

Official Study Title: Gabapentin for Relapse Prevention: Alcohol Withdrawal
Effects

NCT02349477

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RESEARCH STRATEGY

Significance

While it is estimated that up to 20 million people meet criteria for alcohol use disorders only 20% of them receive treatment and, of that number, only 20% (or less than 1 million individuals) receive medication to help them with maintaining abstinence or reducing drinking. There are many reasons for this, but a particularly salient one is that currently available medications are not efficacious for everyone and/or might be costly, contraindicated, or not well tolerated. It has also been recognized that many “treatment failures” occur during the first few months of treatment. So there is a great opportunity to individualize medications based on subject profiles (personalized medicine), and to use medications that are well tolerated and not costly. Some examples of this are the use of serotonin acting drugs for subtypes of alcoholics based on stage of onset (Johnson, 2000; Kranzler, 2011; Pettinati, 2000) and/or genetic predictors of response to these drugs (Johnson, 2011; Kranzler, 2011) or to naltrexone (Anton, 2008; Oslin, 2003).

One “phenotype/subtype” of alcohol dependence that has been understudied is individuals with alcohol withdrawal syndrome (AWS) (Schuckit, 1998). While the quantity and frequency of drinking might predict who is “at risk” for AWS, there is considerable variation (some of which is likely genetic) as to who will, or will not, experience AWS upon the cessation of drinking. Over half of inpatient alcoholics (Caetano, 1998) and 35% of community alcoholics reported AW symptoms, and these individuals had a higher rate of alcohol problems at follow-up (Schuckit, 2003). Similarly, our experience suggests that those with previous medicated detoxes relapse quicker after drinking cessation (Wright, 2007). While up to 30% of alcoholics presenting for clinical trials (who are similar to those seen in addiction outpatient clinics and in primary care) have acute AWS, it has been recognized that lower grade AWS might be present, and last a longer period of time, in considerably more individuals. Many individuals also stop or cut-down drinking prior to initiating treatment, and may experience subtle to florid AWS without being diagnosed. In addition, some undergo self-detoxification by gradually reducing their drinking to avoid AWS. In three of our recent studies, up to 60% of individuals met DSM-IV criteria for past AWS. In individuals with past or current AWS, a constellation of problems including irritability, anxiety, dysphoria, and difficulties with concentration and insomnia might persist for a period of time after the cessation of drinking. Some have labeled these lingering symptoms that occur after the immediate alcohol cessation period as “protracted withdrawal” (Bonnet, 1999). In general, this constellation of symptoms and any “desire to drink” emanating from them is not likely to be impacted by anti-reinforcement or anti-craving medications, such as naltrexone, which is likely to target more reward-based craving. Consistent with this thought, AWS is thought to be mediated by GABA and glutamate brain signaling, in contrast to reward-based craving which is more likely mediated by opioid and dopamine brain signaling (Gass & Olive, 2008; Littleton, 1998). Therefore, medications that target or “normalize” brain GABA and glutamate systems might be particularly useful in the treatment of AWS and, by extension, in those who have previously shown the biological propensity to experience alcohol withdrawal symptoms (Addolorato, 2011; Krystal, 2006).

Studies have repeatedly shown that anticonvulsants (which generally affect GABA-glutamate systems) have been useful for the treatment of acute AWS (Book & Myrick, 2005; Leggio, 2008; Malcolm, 2001) but they have been studied less for relapse prevention and chronic treatment. One exception is topiramate whose mechanism of action remains unclear. While not studied extensively in acute alcohol withdrawal, topiramate has been shown to be efficacious in reducing active drinking (Johnson, 2007). *While not specifically studied, its efficacy might be partially based on suppression of subtle AWS at cessation of drinking. Although efficacious, topiramate might have more cognitive dysfunction compared to gabapentin (Goldberg & Brudick, 2001; Martin et al, 1999), especially during rapid dose-titration, which limit its use. Topiramate is also partially* metabolized by the liver, which may be compromised by chronic alcohol use. On the other hand, our group (Myrick, 2009) and others (Mariani, 2006) have found gabapentin, a medication that putatively modulates both GABA and glutamate tone, to be efficacious for acute AWS and to play a role in relapse prevention (Furieri & Nakamura-Palacios, 2007) - especially in those with either acute or historical AWS (Anton, 2009; Anton, 2011). It is also safe to use in alcoholics, and appears to have limited or no cognitive effects during treatment (Goldberg & Brudick, 2001; Schacht, 2011), no adverse interaction with alcohol (Bisaga, 2006; Myrick, 2007; Myrick, 2009), and total renal excretion.

While the efficacy of gabapentin in alcoholics with AW history based on normalization of GABA/glutamate signaling has considerable appeal and consistency, it should be noted that GABA and glutamate systems are

ubiquitous in the brain and likely sub-serve a number of neurophysiological processes including reward or reinforcement based systems as well. Also, in addition to effects on the GABA/glutamate systems, gabapentin has other pharmacological effects on calcium ion-gated channels, some of which might form the basis of its analgesic effects (Dooley, 2007) and/or its effect on GABA/glutamate transmission (Sills, 2006). Therefore, it is possible that gabapentin could have effects on alcohol cue-induced craving, reward-based drinking, and affective modulation that go beyond its ability to normalize putative protracted abstinence symptoms. For instance, it has been reported that gabapentin blocks affective cue-induced alcohol craving (Mason, 2009) and might reduce drinking through a mechanism of sleep normalization (Karam-Hage & Brower, 2000; Karam-Hage & Brower, 2003), especially in those with repeated medical detoxifications (Malcolm, 2007), although the data on this are not consistent (Brower, 2008). Our initial data (see below) while not testing gabapentin by itself, does point to possible efficacy for relapse prevention but only in those with an AW history. These findings are consistent with our earlier work on gabapentin's efficacy in AWS (Myrick, 2007), its related neurobiological substrate, and potentially lingering effects, and are also consistent with animal work (Roberto, 2008). Nevertheless, these preliminary results need to be replicated, clarified, and expanded in a prospective study directly evaluating the efficacy of gabapentin alone and focusing on those with a history of AW who are most likely to benefit. Finding a unique and efficacious treatment for a subgroup of particularly vulnerable alcohol dependent individuals, those with a history of AWS, is of great need and clinical importance.

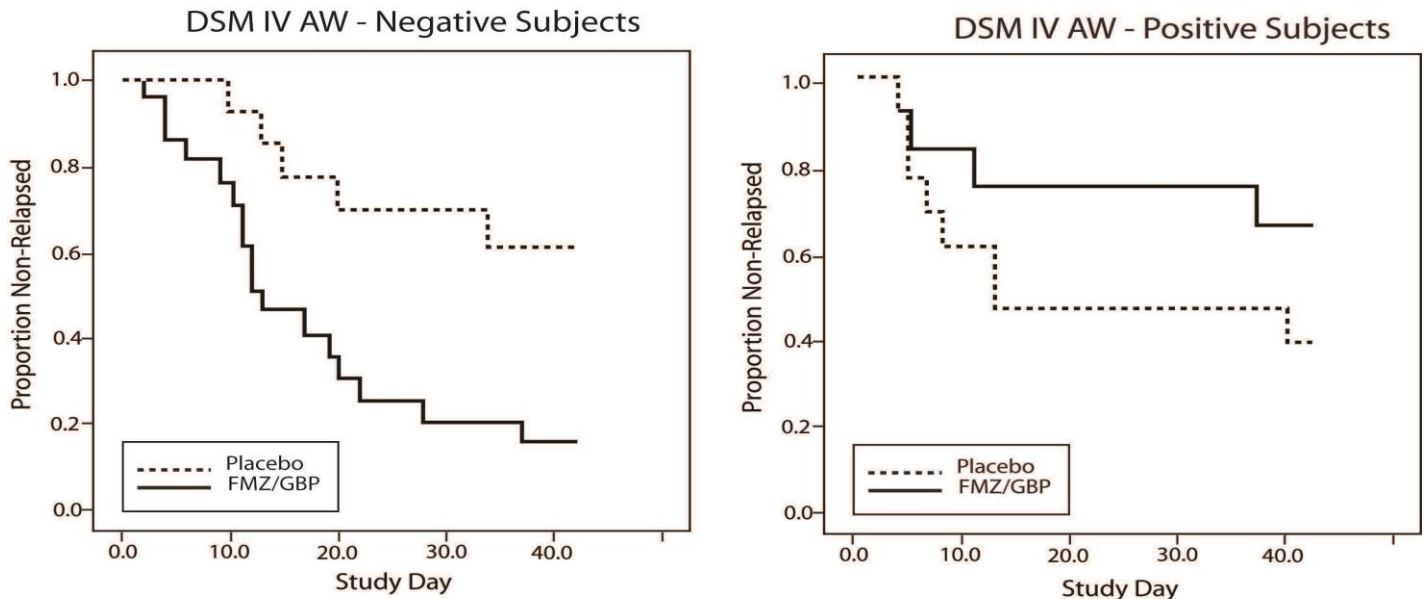
If confirmed in a prospective, placebo-controlled, trial that gabapentin is useful for relapse prevention in a subgroup of alcohol dependent individuals with a history of AWS, it would provide another treatment option for millions of Americans and others worldwide. In addition, if we can determine that changes in brain GABA and glutamate underlie gabapentin's efficacy, it would suggest that other medications having similar effects could also be useful, while at the same time providing translational support for basic science information on the underlying neurobiology of alcohol dependence and withdrawal. Finally, by exploring individual genetic differences in key GABA and glutamate receptor genes already linked to alcoholism, the opportunity to define which individual(s) might respond to gabapentin (personalized medicine) could be evaluated.

Preliminary Data Supporting Significance

In an exploratory study (study 1) our group evaluated a commercial procedure (the combined use of acute flumazenil and six weeks of gabapentin) being marketed for alcohol dependence (Anton, 2009). While initially agnostic about the utility of this approach, it provided the opportunity to explore the use of gabapentin in a structured fashion with the a priori hypothesis that this treatment would work, if at all, only in those with pre-treatment alcohol withdrawal symptoms. The theory underlying the use of IV flumazenil was that 1) it has been shown in a few, but not all, studies to ameliorate AW symptoms and 2) that it reversed the chronic effect of alcohol on GABA-A receptors (Biggio, 2007; Sanna, 2003) allowing them to be more responsive to GABA signaling. Since gabapentin had been shown previously to be useful for the treatment of AW (Myrick, 2009), and as it purportedly normalizes GABA/glutamate balance/tone in the brain, it was hypothesized to work in conjunction with flumazenil to "reset" and "maintain" normal GABA and glutamate tone resulting in less craving and relapse. Armed with this information, we randomized 60 alcohol dependent individuals in a double blind fashion to receive either active flumazenil infusions (2 consecutive days) and gabapentin up to 1200 mg/day or their matching placebos for approximately six weeks. Individuals who drank up to 72 hours prior to randomization were evaluated for alcohol withdrawal using the Clinical Institute Withdrawal Assessment – Revised (CIWA-Ar) (Sullivan, 1989) and urn randomized to the medication groups based on a CIWA-Ar score (below or ≥ 7), *with only 3 people having scores of 10 or more*. Participants were assessed for alcohol withdrawal on the first two days of treatment and drinking was assessed weekly by the timeline follow-back calendar method, with %CDT (a marker of relapse drinking) measured at baseline, week-3, and end of study. We found a significant ($p= 0.021$) effect favoring active treatment in reducing CIWA-Ar scores in the first two days of treatment. Importantly, we found no main effects of medication treatment on any drinking measure but a significant interaction of medication group by pre-randomization AW score, such that percent days abstinent ($p= 0.0006$), percent of individuals completely abstinent ($p=0.002$), time to first heavy drinking day ($p= 0.06$) and %CDT ($p=0.001$) all favored the active medication group in those with high pre-randomization CIWA-Ar scores (about 20% of subjects). Findings were similar when expanding and comparing those with and without a positive DSM-IV AW item (43% of subjects) (see Figure 1). The odds ratio for DSM-IV AW positive individuals not relapsing when treated with active medication vs. placebo was 3.2 (95% CI 1.4 - 7.4). Also, while there was more baseline drinking in those who were DSM-IV AW positive, co-varying various baseline drinking and severity (ADS, OCDS) measures did not impact the significance of the active medication vs.

placebo effect, suggesting it was the AWS history itself that was important in predicting outcome and not recent drinking levels or alcohol severity. This novel finding of DSM-IV positive AW subjects possibly responding to gabapentin alone opens a treatment option for more individuals and is the basis of this proposal.

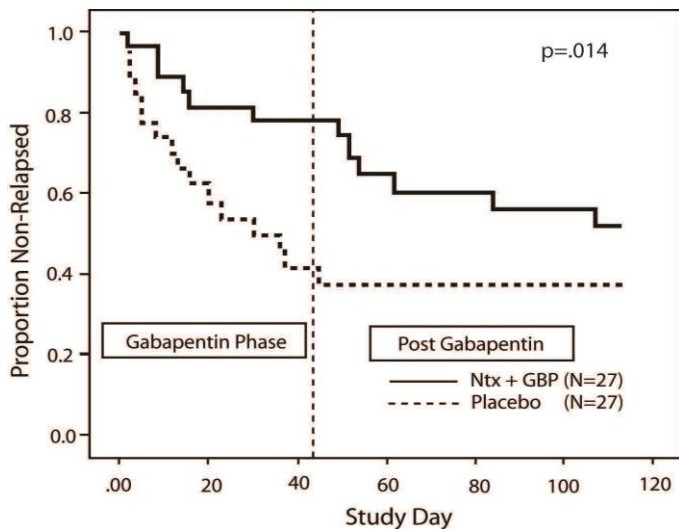
FIGURE 1. Proportion non-relapsed based on pre-randomization DSM IV history of AWS (negative or positive) treated with Placebo or a combination of IV Flumzenil (FMZ) plus oral Gabapentin (GBP)



The Interaction between AW status and medication group ($X^2(1) = 7.61, p = .006$)

In another study (study 2) funded by NIAAA, we evaluated the effect of adding gabapentin to naltrexone, compared to naltrexone alone or placebo with the hypothesis that gabapentin given during the first 6 weeks of a 16-week naltrexone trial would improve drinking outcomes based on reduction of early abstinence effects (sleep problems, irritability, etc.). In this study (Anton, 2011), 150 alcohol dependent individuals were randomized to naltrexone (50 mg/day) and gabapentin (1200 mg/day), naltrexone alone plus placebo, or double placebo. Gabapentin/placebo was given for 6 weeks and naltrexone/placebo was given for 16 weeks. Individuals received Combined Behavioral Intervention (CBI), Medical Management (MM), and assessments as described in the COMBINE Study (Anton, 2006). *The initial results favored the naltrexone-gabapentin group over the other two groups (p values 0.001 to 0.04 for different drinking outcomes), especially during the gabapentin phase (first 6 weeks) of the study.*

FIGURE 2. Proportion non-relapsed with a pre-randomization DSM-IV history of AW treated with placebo or naltrexone plus gabapentin.



However, effects faded over time once gabapentin was stopped. Further exploratory analysis of the data suggested that gabapentin had the greatest efficacy in those with a history of AWS (see Anton, 2011). Based on study 1 results, and to obtain further preliminary data for this proposal, we recently reanalyzed the data based on meeting DSM-IV AW criteria (SCID AW Item, current and historical). Within the group who were positive for a history of AW (56% of subjects), the effect of gabapentin over placebo on the percent subjects with no heavy drinking day was $p = 0.017$ with an OR of 4.4 (95% CI 2.4 – 7.9). For illustration, time to relapse to a heavy drinking day is depicted in **figure 2**. There was no significant effect of ntx/gbp in those without an AW history and/or those with an AW history treated with naltrexone alone ($p > 0.05$, data not shown). Also, although DSM-IV AW positive individuals had higher baseline drinking and severity, co-varying these variables did not significantly alter medication efficacy, findings consistent with study 1.

Also, using a “Reasons for Drinking Scale” that we devised to evaluate why people drink: items dealing with pleasure, liking high, stress, feeling normal, habit, and relief (avoiding feeling bad, nervous, irritable, and insomnia), those scoring higher on “relief drinking” responded better to naltrexone/gabapentin than placebo or naltrexone alone ($p=0.001$). This also supports the idea that gabapentin works in those with a history of AW symptoms compared to those without.

While these studies suggest that gabapentin is likely to be efficacious in relapse prevention and drinking-reduction in those with a history of AW symptoms, they are limited in several important ways: **1) gabapentin was not given by itself, 2) it was given for only 6 weeks in study 2 and relapse occurred after it was stopped, 3) in study 2, individuals were not randomized based on AW history and the analysis was post-hoc.** Therefore, it is important to do a proper 16-week prospective placebo-controlled trial of gabapentin alone in those with a history of AW in order to confirm its efficacy in this important subgroup of alcohol dependent individuals. This is the primary objective of this proposal.

As noted previously, gabapentin is thought to reduce brain glutamate (Cunningham, 2004; Errante & Petroff, 2003) and increase brain GABA (Loscher, 1991) perhaps through its action on voltage sensitive $CA(2+)$ channels (Quintero, 2011; Sills, 2006) thereby potentially rebalancing the dysregulated GABA/glutamate tone evident at the cessation of drinking. *We have the opportunity to examine this issue as it relates change during early abstinence, relapse prediction, and gabapentin effectiveness.* We can do this based on recent advances in magnetic resonance spectroscopy (1H -MRS) that has allowed in vivo measurement of both of these neurotransmitters in humans. Given the recent recruitment of a world-renowned brain-imaging physicist, Dr. Truman Brown, to our Institution and based on our pilot method development data, we are confident that we can apply (1H -MRS) to this aim. Please see the approach-methods section for our pilot work using 1H -MRS to measure brain glutamate and GABA levels.

Previous 1H -MRS studies have generally supported brain glutamate and GABA level disturbances in individuals with alcohol dependence (AD) but timing of measurement in relationship to alcohol use and abstinence as well as alcohol severity, brain area of measurement, sample size, smoking, sex, and recent use of benzodiazepines for detoxification all might influence results. The preponderance of studies have supported elevated cerebrospinal fluid (Tsai, 1998) and anterior cingulate/prefrontal (Frischknecht, 2010; Hermann, 2011; Lee, 2007; Thoma, 2011) glutamate/glutamine levels in recently drinking AD's relative to controls, while several showed changes over time during early abstinence (Frischknecht, 2010; Hermann, 2011; Mon, 2012; Umhau, 2010). Several others showed no differences between AD and controls, perhaps because measurements were made in the occipital lobe, had small sample sizes, used older measurement methods or sampled later in abstinence (Behar, 1999; Mason, 2006). Also, ACC glutamate levels were associated with cognitive function during early abstinence (Lee, 2007) and craving after detoxification (Bauer et al, 2013). Most germane to this proposal, Umhau (2010) reported that glutamate increased during early abstinence (consistent with Mon, 2012) and that this increase was suppressed by acamprosate treatment, a drug approved by the FDA for relapse prevention and thought to stabilize glutamate function.

1H -MRS of GABA is technically more challenging and less studied. Although one early study reported that AD had significantly less occipital GABA than controls (Behar, 1999), two subsequent studies failed to find significant differences in mean brain GABA between AD and controls (Mason, 2006; Mon, 2012). Close inspection of the (Mason, 2006) data, however, suggest that GABA levels might differ by AW symptoms and history, while smoking history was found to also affect GABA level change over time. Intravenous ethanol reportedly decreases 1H -MRS GABA levels in social drinkers - interpreted as a reflection of alcohol-induced GABA receptor sensitivity leading to down regulation of GABA production and release. While, to our knowledge, no one has studied the effect of gabapentin on brain glutamate/GABA in alcoholics, gabapentin has been shown to increase brain GABA levels in humans by 1H -MRS both with acute (Cai, 2012; Petroff, 1996; Petroff, 2000) and chronic dosing (Kuzniecky, 2002). Remarkably, those with the lowest baseline levels (as might be found during AW) showed the most increase (Petroff, 2000). However, there has been very little published data on the effect of gabapentin on human brain glutamate levels. The only existing study found that a single gabapentin dose did not significantly impact glutamate levels in healthy controls (Cai, 2012). Data

generated from the proposed study could therefore add to the literature by elucidating the role of chronic gabapentin on glutamate levels in alcoholics.

In sum, although studies of glutamate and GABA in AD have not been completely consistent, they clearly demonstrate that 1) ¹H-MRS can be successfully applied to study this issue 2) that both systems might change over time with abstinence and/or with gabapentin treatment in a similar fashion to acamprosate (Umhau, 2010) and 3) there is a need to study these systems specifically in those with a history of alcohol withdrawal as a prediction of medication response and in the context of salient genetic differences.

Since more emphasis is being placed on personalized medicine (Collins, 2010) and given our experience with examining the effects of naltrexone based on genetic differences (Anton, 2008; Anton, 2012; Oroszi, 2009) and especially on brain-imaging genetics (Schacht et al, 2013), we also have the ability to explore whether genetic differences in specific GABA and glutamate receptors which have been linked to alcoholism might be associated with gabapentin response and/or, secondarily, with propensity to experience AW. The focus, but not the limit, of this exploration will be on the *GABRA2* gene single nucleotide polymorphism (SNP) rs279858 in exon 5 of the gene that codes for the A2 subunit of the GABA receptor that is important for benzodiazepine binding (Harris, 1998) and shifts during alcohol use and withdrawal (Krystal, 2006; Sanna, 2003). Diversity of this SNP has been associated with alcohol dependence both on its own and as part of a haplotype strongly associated with alcohol dependence (and event related EEG oscillations in alcoholics) (Edenberg, 2004), as well as in a family based alcoholism study (Covault, 2004). Those who were homozygous for the more common A(T) allele had greater responses to oral alcohol (Pierucci-Lagha, 2005) and those with at least one copy of the A(T) allele reported greater alcohol subjective effects of IV alcohol (Roh, 2010). In addition, another study suggested variants at this locus not only corresponded with a family history of alcoholism but also with the level of impulsivity and with reward salience detected by brain imaging (fMRI) (Villafuerte, 2012). Finally, a recent study found that this GABA2A SNP predicted relapse risk (Bauer, 2012). Taken together this SNP, whether it has direct effects on GABA function or as a tag SNP for other genetic variation, is a strong candidate gene to investigate the relationship between AW and gabapentin response. DNA will be available to look at other SNP's and/or for exon sequencing or to examine epigenetic markers/effects in this region of the *GABRA2* gene. In pilot work we have genotyped 83 alcohol dependent Caucasian individuals and found allele frequencies of rs279858 to be similar (A=0.57; G=0.43) to that reported in HapMap (A=0.53; G=0.47) with genotype frequencies (AA=0.33; AG=0.49; GG=0.18) also comparable to HapMap (AA=0.30; AG=0.45; GG=0.25).

For glutamate system genes there is less reported with regards to alcoholism heritability and/or alcohol effects. However, one focus of exploration could be on the glutamate receptor 8 (*GRM8*) gene that codes for a pre-synaptic (group III) glutamate receptor found in brain cortex/hippocampus and expressed at nerve terminals that target subgroups of GABAergic neurons (Ferraguti, 2005). Agonists at this receptor have been shown to have anxiolytic effects (Palucha, 2004) and reduce alcohol intake and cue responding (Backstrom & Hyytia, 2005). Most germane for the current project is a *GRM8* SNP rs1361955 located in intron 6 which was both associated with alcohol dependence and very strongly associated with event related brain wave activity in alcoholics (Chen, 2009) with the T allele carriers having greater EEG Theta activity than CC genotypes. Of interest, using ¹H-MRS (similar to that proposed here), a strong relationship was reported between brain glutamate concentration and frontal theta activity (Gallinat, 2006) suggesting a link between genetics, glutamate activity and brain function. Similar to GABA, the DNA will be available to explore other gene receptor, enzyme, and transporter variants, along with exome sequencing and epigenetic differences that might be reported to have functionality in the future. In pilot work on the same 83 individuals mentioned previously we genotyped the *GRM8* rs1361955 SNP and found allele frequencies to be similar (C=0.60; T=0.40) to that reported in HapMap (C=0.61; T=0.40) with genotype frequencies (CC=0.40; CT=0.41; TT=0.19) also comparable to HapMap (CC=0.35; CT=0.52; TT=0.13).

By combining pharmacology, neurobiological measurement, and genetics in a clinical trial of alcohol use disorder there are scientific and **translational synergies that are larger than the sum of the parts**. This in addition to providing a critical need – better treatments for alcohol dependence – makes this study quite significant.

Innovation

This investigation addresses a number of novel and important issues. First and foremost there has never been a longer-term randomized clinical trial, to our knowledge, that has attempted to improve relapse-risk based on a priori knowledge of AW history. This has **enormous clinical importance for alcoholics that might be biologically (perhaps genetically) prone to experience AW symptoms (a high relapse-risk group)**. Second, this study is novel in that it could begin to **aid in understanding neurobiological underpinnings of relapse in those with AW and link this to medication response** – thereby providing both face and predictive validity for these biologic differences. Furthermore, the **exploration of how several genes in these key biological systems which have been previously linked to alcohol dependence, or alcohol effects, might interact with brain chemical differences and medication efficacy** is highly novel and points to an enhanced “personalized medicine” approach. Finally, this highly translational approach **could add significant information linking clinical and basic science findings**.

Approach

General Design of the Study: *Over a 39-month enrollment period 190 subjects will be recruited from both specific research advertisement and from our clinical program. After a minimum of 3 and maximum of 7 days of abstinence, 90 subjects (about 50% of those screened) who meet DSM-5 criteria for both alcohol use disorder AND a history of AWS (DSM-5 AW Item positive) with CIWA-Ar scores < 10, will receive gabapentin (N=45) or matching placebo (N=45) for 16 weeks while receiving medical management (MM). Outcome will be assessed during this 16-week treatment period and again at one month and three months after the treatment period. Inclusion/exclusion criteria as follows are based on previous studies but modified to focus on AW symptoms.*

Inclusion Criteria:

- Ages 18-70 meeting criteria for primary alcohol use disorder based on current DSM-5 criteria and consuming, on average, 5 standard drinks per day for men and 4 drinks per day for women in the past 30 days, *who also meet a history of DSM-5 alcohol withdrawal criteria*.
- Must be able to maintain sobriety for a minimum of 3 days as determined by self-report, urinary EtG, and breathalyzer measurements

Exclusion Criteria:

- Meets DSM-5 criteria for any other psychoactive moderate to severe substance use disorder except nicotine use disorder. Any mild substance use disorder, except marijuana and nicotine, in the last 30 days by subject report and urine drug screen
- Meets DSM-5 criteria for current axis I disorders of major depression, panic disorder, obsessive-compulsive disorder, post-traumatic stress syndrome, bipolar affective disorder, schizophrenia, or eating disorder or any other psychotic disorder or neurocognitive disorder
- Has current suicidal ideation or homicidal ideation.
- Need for maintenance or acute treatment with any psychoactive medication including anti-seizure medications. Use of alcohol medications (naltrexone, topiramate, acamprosate, disulfiram) in last 30 days.
- Clinically significant medical problems including hepatocellular disease indicated by elevations of ALT and AST of at least 3.0 times normal at screening and/or after 7 days abstinence
- Sexually active females of child-bearing potential who are pregnant (by urine HCG), nursing, or not using a reliable form of birth control
- Pending charges for a violent crime (not including DUI offenses).
- Does not have a stable living situation
- Has taken gabapentin in the last month or experienced adverse effects from it at any time
- *Has a CIWA-Ar score of 10 or more during assessment or history of AW seizures or DT's*
- For ¹H-MRS, 1) metal in, or near, head 2) significant claustrophobia or 3) inability to lie still for up to 1 hour

After initial phone screening, further in person screening will occur after signed informed consent (if their breathalyzer reading is zero). A urine drug screen will be obtained during the initial assessment day, and on all screening days a breathalyzer measurement will be done. Subjects will have their blood drawn for a general health screen, liver function tests, gamma glutamyl-transferase (GGT) and carbohydrate deficient transferrin (%dCDT). Also, on the first day, the DSM-5 criteria for alcohol use disorder will be assessed with special attention to the criteria for AW. Historical symptoms of AW based on SCID interview will be recorded and a CIWA-Ar will be done. The CIWA-Ar will be repeated at each screening visit. Those that meet criteria for the DSM-5 AW item (current or historical) will be included – we expect about 55% of screened subjects to meet

these criteria based on data from our 4 recent clinical trials containing a total of almost 400 subjects. A CIWA-Ar score of 10 or more at any time during screening (5% of AW SCID positives based on recent studies), indicating the need for a more aggressive medical detoxification (Mayo-Smith, 1997; Saitz, 1998), will be exclusionary and we will refer the subject for clinical care and/or provide short-term benzodiazepine (lorazepam) until further care can be arranged. We feel that this is a fair compromise between appropriate subject selection, ethical treatment of subjects, and scientific integrity (referring individuals for clinical care and not including participants taking benzodiazepines which might compromise efficacy measures, GABA/glutamate levels, or interpretation of gabapentin response).

Study subjects will be asked to come into the clinic at least 2 times prior to randomization (with breathalyzer, CIWA-Ar, and urine EtG collected). During this time a full SCID, other questionnaires, a physical history and exam will be done. Subjects who maintain sobriety for at least 3 days will undergo a mass spectroscopy scan (¹H-MRS for glutamate and GABA) immediately before receiving the first MM session and first dose of study medication. We chose 3 days of sobriety based on our past results from previous work (Anton 2011) but not too long (no longer than 7 days) as to effect potential gabapentin efficacy measurement and/or GABA/glutamate levels. If they cannot maintain sobriety, or have a positive EtG on day 3 of abstinence, they will be given an additional chance to make the abstinence criteria before being excluded. For smokers, time of the last cigarette will be recorded and used as a covariate in the glutamate/GABA analysis, if necessary.

Procedure for Pre-Study Evaluation:

Subjects will receive all the pre-study assessment over a several day assessment period during the pre-randomization abstinent period (sobriety days 3-7).

General Assessment (non-hypothesis specific): The purpose of these assessments are to provide a subject database which includes alcohol use history, levels of consumption, smoking status, psychosocial problems which are alcohol related, family history of alcohol and psychiatric problems, employment and social support status, as well as general level of psychiatric symptoms and psychological distress. One purpose of these measurements will be to compare these salient subject variables across the treatment groups and to provide the data for covariate analysis if this becomes necessary because of chance non-random distribution. Another purpose of some of these ratings is to provide data for exploratory and/or explanatory hypotheses regarding intermediary variables to explain gabapentin's actions (such as change in mood, irritability, sleep etc.) on drinking behavior. Some variables such as craving (OCDS) are secondary variables for main efficacy analysis.

Instruments to be used for baseline (pre-study) assessment include:

- Structured Clinical Interview for DSM-IV (SCID) modified for DSM-5
- Addiction Severity Index (ASI)
- Alcohol Dependence Scale (ADS)
- Form 90 (modified time line follow-back method for documenting alcohol consumption)
- Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI)
- The Obsessive Compulsive Drinking Scale (OCDS)
- The Drinker Inventory of Consequences (DrInC)
- Rand SF-36
- Profile of Mood States (POMS)
- Pittsburgh Sleep Quality Index (PSQ)
- Clinical Institute for Withdrawal Assessment Scale – abbreviated-revised (CIWA-Ar)
- SAFTEE Interview for emergent adverse events (side effects)

Subjects will be assessed (see **Table 1** on the following page) by the research assistant and medical management (MM) provider (physician, nurse) on weeks 1, 2, 3, 4, 6, 8, 10, 12, and 16 similar to the COMBINE study (Anton, 2006). The MM provider will evaluate any physical complaints and judge the probability of these being related to the study drugs using the SAFTEE interview. In addition, physicians will be available to see a subject at all times during the study. MM providers will follow the COMBINE Study manual for MM delivery (Pettinati, 2004) that emphasizes medication adherence, alcohol education, health care review, side effect appraisal, and abstinence/non-relapse goals. MM providers will have GGT as well as %dCDT (Helander, 2010) available at specified weeks for progress feedback. The timeline follow-back calendar drinking method will document daily self-reported drinking during the study. If a subject drops out of the study, the reason for this will be recorded on a "reason for termination" checklist. Week-16 drinking data will be collected in person or by phone even if a subject "drops out" prior to that date. We have been successful in collecting up to 95% of all drinking data to minimize missing data biases.

Two post-study follow-up assessment interviews at 1 (week 20) and 3 (week 28) months after study completion will assess alcohol use and psychosocial functioning to evaluate sustained post-treatment efficacy effects. Subjects will be given \$50 for completing the 16-week visit, and 20, 28-week follow-ups (max. total \$150).

Two scales that we have developed and that might be useful to understand gabapentin's mode of action, the Reasons for Drinking Scale (RDS) (see Pilot Data Section) and the Medication Effect Scale (MES) (measures similar constructs as RDS during the course of the study) will be utilized. The OCDS, POMS, and PSQ index will also be used to evaluate the effects of gabapentin on craving and aspects of "protracted withdrawal".

ASSESSMENT	BASELINE	DAY 1-7	Study Weeks												
			1	2	3	4	6	8	10	12	16	20	28		
SCID- ALCOHOL DEP.	X											X		X	X
CIWA-Ar	X	X												X	X
BREATHALYZER	X	X	X	X	X	X	X	X	X	X	X	X		X	X
FORM 90	X						X					X		X	X
TLFB			X	X	X	X	X	X	X	X	X	X		X	X
OCDS	X		X	X	X	X	X	X	X	X	X	X		X	X
REASONS FOR DRINKING	X														
MED. EFFECT SCALE			X	X	X	X	X	X	X	X	X	X			
SAFTEE (for side effects)		X	X	X	X	X	X	X	X	X	X	X			
POMS		X	X	X	X	X	X	X	X	X	X	X			
PITTSBURG SLEEP QUAL.		X	X	X	X	X	X	X	X	X	X	X			
BDI/BAS		X			X		X		X		X		X	X	
SCID-FULL		X													
ALCOHOL DEP. SCALE		X										X	X	X	
ASI		X										X	X	X	
RAND SF-36		X										X	X	X	
FAMILY HX-FROM ASI		X													
DRINC*		X										X	X	X	
MED. HX AND PHYSICAL		X													
BIOLOGICAL TESTS															
HEALTH PANEL	X						X					X			
LIVER FUNCTION TESTS	X				X		X		X		X		X	X	
CBC	X						X				X				
DRUG SCREEN	X						X				X				
%dCDT	X				X		X		X		X		X	X	
Urinary EtG		X													
URINE RIBOFLAVIN	X	X		X	X	X	X	X	X	X	X				
PREGNANCY TEST	X		AS NEEDED -----												
DNA COLLECTION	X														
Mass Spectroscopy Imaging		X		X											
MEDICAL MANAGEMENT															
GROUP A	BASELINE-----		PLACEBO -----									-FOLLOWUP-----			
GROUP B	BASELINE-----		GABAPENTIN UP TO 1200 MG DAILY--									-FOLLOWUP-----			
<i>The following assessments are for inclusion/exclusion criteria: SCID, CIWA-Ar, Form 90, LFT's, CBC, DRUG SCREEN, PREGNANCY TEST, EtG, HX AND PHYSICAL. The following for subject characterization: ASI, DrInC, ADS, FAM-HX, DNA; and the following for efficacy (TLFB, OCDS, RAND SF-36, PSQ index, POMS, GGT, %dCDT, RIBOFLAVIN) or safety (SAFTEE interview and BLOOD TESTS).</i>															

A Survey System Data Capture program that reduces the risk of data entry errors by utilizing a tablet PC (rather than paper) and stylus (rather than pen) will be used for data collection of questionnaires and interviews. The system ensures that all necessary items on a questionnaire must be answered, as "business rules" can be implemented, which alert the participant to complete the missing item before the next questionnaire is administered. Data collected using the Survey System data capture program are easily exported into SPSS data files, and exporting of data will occur immediately following each participant's visit and checked for completeness, outliers, and adequacy of transfer. SPSS data files are backed-up daily.

Gabapentin Dosing: Our group has experience with the use of gabapentin, as detailed in the background section, in alcohol withdrawal studies (Myrick, 2007) and clinical trials (Anton, 2009; Anton, 2011). In our

experience it is well tolerated at the doses (1200 mg/day) utilized with no differential drop out between active medication and placebo groups. Gabapentin is currently a generic drug, FDA approved for the treatment of seizure disorders and post-herpetic neuralgia and has been utilized for many years and given to millions of individuals. The elimination half-life of gabapentin is 5 to 9 hours (McLean, 1994). Therefore, in most studies of seizure subjects (Ramsay, 1994) and anxiety disorder subjects (Pande, 1999), gabapentin is given on a three times a day dosing schedule. While the medication is generally well tolerated, sedation could be a limiting factor and titration of dosing is recommended. Gabapentin will be purchased through our research pharmacy and over-encapsulated. Active gabapentin capsules (300 mg) and matching placebo capsules will have riboflavin added (see below) for compliance monitoring. During the first week gabapentin will be titrated to a maximum dosage of 1200 mg as diagramed below. While we have found this titration and dosing schedule to be well tolerated the study physician can lower the gabapentin dose by one pill (300mg) at noon and, if necessary, next remove the dose scheduled at night (300mg). This allows a downward titration, first to 900 mg/day then to 600 mg/day, to occur if side effect or tolerance concerns emerge in some individuals. If a person cannot tolerate 600 mg a day he/she will be terminated from the medication aspects of the trial but data will be collected for an intent-to-treat analysis. Study physicians will also be able to instruct a willing subject to attempt an increase of the GBP pills back up towards 1200 mg per day as tolerated. Anticonvulsants drugs might increase risk of suicidal ideation. While we have not observed this previously, to minimize this risk, individuals with active suicidal ideation will be excluded from study participation. In addition, we will assess emergent suicidal ideation in two ways 1) by adding an item to the SAFTEE interview about suicidal ideation, and 2) by evaluating emergent depression and suicidal ideation (item 9) on the Beck Depression Scale at weeks 3,6,10, and 16.

Dosing and Randomization Schedule: A double dummy placebo controlled design and dosing schedule will be employed (as in **Table 2**). Randomization will occur in two strata based on history of AW or no-history of AW. Within each stratum, subjects will be further urn randomized by sex and smoking status.

TABLE 2

	A. Placebo Group			B. Gabapentin Group		
	AM	Noon	PM	AM	Noon	PM
Day 1	PLC	PLC	PLC/PLC	PLC	PLC	PLC/GBP
Day 2	PLC	PLC	PLC/ PLC	GBP	PLC	PLC/GBP
Day 3	PLC	PLC	PLC/ PLC	GBP	GBP	PLC/GBP
Day 4	PLC	PLC	PLC/ PLC	GBP	GBP	PLC/GBP
Day 5 - 112	PLC	PLC	PLC/PLC	GBP	GBP	GBP/GBP
<i>PLC =Placebo GBP =Gabapentin 300 mg</i>						

Compliance with gabapentin and placebo will be monitored by the addition of 25 mg of riboflavin to each of the four daily study medication capsules. Subjects will be informed not to take any vitamins other than those that do not contain riboflavin. Baseline urine will be collected on each subject at two times to establish a reference

point for each subject. Urine riboflavin measurements will be obtained at each research assessment time, as stated above, for a total of 11 samples per subject to provide an overall assessment of study medication compliance and to examine for differential effects between treatment groups. Although not as reliable, pill counts of remaining medication at each treatment visit will be done as an ancillary measure of subject compliance and analyzed separately. All medications will be placed in blister cards with days of the week printed to assist in subject compliance. The MM provider will review these blister packs with each subject at study visits to monitor and enhance compliance.

Proton Magnetic Resonance Spectroscopy (¹H-MRS) for Glutamate and GABA: This will occur prior to randomization (see above) and again at week 3 (window between days 17-24) of study medication between 2-4 hours after the last gabapentin/placebo dose to standardize acute dosing effects. Subjects will be breathalyzed prior to the scan and need to be zero (or will be rescheduled ASAP), and, if smokers, the time of last cigarette will be recorded. In an ongoing trial we have found no problems with imaging (fMRI) clinical trial participants twice (baseline/week 3) – very few have to be rescheduled due to drinking and some subjects find it intriguing. Each scan takes about 60 minutes and uses the following protocol: A structural scan will be taken for ¹H-MRS voxel placement and tissue segmentation (256 sagittal slices; 1mm thick/50% gap). The ACC voxel for ¹H-MRS will be placed on midsagittal T1-weighted images, anterior to the genu of the corpus callosum, with the ventral edge of the voxel aligned with the dorsal edge of the genu and a voxel size of 3 x 2.5 x 2.5 cm³ will be selected (consistent with Umhau, 2010 and Mon, 2012). Following auto-shimming, single-voxel water-suppressed ¹H-MRS spectra will be acquired using a Point Resolved Spectroscopy (PRESS) sequence: Repetition Time (TR) = 2000ms; Echo Time (TE) = 40ms; number of averages = 256); an unsuppressed water spectrum will be co-acquired (TE = 40ms, number of averages = 16), scaled for partial volume effects and relaxation, and used as a concentration reference. This sequence has been shown to

perform superiorly to other PRESS sequences (TE = 30ms, 80ms; TE-averaged; (Mullins, 2008)) for imaging glutamate. Analysis of PRESS data will be conducted using LC Model 6.3 (Provencher, 1993), an operator-independent curve-fitting software package that uses least-squares estimation for quantifying metabolite concentrations; the basis set for TE = 40ms is provided by the vendor and includes myo-inositol, gamma-aminobutyric acid (GABA), lactate, taurine and aspartate, creatine, glutamine, glutamate, N-acetylaspartate (NAA), N-acetylaspartate-glutamate (NAAG), and phosphocholine (Cho). Although glutamate resonances are historically challenging to isolate, simulation research has demonstrated that glutamate can be measured with a similar level of reliability as NAA using the proposed acquisition parameters (PRESS with TE = 40ms, VOI = ACC, field strength = 3T) (Mullins, 2008). Although a TE = 40 PRESS acquisition sequence is ideal for imaging glutamate, it is not suitable for imaging GABA because, using standard acquisition protocols, GABA MRS peaks are greatly overshadowed by nearby creatine and choline MRS peaks. As a result, we will acquire a separate GABA MRS scan using the state-of-the-art MEGA-PRESS j-editing technique for isolation of GABA (Mescher, 1998). This sequence (TR = 1500ms; TE = 68ms; number of averages = 256) alternately applies a frequency-selective editing pulse at 1.9 ppm and 7.5 ppm, in an interleaved fashion; subtracting these spectral pairs produces a spectrum with a GABA peak that is clearly separated from the larger surrounding creatine and choline signals. *Analysis of MEGA-PRESS data will also be conducted using LC Model; the latest version of the software (i.e., 6.3) provides a simulated basis set and specialized routines for quantifying GABA metabolite concentrations.*

Only metabolites with fitting uncertainties (Cramer-Rao Lower Bound values) < 20% of SD in the LC Model output will be retained for analysis. LC Model includes standardized zero filling, Fourier transformation, and automated phase, baseline, and eddy current correction. To address variability in within-voxel tissue composition we will: 1) co-register T₁-weighted images to their scout images and correct for b₁ field bias, 2) extract and segment T₁-weighted images into partial volume maps of gray matter (GM), white matter (WM), and CSF using FMRIB Software Library (FSL) tools, 3) match the coordinates and size of the ¹H-MRS voxel with the segmented images and extract the tissue fractions within the voxel using FSL tools, 4) correct the raw values obtained from the LC model (scaled to water) for CSF and coil loading (Michaelis, 1993), and 5) calculate each participant's GM to brain matter (BM) ratio (i.e., GM:BM = GM/[GM+WM]) for use as a covariate in statistical analyses. This calculation will be made separately for each metabolite of interest because the spatial region that each metabolite is localized to is not the same due to the chemical shift artifact. The particular displacement of a given metabolite's localization region is determined by the metabolite's chemical shift divided by the product of the strength of the gradient used to localize the voxel and the gyromagnetic ratio for protons. For each metabolite, in each participant, we will match the coordinates and size of the voxel (accounting for chemical shift displacement) with the segmented images and extract the tissue fractions using FSL tools. Metabolite concentration estimates will be corrected for T₁ and T₂ relaxation effects using established literature values (Mlynarik, 2001; Wansapura, 1999) and scaled to water. Following this procedure we have produced the following example spectra:

FIGURE 3. LC Model Spectra for Glutamate

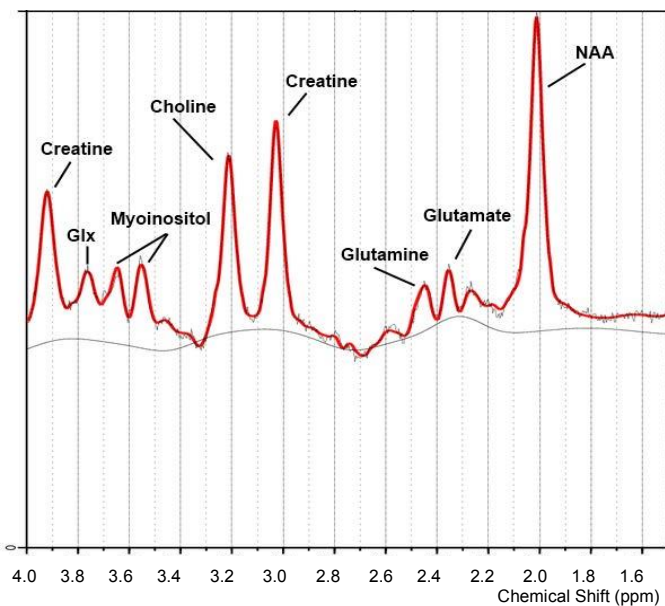
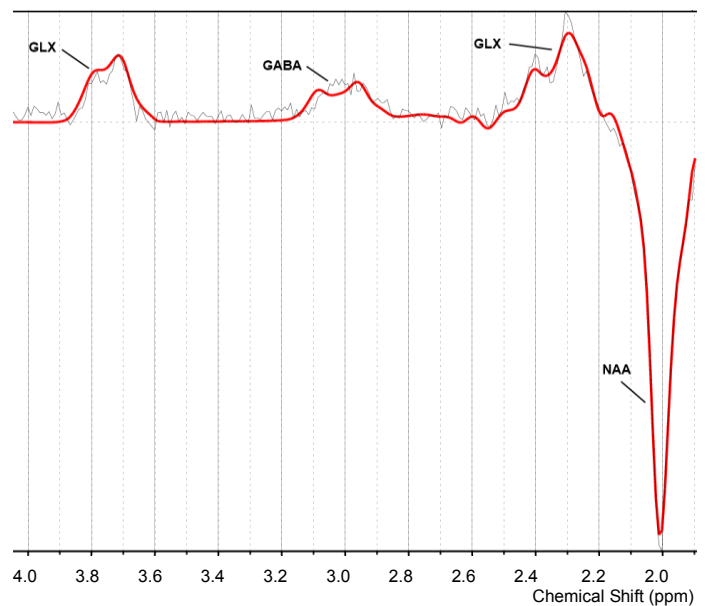


FIGURE 4. LC Model Spectra for GABA



We have run 6 healthy controls *through the PRESS MRS sequence* and found a mean (SD) glutamate, as a ratio to water, of 7.66 (1.24) or as a ratio to creatine, 1.38 (0.15). *We have run an additional 7 healthy controls through the MEGA-PRESS MRS sequence and found a mean (SD) GABA, as a ratio to water, of 1.75 (0.19). These values are consistent with published levels (where there is a range of values based on quantification method and reference analyte).*

Lab and Genetic Assays: The CNL lab directed by Dr. Anton has 20 years of experience in measuring carbohydrate deficient transferrin (CDT) and currently runs a state-of-the-art HPLC based assay that is recognized by the IFCC as a candidate reference method (Helander, 2010). The analyte of interest is disialo-CDT as a percentage of total transferrin (%dCDT). Extensive quality control and proficiency testing is routinely performed. His lab also assays urinary Ethylglucuronide (EtG), a sensitive measure of any/low alcohol consumption using a Microgenics immunoassay which correlates highly with LC-MS. Qiagen DNA extraction kits are used to extract DNA from whole blood by a dedicated genetic technician in his lab, tested for DNA purity, and used in Taqman PCR analysis with specific probes and primers purchased from Life Science Technologies using a Step One analyzer (Applied Biosystems). His K05 funding allowed him to become proficient in this type of analysis (Anton, 2012) and he has genotyped hundreds of samples with various SNP's and VNTR's including the glutamate and GABA genes of interest (see background section).

Outcome Variables and Power Analyses: The primary drinking outcome variable will be the percent of subjects with no heavy drinking days (PSNHDD) as defined by Falk (2010), chosen because it is currently accepted by the FDA in its guidance for clinical trials and also is consistent with our prior work with gabapentin. However, other secondary drinking variables such as percent days abstinent, % heavy drinking days, and drinks per day, as well as craving (OCDS) and biomarkers (%dCDT and GGT) will be calculated and reported for completeness sake. Urinary riboflavin and pill counts will be used as measures of compliance and compared between groups while potentially serving to define compliers for independent analysis. PSQ, POMS, and MES will evaluate sleep quality, mood state, and medication effect differences between medication groups. Pre-randomization and week-3 brain glutamate and GABA levels will be calculated for each subject and their change will be the unit of measurement for the treatment effect mediator analysis (see below).

A statistical power calculation was conducted using data from our past two studies (background section) using the treatment effect in subjects with a DSM-IV history of AW on the PSNHDD outcome variable. With 45 subjects per medication group (total N=90) the power to detect the gabapentin treatment effect on PSHDD is between .83 (study 1 calculation) and .98 (study 2 calculation). While significant covariate effects in our earlier studies have not been observed with this outcome variable, additional power analysis using Monte Carlo simulation (n=500) in the presence of covariates modeled, (e.g. pretrial drinking, OCDS, ADS, etc.) on our previous studies was conducted. Power values comparable to those in the simpler analysis were found in all cases ranging from .81 to .87 (study 1) to .95 to .99 (study 2).

The statistical power calculation for gabapentin's effect on brain glutamate change from baseline to week-3 is based on the work of Umhau (2010), the study design closest to our own, where an acamprosate-induced change in brain glutamate levels (vs. placebo) was detected with an effect size of .95. *The power to detect a similar gabapentin vs. placebo difference in the current proposal with an n=45/group is in excess of .99 with the ability to detect an effect size as low as $d = .6$ with power of .8. Since the reported effect of gabapentin on GABA is quite large (Cai, 2012; Kuzniecky, 2002; Petroff, 1996; Petroff, 2000) the power to detect a gabapentin vs. placebo difference with an n=45/group is in excess of .9. Given this large statistical power, an expected maximal 10% attrition due to inadequate scans should not materially affect our ability to detect a medication effect on glutamate or GABA. Additionally, there is likely to be sufficient variation in both medication response and brain glutamate/GABA level change to allow acceptable use of a mediation analysis to examine the effect of this glutamate/GABA change on treatment response.*

Power has not been calculated for the exploratory genetic analyses. The genetic SNP's we have chosen to explore are not only indicated by the literature but also, importantly, have a reasonable genotypic distribution. Given our past work and HapMap data (see Preliminary Data section) we expect the distribution of both the *GABRA2* and *GM8* allele frequencies to be about 60:40 with not much difference between Euro-Caucasians and African Americans. This should provide a good balance between treatment groups. Modifier effects are likely to be observable if the effect size is moderate to large. In any case, this data will allow more appropriate power calculations for subsequent studies. Effects of genetic variation on brain glutamate/GABA are unknown and completely novel but as a main effect with 90 subjects (even accounting for drop-outs or unusable scans) the power to detect a gene by analyte effect should be sufficient if clinically meaningful.

Data Analysis Plan: *The PSNHDD (with baseline percent heavy drinking days as a covariate) will be analyzed with a multiple logistic regression with indicator coding medication group as the primary independent variable. All hypotheses (primary, secondary) will be assessed with likelihood ratios. Other possible pre-randomization variables, e.g. sex, smoking status, ADS score, pre-randomization CIWA-Ar score, etc. will be evaluated as possible covariates in the final analysis. Missing data (assuming monotonicity) will be imputed as heavy drinking days. In addition, the relapse time-course will be analyzed by GEE techniques for the full treatment X time factorial design. Secondary outcome variables, e.g. other drinking variables, OCDS, POMS, PSQ index, MES and biomarkers (%dCDT and GGT), will be analyzed as either mixed models (SAS PROC MIXED) or in GEE analyses (SAS GENMOD) depending upon the nature of the variable (parametric or categorical).*

Change in glutamate and GABA levels will be analyzed as a mixed model (SAS PROC MIXED) with time as a within subject variable and medication group as between subject variables. Significant interactions will be interpreted with simple effects and main effects will be assessed in the absence of the interaction. If sex and smoking status (reported by some to effect brain GABA levels), or pre-randomization CIWA-Ar score are found to significantly influence glutamate or GABA levels, or if their inclusion reduces residual variance, they will be included in the model as covariates.

In order to assess the degree to which changes in brain GABA and glutamate account for drinking outcomes, neurochemical variables (as difference scores) will be entered into the main logistic regression model. A reduction in the significance of the main treatment effect or the treatment by time interaction will be suggestive of a mediation effect. Path analysis will be used to test the significance of the indirect path from treatment to GABA/glutamate levels to relapse status and/or other drinking measures.

Timeline: Since our staff is trained in all procedures and scales are digitalized, the first 3-4 months will be used for medication production, database building, and data collection computer tablet study specific program production. In the first year we will screen/randomize 38/18 subjects and then 57/27 subjects in year 2-3 and 38/18 subjects in year 4. The last five months will be used for data analysis and publication production.

Interpretations and Future Directions: While this study will focus on gabapentin efficacy in those with a history of AWS, it is possible that it might have anti-craving and reinforcement effects *independent of effects based on AWS history*. Since this proposal will not include those without a history of AWS we will not be able to address the issue of broader efficacy directly. However, using exploratory analysis on craving effects, reasons for drinking and medication effects scales, as well as sleep, we might discover mediators of treatment efficacy that could be studied in expanded populations in the future. Also, baseline levels of brain glutamate/GABA cannot be compared with levels in alcohol dependent individuals without a history of AW. Nevertheless, the ability to relate drinking outcome to changes in these levels and ability to relate the efficacy of gabapentin to glutamate/GABA brain changes would still be salient and important, as would the pharmacogenetic exploration. Since the complete understanding of brain glutamate/GABA levels as measured by ¹H-MRS is evolving, it is possible that those with AW might have less glutamate and more GABA prior to randomization since the pools measured might exist mostly in the intra-cellular space due to auto-regulatory mechanisms and/or sequestration, but still might change during treatment. The directionality of the effect will not negate the potential to observe changes based on gabapentin treatment and the relationship of those changes to efficacy. It will add data to the growing literature that could, in fact, assist in correct interpretation of the localization of these brain neurotransmitters. Furthermore, even if gabapentin is not found efficacious, the difference in brain glutamate and GABA levels change over time, especially in relationship to relapse status, would still add new information about AW biology and prognosis *that could be useful for future drug development*. The collection of DNA on all subjects will allow future explorations including deeper sequencing and epigenetic/regulatory changes of the candidate genes proposed, as well as exploration of other genetic variation that might emerge in relationship to relapse and medication response. *If these results are positive, future studies might investigate more specific pharmacological treatment matching between those with and without a history of alcohol withdrawal symptoms.*