observed in the step function study.

In both studies, a sharp peak is observed as long as a minimum threshold of ethanol is achieved for a relatively short duration or low frequency. This threshold is an amplitude of approximately 0.10 for this set of controller parameters and conditions. It is hypothesized that the slope upwards towards the peak represents the onset and development of dependence, while all points after the peak tend to have high measures of both withdrawal extent and severity, representative of a fully dependent system.

The periodic ethanol disturbance function shows a response that decays with frequency. This is believed to be representative of the limitations of the controller for the given set of parameters. That is to say that beyond the excitotoxic peak, the frequency of alcohol consumption is high enough that the controller cannot effectively keep up with the fluctuations. This may be representative of the kinds of acute consumption that do not lead to maladaptive alterations in synaptic function and composition.

The similar behaviors observed in these plots is believed to be due to comparable areas under the alcohol curve, which can be taken as similar total exposure over time. This result implies that it should be possible to come to a mathematical description of dependence using area under the alcohol curve as a baseline for exposure, with modifications to consider the duration and frequency of exposure.

In order to gain a better understanding of exactly how these parameters interact, the data was plotted in three-dimensions, rather than as a series of superimposed datasets. The results, shown in Figures 22 and 23, show that a similar peak is observed in all four cases. However the shape of the curve is slightly different depending on parameter

limitations, which shows that each parameter affects the development of dependence differently.

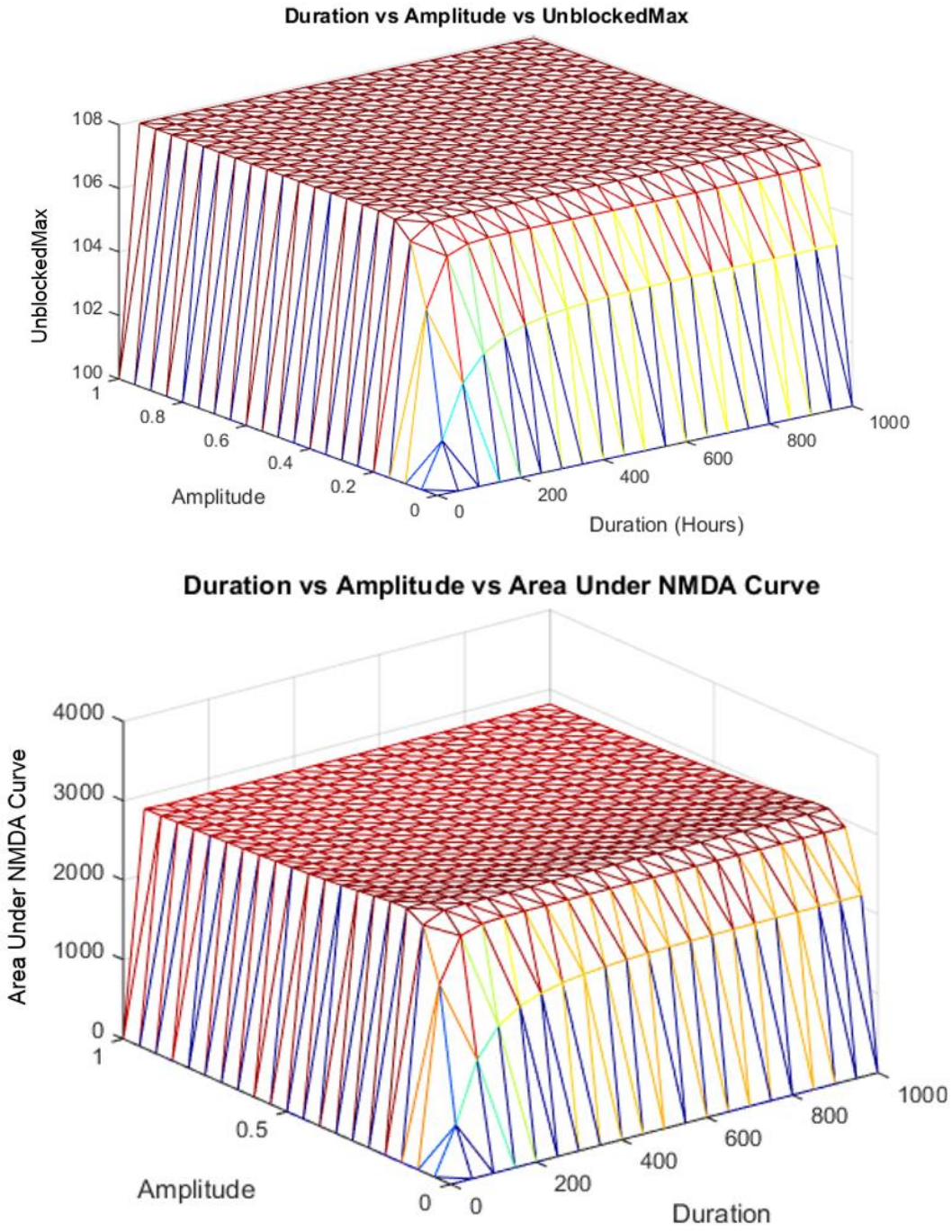


Figure 22. Surface mesh plots of measures of withdrawal versus duration and amplitude. Mesh surface plots of the duration and amplitude of ethanol consumption against the maximum number of unblocked receptors (top) and area under the NMDA curve (bottom) show that measures of withdrawal are largely independent of these parameters as long as a minimum threshold of exposure is met.

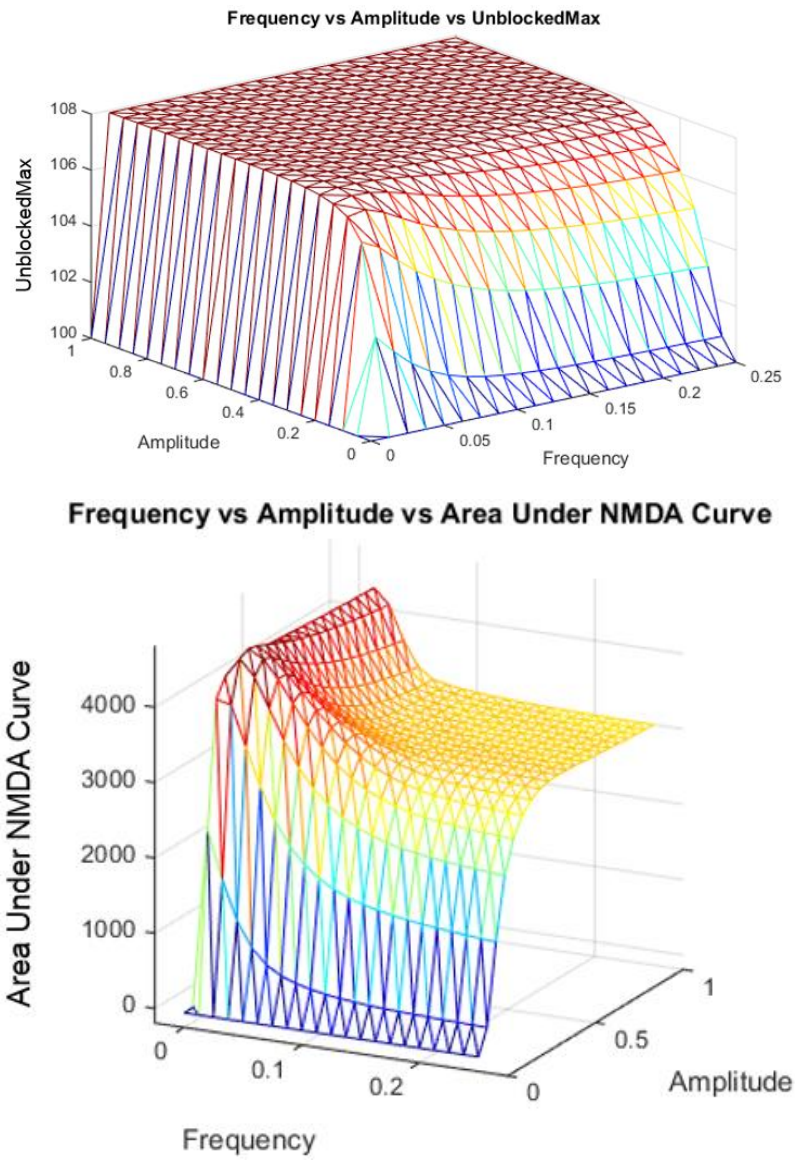


Figure 23. Surface mesh plots of measures of withdrawal versus amplitude, and frequency. Mesh surface plots of the duration and amplitude of ethanol consumption against the maximum number of unblocked receptors (top) and area under the NMDA curve (bottom) show that measures of withdrawal are largely independent of these parameters as long as a minimum threshold of exposure is met. Plots of frequency and amplitude against maximum number of unblocked receptors (top right) and area under the NMDA curve (bottom right) show that frequency of consumption has a more dramatic effect on the shape of the curve describing the development of dependence.

The results obtained in this study are consistent with previous results in that the development of physical dependence does not vary substantially with duration or amplitude. As long as enough ethanol is present for a minimum threshold of time, dependence will be developed and withdrawal symptoms will be observed. It can, however, be observed that variations in frequency can dramatically changes the curvature of the cross-sections describing the development of dependence. This suggests that any mathematical measure of dependence will be largely based on a combination of total ethanol exposure, measured by area under the ethanol curve, and the frequency at which the individual is exposed. It is important to note that the time scales used in this study are arbitrary, and are used to show trends in behaviors which could be fitted to future clinical and experimental data.

4.6 Investigation of the Potential for Patient-Specific Modeling

One of the goals of this study is to demonstrate the possibility of developing plans of care that take into account patient-specific degrees of dependence and unique neurochemical dynamics. In this case, differences in neurochemical behavior are accounted for exclusively through changes in controller parameters. Of particular interest is the balance between the parameters y_{max1} and y_{max2} as well as a_1 and a_2. This is because y_{max1} and y_{max2} represent the maximum controller action attainable by the activity and density controllers respectively, and changes in the balance between these controllers will significantly affect controller behavior when both subcontrollers are active. Similarly, a_1 and a_2 represent the threshold at which the subcontrollers become active, and could

determine the extent to which dependence can develop and the likelihood of emergent withdrawal symptoms.

In order to study the effects that variations in these controller parameters have on measures of withdrawal, a trial was performed where y_{max1} and y_{max2} were varied from 10 to 150 in steps of 7 using Parameter Set E (Table 1) and the periodic ethanol profile with abrupt withdrawal shown in Figures 17A and 18A. Similarly, a second trial was performed where a_1 and a_2 were varied from 50 to 150 in steps of 5. For the purposes of better understanding the controller dynamics, the effect of alcohol on a_1 was ignored, and the final value of a_1 was varied directly. The results of these trials are in Figures 24 and 25.

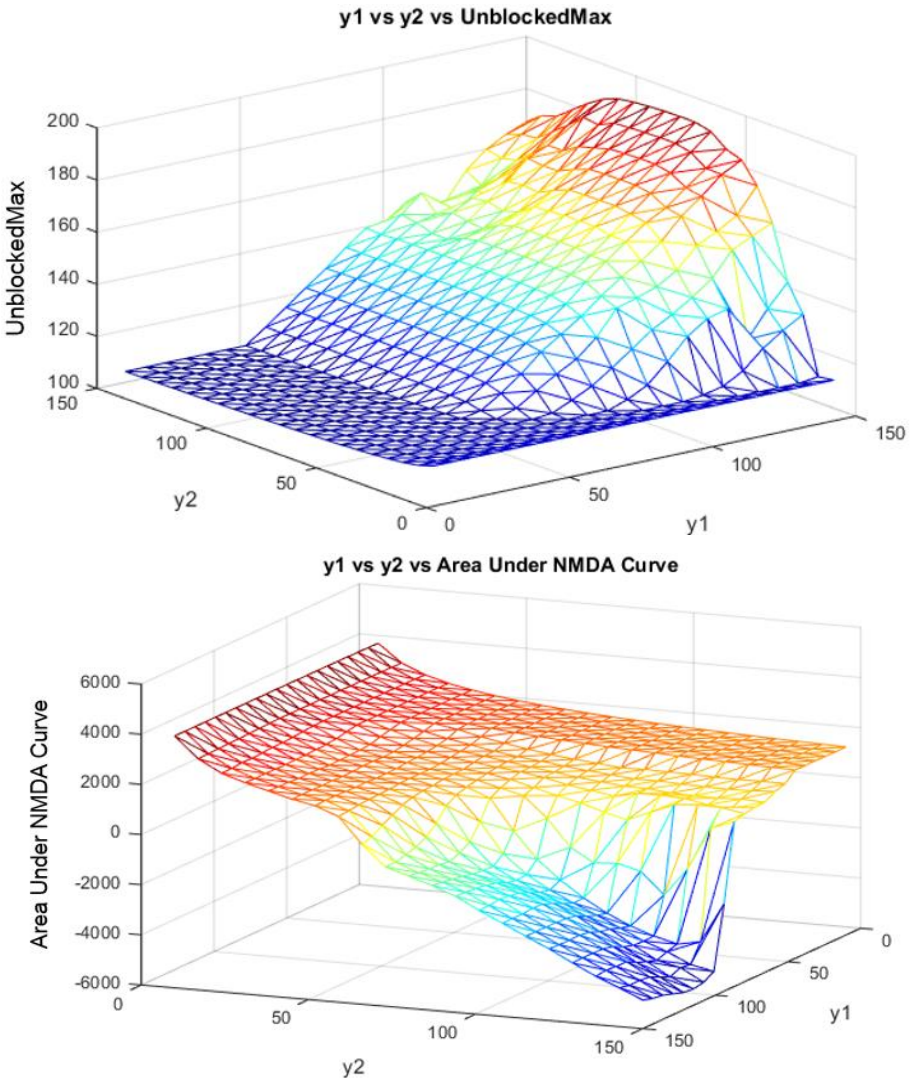


Figure 24. Surface mesh plots of measures of withdrawal versus y_{max1} and y_{max2}. When y_{max1} and y_{max2} are small, there is no observed deviation in the number of unblocked receptors (top) due to the system's inability to respond to the ethanol disturbance. As y_{max1} increases, it is expected that synaptic trafficking will increase. For fixed y_{max1}, increasing y_{max2} results in an initial increase, followed by a plateau as controller actions are balanced, and finally a decay as the density controller becomes dominant. Area under the NMDA curve (bottom) is minimized at high controller actions for both y_{max1} and y_{max2}, with the dependence on y_{max2} appearing to be almost linearly decreasing and dependence on y_{max1} having a sharp change in regime around $y_{max1} = 100$.

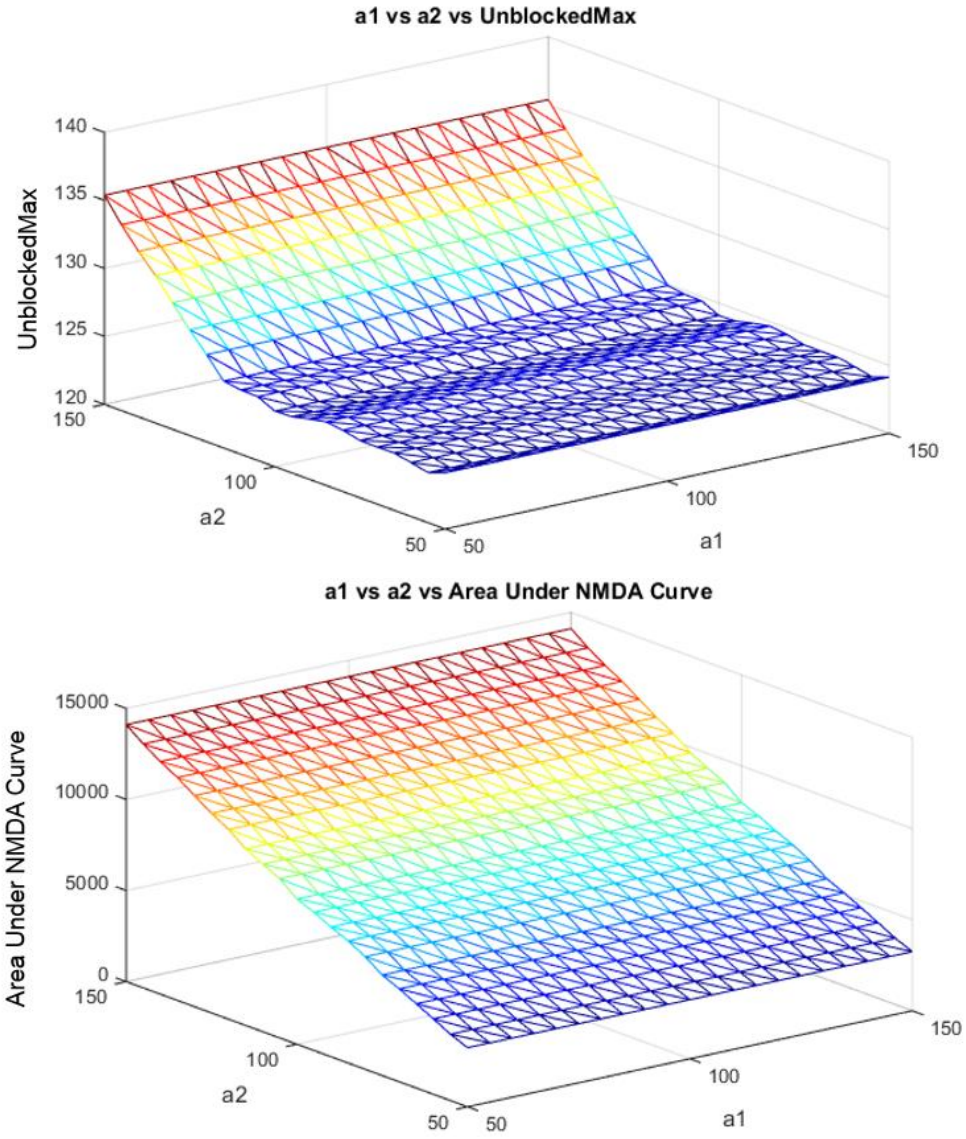


Figure 25. Surface mesh plots of measures of withdrawal versus controller parameters a_1 and a_2. It is observed that both maximum unblocked receptors (top) and area under the NMDA curve (bottom) are both effectively independent of a_1. This is reasonable because the system is given time to reach a stable state of ethanol dependence such that there was time for the controller to overcome deviations in a_1. Both measures show regions of linear dependence with respect to a_2, implying that trafficking away from the synapse is the process which determines the development of withdrawal symptoms.

In these figures it can be seen that the balance between y_{max1} and y_{max2} has a much more dramatic effect on the observed emergence of withdrawal symptom than does a_1 or a_2. This is reasonable because of the time scale over which this system is being observed. Given enough time, the system is able to overcome limitations on controller activity imposed by a_1. It is likely that in trials where the system is not allowed to equilibrate in a dependence state, that the impact of deviations in a1 would be more substantial.

The observed behaviors with respect to a_2 are consistent with expected behaviors. As the threshold at which the controller begins taking action is increased, the system is allowed to spend more time in a hyperexcited state before the controller begins taking corrective actions, resulting in an increased quantity of unblocked receptors and area under the NMDA curve.

The balance between y_{max1} and y_{max2} however, has a significant effect on the system at points where both controllers are active. In particular, this means that the balance between the maximum actions of these controllers determines the degree to which dependence is developed and the severity of controller action as ethanol concentrations are decreasing. It is observed that there are a number of different regimes of behavior in both maximum number of unblocked receptors and area under the NMDA curve. Being able to predict these changes in behavior by mapping a patient's neurological dynamics to model parameters could be useful in developing a withdrawal treatment which minimizes the likelihood of severe withdrawal symptoms based on the degree of dependence, consumption patterns, and predicted regime of controller behavior.

4.7 Limitations and Caveats

No experimental data measuring the translocation of NMDARs in human brain tissue was found over the duration of this study. Therefore, the kinetics shown here are only hypothetical realizations of the control system hypothesis. Wherever possible, dimensionless (e.g. alcohol level) or easily scalable (e.g. y_{max}) functions and parameters were used so that the model could be adapted easily to fit experimental data.

Furthermore, the predicted control actions do not reveal mechanistic information. For example, it has been established that NMDAR subunit composition changes in response to alcohol[13, 30, 32, 54], promoting a removal NMDARs from the synapse. In the model, this is represented in the bulk sensing of blocked NMDARs and removal of receptors by the density subcontroller.

Finally, it is recognized that the severity of alcohol withdrawal cannot be predicted by the levels of unblocked NMDARs alone. For example, the neuroinhibitory system (especially $GABA_A$ receptors) has been implicated in the brain's response to alcohol[6, 20, 63]. A complete representation of withdrawal would require incorporation of these additional systems. However, given the excitotoxic nature of the most detrimental symptoms of alcohol withdrawal (delirium tremens, seizures, etc.), this study has focused on describing the neuroexcitatory effects of alcohol via NMDARs.

Chapter 5

Conclusions and Recommendations[3]

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In this work, a computational model was developed that is based on a negative feedback control system hypothesis of NMDAR regulation at the synapse in the presence of alcohol. The focus of this study is on the hypothesis of the lateral translocation of NMDARs between the synapse and extrasynaptic regions. The model accomplishes this via a dual-action control system whereby NMDARs are translocated from an extrasynaptic pool to the synapse by an activity subcontroller in order to maintain a set number of unblocked, active NMDARs at the synapse while, simultaneously, NMDARs are removed from the synapse by a density subcontroller to maintain a constant density of total NMDARs at the synapse. The composite action of the two subcontrollers aims to maintain glutamatergic signaling even when NMDARs are blocked by ethanol molecules.

The results show that the proposed composite controller produces simulated dynamics consistent with qualitative experimental data describing the biophysical causes of both dependence and withdrawal across a range of values for controller parameters. This means that the model is highly tunable, containing seven parameters which can change the maximum controller response (y_{max}), the threshold of activity (a), the curvature (n), and sensitivity to ethanol (a_{max}). Consequently, it could be fit to any experimental data published in the future, and could subsequently provide powerful insights into individualized dependence and withdrawal dynamics.

[3] Substantial portions of this chapter were adapted from Gutierrez and Staehle[57]

Ethanol functions as a disturbance to this system, engaging homeostatic controller action to maintain a desired neruoexcitatory state. A variety of disturbance functions were imposed during both dependence and withdrawal (Figures 12 and 13) to determine how the emergence of withdrawal symptoms changed in response. Results suggest that withdrawal severity is not influenced by the manner in which alcohol dependence is achieved, provided that the state of dependence is similar. That is to say, as long as the levels of ethanol exposure are sufficiently high for any extended period of time, dependence will develop. This suggests that for a particular individual (analogously, a particular set of controller parameters), the prediction of withdrawal severity depends on the characterization of the current state of dependence (frequency, quantity, and duration of alcohol consumption) and the specific parameters of the individual's NMDAR controller activity. It is important to note that this study assumes that the controller parameters (a, y_{max}, n) remain constant throughout a simulation. It is possible, even likely, that these parameters are dependent on patient-specific factors such as age, gender, and general health, or even parallel neuroexcitatory signaling cascades. Furthermore, prolonged ethanol exposure could modulate parameter values. These effects and the resultant behaviors warrant future investigation.

The severity of alcohol withdrawal is substantially influenced by the alcohol input during withdrawal. This is consistent with experimental results that showed that administration of NMDAR antagonists such as ethanol reduce the negative effects of alcohol withdrawal[8], while the administration of NMDAR agonists such as NMDA increase the severity of withdrawal symptoms[7,27]. The results of this work show that the

most effective means of reducing excitotoxicity involve exacerbating the response with increased total alcohol. This is then followed by carefully decreasing alcohol levels over a prolonged period of time. This is not likely to be a viable option clinically, but the model provides tremendous flexibility for conducting *in silico* investigations of alternative withdrawal profiles, which provides the opportunity to gain a better understanding of how changes in the dependence and withdrawal profiles can affect the outcomes of excitotoxic withdrawal and long-term changes to system dynamics and to generate testable hypotheses.

However, all of this data was determined for a system which was "sufficiently ethanol-dependent," meaning that the system had equilibrated at a state of inhibition due to prolonged ethanol exposure. That means that there is interest in determining if there is a means by which the extent of dependence can be measured, as this weighs heavily in the determination of appropriate treatment. To that end, the system was studied using a Heaviside step function and sine wave to study the effects of amplitude, duration, and frequency of ethanol consumption on the development of dependence.

The results obtained in the study of the development of dependence are consistent with previous results that all paths to dependence lead to a similar equilibrated dependence state. There are three regimes which can be identified. First, there are regions where too little alcohol is consumed to substantially change the development of withdrawal symptoms. Second, there are planar regions at which an equilibrated state of dependence has been achieved. Third, there are regions between, the curvature of which is highly dependent on the frequency of consumption moreso than the magnitude or

duration of ethanol exposure. This kind of multivariate determination of a dependency surface is unlikely to have direct clinical applications, but means that there is room for additional work in developing a simpler and more comprehensive measure of dependence.

Finally, the model was studied to determine the effect that model parameters have on the development of withdrawal symptoms when the ethanol disturbance is held constant. This is intended to simulate the substantial variations in a population's susceptibility to becoming dependent and subsequently developing withdrawal symptoms. These differences in expected outcomes are a consequence of differences in age, gender, general health, and unique neuroexcitatory dynamics. For baseline analysis, it is hypothesized that these differences can be considered as a change in the balance between the minimum threshold of action and the gains of the activity and density controllers. To that end, two pairs of controller parameters, y_{max1} and y_{max2} as well as a_1 and a_2, were varied and the severity of withdrawal symptoms were determined.

It was observed that a_2 and the balance of y_{max1} and y_{max2} were found to have a substantial impact on the severity of withdrawal for a given state of dependence. This means that from the same state of equilibrated dependence, the withdrawal profile that minimizes the development of symptoms will not necessarily be the same for two unique sets of controller parameters (patients).

To summarize, the results of this study are a robust, tuneable, dual-action control system model for the relocation of NMDARs in response to neuroexcitatory ethanol disturbances. Baseline results are consistent with anticipated behaviors. The model

provides the ability to predict proposed measures of withdrawal severity, and shows, for a given set of controller parameters, that these measures depend primarily on the path taken to reduce ethanol exposure from a state of equilibrated dependence. Further analysis of the paths taken to achieve dependence show that a dependence surface can be generated for a given set of controller parameters to show various degrees of the development of equilibrated dependence and that the shape of this surface changes dramatically with the frequency of consumption. Finally, it is shown that changes in the balance of controller parameters have dramatic effects on withdrawal outcomes, meaning that the model parameters can be varied to more closely match an individual patient.

The goal of this study was to build upon previous work towards developing a more comprehensive model of the neuroexcitatory control of NMDARs in response to ethanol disturbances for the purposes of better understanding withdrawal dynamics and the potential for proposing proactive, personalized treatment paths for patients. To that end, there is still substantial work left to be done.

First, there are a multitude of additional layers of neuroexcitatory complexity which can be considered and implemented. Various parallel mechanisms for modulation of NMDAR population composition and activity were outlined, including modulation by nitric oxides and phosphorylation. This study assumes that the controller parameters (a, y_{max}, n) are constants. As additional clinical and experimental data becomes available, it is possible that the effects of these parallel neuroexcitatory processes could be captured by introducing some functionality of these controller parmaeters with respect to phosphorylation, nitric oxides, and presence of other ions and neuroexcitatory agents.

The model has not been tested to see if behaviors of physical dependence can be accurately modeled. To test this, a second, independent control system could be developed which tries to control the neuroexcitatory state by varying the alcohol disturbance function once a state of dependence has been achieved. This controller would measure the number of unblocked NMDARs at the synapse, and would "drink" whenever the excitatory state exceeded a given number of unblocked receptors to maintain an ethanol-dependent homeostasis. Critical variables include the threshold for controller activity, and the shape and magnitude of the subsequent ethanol disturbance. These results could allow for the prediction of the behaviors of individuals who have developed physical dependence on ethanol.

Current analysis of the development of dependence is based on two individual sets of two parameters: the duration and amplitude of a Heaviside step function and the amplitude and frequency of a sine wave. Based on the shapes of the surfaces obtained from these analyses, it seems likely that duration and amplitude could be grouped together and measured by the area under the alcohol curve. This could allow for a more robust, two-component model whereby dependence is measured by a frequency of consumption and area under the ethanol curve. This hypothesis can be tested by generating dependence surfaces for an extensive array of disturbance profiles to determine if the surfaces are sufficiently similar.

This dependence analysis would also allow for further study of withdrawal dynamics. If a reasonable measure of the extent of dependence can be developed, this

allows for the analysis of withdrawal dynamics beginning at different points on the dependence surface.

This study only considered two pairs of parameters: y_{max1} and y_{max2} as well as a_1 and a_2. The parameters n_1 and n_2 were not considered in this study; the reasons for this are two-fold. The first is that the effect that these changes have on the control system are easy to understand as a shift in either the maximum control action or threshold at which the control begins taking homeostatic actions, while changes in n_1 and n_2 result in changed curvature. The second reason is that analysis of n1 and n2 resulted in issues with discontinuity which have not yet been satisfactorily resolved.

Finally, the end goal of future work in this vein is to develop a mechanism by which the withdrawal profile that minimizes the emergence of excitotoxic behaviors for a given dependence state and set of controller parameters can be identified. If this model can be fitted to data for clinical or experimental withdrawal dynamics, this would allow for the prediction of treatment plans which would minimize withdrawal outcomes.

There are several means by which this might be accomplished. A simple solution would be to test changes in ethanol content and use the model to identify which change minimizes the deviation variable in the next time step. If each time step is optimized in this way, the results should roughly approximate a path which minimizes withdrawal parameters. Alternatively, a database of withdrawal profiles and curvatures could be developed as part of the process for testing dependence surfaces, and unique problems could be matched to prior cases which most closely match the current problem. Finally, the most rigorous solution likely involves the generation of individualized dependence and withdrawal surfaces, with the use of gradients to determine the path by which

dependence can be most quickly reduced with minimal emergence of excitotoxic symptoms.

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Appendix A

Raw Code for Varying Withdrawal and Dependence Profiles in Visual Basic Applications

This is the code for the base case of the bimodal controller hypothesis for NMDAR-mediated ethanol withdrawal as described in Figures 17A and 18A.

A.1 Sinusoid with Abrupt Withdrawal – Base Case

```
'Initial Conditions + Variables to track
Public Alcohol(0 To 200000) As Single 'Array for tracking
Alcohol Content with Time
Public C1Action(0 To 200000) As Single 'Array for tracking
Controller 1 Action with Time.
Public C2Action(0 To 200000) As Single 'Array for tracking
Controller 2 Action with Time.
Public CTAction(-5 To 200000) As Single 'Array for tracking
Total Controller Action with Time.
Public UnblockedS(0 To 200000) As Single 'Array for
tracking Unblocked receptors at the synapse.
Public BlockedS(0 To 200000) As Single 'Array for tracking
Blocked receptors at the synapse.
Public UnblockedP(0 To 200000) As Single 'Array for
tracking Unblocked receptors in the extrasynaptic pool.
Public TotalS(0 To 200000) As Single 'Array for tracking
Total receptors at the synapse.
Public T As Single              'Loop Counter
'Parameters for elementary kinetics.
Public k1 As Single
Public k2 As Single


Public UsDesired As Single 'Desired number of receptors at
the synapse.
Public Deviation1 As Single 'Deviation from set point for
Activity Controller (1)
Public Deviation2 As Single 'Deviation from set point for
Density Controller (2)
Dim AreaUnderCurve As Single 'Used to track area under
alcohol curve after withdrawal is imposed.
Dim UsMax As Single          'Used to track the maximum
number of unblocked receptors at the synapse after
withdrawal is imposed.
```

```
'Controller One Parameters (Activity). Controls relocation
TO synapse.
Public n1 As Single
Public anorm As Single
Public amax As Single
Public ka As Single
Public ymax1 As Single
'Controller two parameters (Density). Controls relocation
FROM synapse.

Public ymax2 As Single
Public a2 As Single
Public n2 As Single

Public Coverage As Single 'Geometric/Physical limitation on
the number of receptors which can be fit at the synapse.
Public Excess As Single 'The number of receptors in excess
of the coverage constraint which the controller attempted
to insert.

Sub RelocationOnly()
'Define and Read Alcohol Parameters

Dim P As Single              'Periodicity
Dim G As Single
Dim tend As Single           'total length of simulation
Public Amplitude As Single 'Amplitude of Sinusoid
Dim withdrawtime As Single  'time at which withdrawal
occurs
P = Sheet1.Cells(9, 2).Value
G = Sheet1.Cells(10, 2).Value
tend = Sheet1.Cells(11, 2).Value
withdrawtime = Sheet1.Cells(13, 2).Value
Amplitude = Sheet1.Cells(14, 2).Value

'Define and Read Kinetics, Controller Parameters,and
Initial Conditions
k1 = Sheet1.Cells(2, 2).Value
k2 = Sheet1.Cells(3, 2).Value
n1 = Sheet1.Cells(22, 2).Value
anorm = Sheet1.Cells(23, 2).Value
amax = Sheet1.Cells(24, 2).Value
ymax1 = Sheet1.Cells(25, 2).Value
ka = Sheet1.Cells(26, 2).Value
```

```
ymax2 = Sheet1.Cells(28, 2).Value
a2 = Sheet1.Cells(29, 2).Value
n2 = Sheet1.Cells(30, 2).Value
Coverage = Sheet1.Cells(32, 2).Value
UsDesired = Sheet1.Cells(27, 2).Value

UnblockedS(0) = Sheet1.Cells(35, 2).Value
BlockedS(0) = Sheet1.Cells(37, 2).Value
UnblockedP(0) = Sheet1.Cells(36, 2).Value
TotalS(0) = UnblockedS(0) + BlockedS(0)
UsMax = UnblockedS(0)
AreaUnderCurve = 0

'Iterate for the full time interval
For T = 0 To tend Step 0.1

'Calculate Current Alcohol
Alcohol(T) = Amplitude * Sin(P * T) * Exp(G * T)
    If T > withdrawtime Then 'Imposes abrupt withdrawal at
withdrawtime.
        Alcohol(T) = 0
    ElseIf Alcohol(T) < 0 Then 'Forces alcohol to be >=
zero.
        Alcohol(T) = 0
    End If

'Calculate Current Controller Action
C1Action(T) = Controller1Action(UnblockedS(T), Alcohol(T))
'Calls a function which calculates Controller 1 Action.
C2Action(T) = Controller2Action(TotalS(T)) 'Calls a
function which calculates Controller 2 Action
CTAction(T) = C1Action(T) - C2Action(T)

'Calculate new values for NMDARs at various positions for
next step
UnblockedS(T + 1) = UnblockedS(T) + (-k1 * Alcohol(T) *
UnblockedS(T)) + k2 * BlockedS(T) + CTAction(T)
UnblockedP(T + 1) = UnblockedP(T) - CTAction(T)
BlockedS(T + 1) = BlockedS(T) + k1 * UnblockedS(T) *
Alcohol(T) - k2 * BlockedS(T)
UnblockedP(T + 1) = UnblockedP(T) - CTAction(T)
TotalS(T + 1) = BlockedS(T + 1) + UnblockedS(T + 1)

'Check for new Unblocked Max
If UnblockedS(T + 1) > UsMax Then
    UsMax = UnblockedS(T + 1)
End If
```

```
'Check for coverage, send excess back to pool
If TotalS(T + 1) > 175 Then
    Excess = TotalS(T + 1) - 175
    UnblockedS(T + 1) = UnblockedS(T + 1) - Excess
    TotalS(T + 1) = 175
    UnblockedP(T + 1) = UnblockedP(T + 1) + Excess
End If
'Area Under the Curve Approximation
If T > withdrawtime Then
If UnblockedS(T - 1) > UsDesired Then
    If UnblockedS(T) > UsDesired Then 'Check if both this
time step and previous were above set point.

    IntegralCheck = 0.05 * (UnblockedS(T - 1) +
UnblockedS(T) - 2 * UsDesired) 'Midpoint approximation of
area between those time steps
    AreaUnderCurve = AreaUnderCurve + IntegralCheck
    End If
End If
End If

'Record in Spreadsheet
Sheet2.Cells(10 * T + 2, 1).Value = T
Sheet2.Cells(10 * T + 2, 2).Value = Alcohol(T)
Sheet2.Cells(10 * T + 2, 3).Value = BlockedS(T)
Sheet2.Cells(10 * T + 2, 4).Value = UnblockedS(T)
Sheet2.Cells(10 * T + 2, 5).Value = UnblockedP(T)
Sheet2.Cells(10 * T + 2, 6).Value = C1Action(T)
Sheet2.Cells(10 * T + 2, 7).Value = C2Action(T)
Sheet2.Cells(10 * T + 2, 8).Value = CTAction(T)
Sheet6.Cells(10 * T + 2, 1).Value = T
Sheet6.Cells(10 * T + 2, 2).Value = Deviation1
Sheet6.Cells(10 * T + 2, 3).Value = Deviation2

Next T

'Report Area Under Curve and UsMax
Sheet1.Cells(13, 6).Value = AreaUnderCurve
Sheet1.Cells(14, 6).Value = UsMax

End Sub

‘Definition of Functions which calculate controller actions

Function Controller1Action(Unblocked As Single, Alcohol As
Single) As Single
```

```
Dim a1 As Single

Deviation1 = UsDesired - Unblocked
If Deviation1 > 0 Then 'Controller Actions are defined
positive until net controller action is calculated.
a1 = anorm + amax * Exp(-ka * Alcohol)
Controller1Action = ymax1 * (1 - (a1 ^ n1 / (Deviation1 ^
n1 + a1 ^ n1))) 'Calculate Controller 1 Action
Else
Controller1Action = 0

End If

End Function

Function Controller2Action(Total As Single) As Single
Deviation2 = Total - UsDesired
If Deviation2 > 0 Then
Controller2Action = ymax2 * (Deviation2 ^ n2 / (a2 ^ n2 +
Deviation2 ^ n2))
Else
Controller2Action = 0
End If

End Function
```

A.2 Code for Dependence Profiles

These lines are inserted in place of the Sinusoid in the previous code under 'Calculate Current Alcohol," and are the profiles generated in Figure 12.

Linear Alcohol Profile

```
'Calculate Current Alcohol
Alcohol(T) = M * T + B
    If T > withdrawtime Then
        Alcohol(T) = 0
    ElseIf Alcohol(T) < 0 Then
        Alcohol(T) = 0
    End If
```

Step-Up Alcohol Profile

```
'Calculate Current Alcohol
If T < 1 Then
    Alcohol(T) = 0

ElseIf StepCount = StepFrequency * 10 Then  'Count step
frequency.

    Alcohol(T) = Alcohol(T - 0.1) + StepSize 'Add StepSize
to previous alcohol at step frequency.
    StepCount = 0   'Reset Step Count.

Else
    Alcohol(T) = Alcohol(T - 0.1)   'Otherwise, alcohol
says constant, step count increments.
    StepCount = StepCount + 1
End If
If T > withdrawtime Then
    Alcohol(T) = 0
End If
```

Random Consumption with Exponential Decay

```
'Calculate Current Alcohol

Randomize
Flag = Int(100 * Rnd) + 1 'generate a random integer
If Flag <= Probability Then 'Check probability
```

```
    Alcohol(T) = Alcohol(T - 1) + PlusAlcohol 'Subject
"drinks" a fixed amount of alcohol.
    LastAlcoholMax = Alcohol(T) 'Store local alcohol
maximum.
    TimeLast = T 'Store time of local alcohol maximum

'If there is no "drink," ethanol exposure begins to decay
exponentially.
Else
    Alcohol(T) = LastAlcoholMax * Exp(-Decay * (T -
TimeLast))
End If
If T > withdrawtime Then
Alcohol(T) = 0
End If
```

A.3 Code for Withdrawal Profiles

These lines are inserted in after the statement "if t < withdrawtime," and represent the imposed withdrawal profiles generated in Figure 13.

Abrupt Withdrawal Profile

```
If T > withdrawtime Then
    Alcohol(T) = 0
End If
```

Step-Down Withdrawal Profile

```
If T > withdrawtime Then
        If StepCount = StepFrequency Then
            Alcohol(T) = Alcohol(T - 1) - StepDown  'Take a
fixed step down every StepCount
            StepCount = 0
        Else
            Alcohol(T) = Alcohol(T - 1) 'Otherwise Alcohol
is constant and StepCount is incremented.
            StepCount = StepCount + 1
        End If
    End If
```

Linear Withdrawal Profile

```
If T > withdrawtime Then
        Alcohol(T) = AlcoholMax - SlopeDown * (T -
withdrawtime)
    End If
```

Exponential Decay Withdrawal Profile

```
    If T > withdrawtime Then
        Alcohol(T) = AlcoholMax * Exp(-Decay * (T -
withdrawtime))
    End If
```

Appendix B

Raw Code for Varying Amplitude and Duration of Dependence of Step Function

```
'Iterate for Various Time intervals and Alcohol Amplitudes

For K = 0 To 20
    amax = 0.05 * K 'Varies amplitude for each trial

    For J = 0 To 20
        withdrawtime = 50 * J   'Varies Withdrawal time for
each trial.

        'Reset Checks
        Usmax = 0
        BlockedSMax = 0
        TotalSMax = 0
        AreaUnderCurve = 0
        AlcoholAreaUnderCurve = 0
            For T = 0 To withdrawtime + 500 Step 0.1

    'Calculate Alcohol For Step
    If T < withdrawtime Then
        Alcohol(T) = amax
    Else
        Alcohol(T) = 0
    End If

'Calculate Current Controller Action
C1Action(T) = Controller1Action(UnblockedS(T), Alcohol(T))
C2Action(T) = Controller2Action(TotalS(T))
CTAction(T) = C1Action(T) - C2Action(T)

'Calculate new values for NMDARs at various positions for
next step
UnblockedS(T + 1) = UnblockedS(T) + (-k1 * Alcohol(T) *
UnblockedS(T)) + k2 * BlockedS(T) + CTAction(T) + kd *
(CTAction(T - 1) - CTAction(T - 2)) + ki * (CTAction(T - 1)
+ CTAction(T - 2) + CTAction(T - 3))
UnblockedP(T + 1) = UnblockedP(T) - CTAction(T)
BlockedS(T + 1) = BlockedS(T) + k1 * UnblockedS(T) *
Alcohol(T) - k2 * BlockedS(T)
UnblockedP(T + 1) = UnblockedP(T) - CTAction(T)
TotalS(T + 1) = BlockedS(T + 1) + UnblockedS(T + 1)
```

```
'Check USMax
If UnblockedS(T + 1) > Usmax Then
    Usmax = UnblockedS(T + 1)
End If
If BlockedS(T + 1) > BlockedSMax Then
    BlockedSMax = BlockedS(T + 1)
End If
If TotalS(T + 1) > TotalSMax Then
    TotalSMax = TotalS(T + 1)
End If


'Record Alcohol Profile for Middle Case
If J = 10 Then
If K = 10 Then

'Record in Spreadsheet
Sheet2.Cells(10 * T + 2, 1).Value = T
Sheet2.Cells(10 * T + 2, 2).Value = Alcohol(T)
Sheet2.Cells(10 * T + 2, 3).Value = BlockedS(T)
Sheet2.Cells(10 * T + 2, 4).Value = UnblockedS(T)
Sheet2.Cells(10 * T + 2, 5).Value = UnblockedP(T)
Sheet2.Cells(10 * T + 2, 6).Value = C1Action(T)
Sheet2.Cells(10 * T + 2, 7).Value = C2Action(T)
Sheet2.Cells(10 * T + 2, 8).Value = CTAction(T)
Sheet6.Cells(10 * T + 2, 1).Value = T
Sheet6.Cells(10 * T + 2, 2).Value = Deviation1
Sheet6.Cells(10 * T + 2, 3).Value = Deviation2
End If
End If

'Area Under the Curve Approximation for Trial
If T > withdrawtime Then
If UnblockedS(T - 1) > UsDesired Then
    If UnblockedS(T) > UsDesired Then 'Check if both this
time step and previous were above set point.

    IntegralCheck = 0.05 * (UnblockedS(T - 1) +
UnblockedS(T) - 2 * UsDesired) 'Midpoint approximation of
area between those time steps
    AreaUnderCurve = AreaUnderCurve + IntegralCheck
    End If
End If
End If
```

```
Next T

'Store Data for Trial
Sheet8.Cells(21 * K + J + 2, 1).Value = withdrawtime
Sheet8.Cells(21 * K + J + 2, 2).Value = amax
Sheet8.Cells(21 * K + J + 2, 3).Value =
AlcoholAreaUnderCurve
Sheet8.Cells(21 * K + J + 2, 4).Value = Usmax
Sheet8.Cells(21 * K + J + 2, 5).Value = BlockedSMax
Sheet8.Cells(21 * K + J + 2, 6).Value = TotalSMax
Sheet8.Cells(21 * K + J + 2, 7).Value = AreaUnderCurve

Next J
Next K

End Sub
```

Appendix C

Raw Code for Varying Amplitude and Frequency of Sinusoidal Dependence

```
'Iterate for Various Time intervals and Alcohol Amounts
For K = 0 To 20
    amax = 0.05 * K 'Vary Amplitude for each trial

    For J = 0 To 20
        P = J * 0.0125 'Vary Frequency for each trial

        'Reset Checks
        Usmax = 0
        BlockedSMax = 0
        TotalSMax = 0
        AreaUnderCurve = 0
        AlcoholAreaUnderCurve = 0
            For T = 0 To 1000 Step 0.1

    'Calculate Alcohol For Step
   Alcohol(T) = amax * Sin(P * T)
        If Alcohol(T) < 0 Then
            Alcohol(T) = 0
        End If
        If T > 500 Then
            Alcohol(T) = 0
        End If

'Calculate Current Controller Action
C1Action(T) = Controller1Action(UnblockedS(T), Alcohol(T))
C2Action(T) = Controller2Action(TotalS(T))
CTAction(T) = C1Action(T) - C2Action(T)

'Calculate new values for NMDARs at various positions for
next step
UnblockedS(T + 1) = UnblockedS(T) + (-k1 * Alcohol(T) *
UnblockedS(T)) + k2 * BlockedS(T) + CTAction(T) + kd *
(CTAction(T - 1) - CTAction(T - 2)) + ki * (CTAction(T - 1)
+ CTAction(T - 2) + CTAction(T - 3))
UnblockedP(T + 1) = UnblockedP(T) - CTAction(T)
BlockedS(T + 1) = BlockedS(T) + k1 * UnblockedS(T) *
Alcohol(T) - k2 * BlockedS(T)
UnblockedP(T + 1) = UnblockedP(T) - CTAction(T)
TotalS(T + 1) = BlockedS(T + 1) + UnblockedS(T + 1)
```

```
'Check USMax
If UnblockedS(T + 1) > Usmax Then
    Usmax = UnblockedS(T + 1)
End If
If BlockedS(T + 1) > BlockedSMax Then
    BlockedSMax = BlockedS(T + 1)
End If
If TotalS(T + 1) > TotalSMax Then
    TotalSMax = TotalS(T + 1)
End If

'Record Alcohol Profile for Middle Case
If J = 10 Then
If K = 10 Then

'Record in Spreadsheet
Sheet2.Cells(10 * T + 2, 1).Value = T
Sheet2.Cells(10 * T + 2, 2).Value = Alcohol(T)
Sheet2.Cells(10 * T + 2, 3).Value = BlockedS(T)
Sheet2.Cells(10 * T + 2, 4).Value = UnblockedS(T)
Sheet2.Cells(10 * T + 2, 5).Value = UnblockedP(T)
Sheet2.Cells(10 * T + 2, 6).Value = C1Action(T)
Sheet2.Cells(10 * T + 2, 7).Value = C2Action(T)
Sheet2.Cells(10 * T + 2, 8).Value = CTAction(T)
Sheet6.Cells(10 * T + 2, 1).Value = T
Sheet6.Cells(10 * T + 2, 2).Value = Deviation1
Sheet6.Cells(10 * T + 2, 3).Value = Deviation2
End If
End If

'Area Under the Curve Approximation for Trial
If T > withdrawtime Then
If UnblockedS(T - 1) > UsDesired Then
    If UnblockedS(T) > UsDesired Then 'Check if both this
time step and previous were above set point.

    IntegralCheck = 0.05 * (UnblockedS(T - 1) +
UnblockedS(T) - 2 * UsDesired) 'Midpoint approximation of
area between those time steps
    AreaUnderCurve = AreaUnderCurve + IntegralCheck
    End If
End If
End If
```

```
Next T

'Store Data for Trial
Sheet8.Cells(21 * K + J + 2, 1).Value = withdrawtime
Sheet8.Cells(21 * K + J + 2, 2).Value = amax
Sheet8.Cells(21 * K + J + 2, 3).Value =
AlcoholAreaUnderCurve
Sheet8.Cells(21 * K + J + 2, 4).Value = Usmax
Sheet8.Cells(21 * K + J + 2, 5).Value = BlockedSMax
Sheet8.Cells(21 * K + J + 2, 6).Value = TotalSMax
Sheet8.Cells(21 * K + J + 2, 7).Value = AreaUnderCurve

Next J
Next K

End Sub
```

Appendix D

Raw Code for Varying Controller Parameters y_{max1} and y_{max2}

```
'Iterate for Combinations of ymax1 and ymax2
For K = 0 To 20
    ymax1 = 10 + 7 * K

    For J = 0 To 20
        ymax2 = 10 + 7 * J

        'Reset Checks
        Usmax = 0
        BlockedSMax = 0
        TotalSMax = 0
        AreaUnderCurve = 0
        AlcoholAreaUnderCurve = 0
            For T = 0 To 1000 Step 0.1


    'Calculate Alcohol For Step
   Alcohol(T) = Sin(0.75 * T) * Exp(0.0005 * T)
    If T >= 500 Then
        Alcohol(T) = 0
    ElseIf Alcohol(T) < 0 Then
        Alcohol(T) = 0
    End If


'Calculate Current Controller Action
C1Action(T) = Controller1Action(UnblockedS(T), Alcohol(T))
C2Action(T) = Controller2Action(TotalS(T))
CTAction(T) = C1Action(T) - C2Action(T)

'Calculate new values for NMDARs at various positions for
next step
UnblockedS(T + 1) = UnblockedS(T) + (-k1 * Alcohol(T) *
UnblockedS(T)) + k2 * BlockedS(T) + CTAction(T) + kd *
(CTAction(T - 1) - CTAction(T - 2)) + ki * (CTAction(T - 1)
+ CTAction(T - 2) + CTAction(T - 3))
UnblockedP(T + 1) = UnblockedP(T) - CTAction(T)
BlockedS(T + 1) = BlockedS(T) + k1 * UnblockedS(T) *
Alcohol(T) - k2 * BlockedS(T)
UnblockedP(T + 1) = UnblockedP(T) - CTAction(T)
```

```
TotalS(T + 1) = BlockedS(T + 1) + UnblockedS(T + 1)

'Check USMax

If UnblockedS(T + 1) > Usmax Then
    Usmax = UnblockedS(T + 1)
End If
If BlockedS(T + 1) > BlockedSMax Then
    BlockedSMax = BlockedS(T + 1)
End If
If TotalS(T + 1) > TotalSMax Then
    TotalSMax = TotalS(T + 1)
End If


 'Record Alcohol Profile for Middle Case
If J = 10 Then
If K = 10 Then

'Record in Spreadsheet
Sheet2.Cells(10 * T + 2, 1).Value = T
Sheet2.Cells(10 * T + 2, 2).Value = Alcohol(T)
Sheet2.Cells(10 * T + 2, 3).Value = BlockedS(T)
Sheet2.Cells(10 * T + 2, 4).Value = UnblockedS(T)
Sheet2.Cells(10 * T + 2, 5).Value = UnblockedP(T)
Sheet2.Cells(10 * T + 2, 6).Value = C1Action(T)
Sheet2.Cells(10 * T + 2, 7).Value = C2Action(T)
Sheet2.Cells(10 * T + 2, 8).Value = CTAction(T)
Sheet6.Cells(10 * T + 2, 1).Value = T
Sheet6.Cells(10 * T + 2, 2).Value = Deviation1
Sheet6.Cells(10 * T + 2, 3).Value = Deviation2
End If
End If

'Area Under the Curve Approximation for Trial
If T > withdrawtime Then
If UnblockedS(T - 1) > UsDesired Then
    If UnblockedS(T) > UsDesired Then 'Check if both this
time step and previous were above set point.

    IntegralCheck = 0.05 * (UnblockedS(T - 1) +
UnblockedS(T) - 2 * UsDesired) 'Midpoint approximation of
area between those time steps
    AreaUnderCurve = AreaUnderCurve + IntegralCheck
    End If
End If
End If
```

```
Next T

'Store Data for Trial
Sheet8.Cells(21 * K + J + 2, 1).Value = withdrawtime
Sheet8.Cells(21 * K + J + 2, 2).Value = amax
Sheet8.Cells(21 * K + J + 2, 3).Value =
AlcoholAreaUnderCurve
Sheet8.Cells(21 * K + J + 2, 4).Value = Usmax
Sheet8.Cells(21 * K + J + 2, 5).Value = BlockedSMax
Sheet8.Cells(21 * K + J + 2, 6).Value = TotalSMax
Sheet8.Cells(21 * K + J + 2, 7).Value = AreaUnderCurve

Next J
Next K

End Sub
```

Appendix E

Raw Code for Varying Controller Parameters a_1 and a_2

```
'Iterate for Various Combinations of a1 and a2
For K = 0 To 20
    a1 = 50 + 5 * K

    For J = 0 To 20
        a2 = 50 + 5 * J

        'Reset Checks
        Usmax = 0
        BlockedSMax = 0
        TotalSMax = 0
        AreaUnderCurve = 0
        AlcoholAreaUnderCurve = 0
            For T = 0 To 1000 Step 0.1

    'Calculate Alcohol For Step
   Alcohol(T) = Sin(0.75 * T) * Exp(0.0005 * T)
    If T >= 500 Then
        Alcohol(T) = 0
    ElseIf Alcohol(T) < 0 Then
        Alcohol(T) = 0
    End If


'Calculate Current Controller Action
C1Action(T) = Controller1Action(UnblockedS(T), Alcohol(T))
C2Action(T) = Controller2Action(TotalS(T))
CTAction(T) = C1Action(T) - C2Action(T)

'Calculate new values for NMDARs at various positions for
next step
UnblockedS(T + 1) = UnblockedS(T) + (-k1 * Alcohol(T) *
UnblockedS(T)) + k2 * BlockedS(T) + CTAction(T) + kd *
(CTAction(T - 1) - CTAction(T - 2)) + ki * (CTAction(T - 1)
+ CTAction(T - 2) + CTAction(T - 3))
UnblockedP(T + 1) = UnblockedP(T) - CTAction(T)
BlockedS(T + 1) = BlockedS(T) + k1 * UnblockedS(T) *
Alcohol(T) - k2 * BlockedS(T)
UnblockedP(T + 1) = UnblockedP(T) - CTAction(T)
TotalS(T + 1) = BlockedS(T + 1) + UnblockedS(T + 1)
```

```
'Check USMax

If UnblockedS(T + 1) > Usmax Then
    Usmax = UnblockedS(T + 1)
End If
If BlockedS(T + 1) > BlockedSMax Then
    BlockedSMax = BlockedS(T + 1)
End If
If TotalS(T + 1) > TotalSMax Then
    TotalSMax = TotalS(T + 1)
End If


'Record Alcohol Profile for Middle Case
If J = 10 Then
If K = 10 Then

'Record in Spreadsheet
Sheet2.Cells(10 * T + 2, 1).Value = T
Sheet2.Cells(10 * T + 2, 2).Value = Alcohol(T)
Sheet2.Cells(10 * T + 2, 3).Value = BlockedS(T)
Sheet2.Cells(10 * T + 2, 4).Value = UnblockedS(T)
Sheet2.Cells(10 * T + 2, 5).Value = UnblockedP(T)
Sheet2.Cells(10 * T + 2, 6).Value = C1Action(T)
Sheet2.Cells(10 * T + 2, 7).Value = C2Action(T)
Sheet2.Cells(10 * T + 2, 8).Value = CTAction(T)
Sheet6.Cells(10 * T + 2, 1).Value = T
Sheet6.Cells(10 * T + 2, 2).Value = Deviation1
Sheet6.Cells(10 * T + 2, 3).Value = Deviation2
End If
End If

'Area Under the Curve Approximation for Trial
If T > withdrawtime Then
If UnblockedS(T - 1) > UsDesired Then
    If UnblockedS(T) > UsDesired Then 'Check if both this
time step and previous were above set point.

    IntegralCheck = 0.05 * (UnblockedS(T - 1) +
UnblockedS(T) - 2 * UsDesired) 'Midpoint approximation of
area between those time steps
    AreaUnderCurve = AreaUnderCurve + IntegralCheck
    End If
End If
End If
```

```
Next T

'Store Data for Trial
Sheet8.Cells(21 * K + J + 2, 1).Value = withdrawtime
Sheet8.Cells(21 * K + J + 2, 2).Value = amax
Sheet8.Cells(21 * K + J + 2, 3).Value =
AlcoholAreaUnderCurve
Sheet8.Cells(21 * K + J + 2, 4).Value = Usmax
Sheet8.Cells(21 * K + J + 2, 5).Value = BlockedSMax
Sheet8.Cells(21 * K + J + 2, 6).Value = TotalSMax
Sheet8.Cells(21 * K + J + 2, 7).Value = AreaUnderCurve

Next J
Next K

End Sub
```