behavior. The applicant will seek to complete a first author publication during this semester, under the supervision of the sponsor and with the assistance of the co-sponsors (i.e., Dr. Bryan; Dr. Willcutt). Career Development – The applicant will complete the Graduate Teacher Certificate Program and will continue to meet weekly with sponsor and bi-monthly with co-sponsors. These meetings will provide the applicant with manuscript preparation supervision in a publication where the applicant will be the primary author.

Fall of 2005
Coursework – Courses will focus on Doctoral Dissertation (Research Practicum; Dr. Hutchison) and additional statistical training (Biometrical Methods in Behavioral Genetics; Dr. Hewitt). Research – The applicant will focus on preliminary data analysis resulting from the proposed research. Preliminary results will be prepared for presentation at national conferences such as AABT and RSA. Meetings with sponsor and co-sponsors will be designed to help the applicant complete her dissertation in the spring of 2006.
Career Development – The applicant will begin to evaluate possible internship sites with a strong research component in the field of alcohol (e.g., University of California at San Diego, Brown University). Meetings with sponsors will also provide guidance in terms of internship applications, networking, etc. The applicant will present her research at AABT and will actively engage in networking activities designed to identify the best fit internship for the applicant’s career goals.

Spring of 2006
Coursework – The applicant will have completed all required courses by the spring of 2006. The applicant will devote most of her time to the preparation and defense of her dissertation, which will be completed prior to going on internship.
Research – The applicant will perform the final analysis and manuscript preparation for the proposed research. The applicant will successfully defend her doctoral dissertation. The applicant will submit manuscripts to either Journal of Studies on Alcohol or to Alcoholism: Clinical and Experimental Research.
Career Development – Meetings with sponsor and co-sponsors will focus on manuscript (dissertation) preparation and submission. Sponsors will also discuss future post-doctoral and job opportunities that best fit the applicant’s expertise and help her reach the ultimate goal of this proposal: to achieve an assistant professorship position at a Research I University or Medical Center where the applicant will continue to work on her independent line of research in the field of alcohol addiction.

Item 40b. Research Training Proposal

1. SPECIFIC AIMS

Several risk factors for developing alcohol use disorders have been identified to date. Some of the most consistent findings include family history of alcoholism (Kendler et al., 1997), sensitivity to the effects of alcohol (Schuckit, 1996; Schuckit et al., 1997), personality traits such as novelty seeking, impulsivity, and sensation seeking (Finn et al., 2002; Finn, Earleywine, and Pihl, 1992; Earleywine and Finn, 1991) and craving response following alcohol consumption (Sinha & O’Malley, 1999). Recently the field of alcohol addiction has moved from the identification of traits (phenotypes) associated with problem drinking, to the search for genotypes that underlie the expression of these behavioral markers. For example, the VNTR polymorphism of the D4 dopamine receptor gene has been identified as a potential genetic risk factor for developing alcohol addiction (Hutchison, et al., 2002). The phenotype under study was craving for alcohol during a cue exposure paradigm. Likewise, Marc Schuckit and colleagues (1999) have recently linked the GABA alpha 6 Pro385/Ser SNP to the phenotype of sensitivity to the subjective and physiological effects of alcohol (i.e., level of response to alcohol).

The specific objectives of this proposal are three-fold: (1) to test the relationship between the DRD4 VNTR polymorphism and craving for alcohol; (2) to investigate personality factors that may mediate the
relationship between craving for alcohol and the DRD4 candidate gene polymorphism; (3) to test the association between the GABA alpha 6 Pro385/Ser single nucleotide polymorphism (Pro385Ser SNP) and acute responses to alcohol. The proposed study will compare 40 individuals with the 7 repeat allele of the DRD4 VNTR versus 40 individuals without the risk allele using an intravenous alcohol challenge paradigm. Likewise, our matched research design will include 40 individuals with the Pro/Pro genotype of the Pro385Ser SNP and 40 individuals with the Pro/Ser genotype. Our dependent measures will include multiple assessments of craving for alcohol and acute subjective and physiological responses to alcohol. Personality/cognitive factors such as impulse control and sensation seeking will be examined as potential mediating/moderating variables.

The long-term objective of this proposal is to advance our understanding of the genetic, biological, and behavioral determinants of alcohol addiction by utilizing a highly controlled laboratory design to test preliminary genetic findings. The specific aims and hypotheses are as follows:

1. Specific Aim 1: To test whether individuals with the DRD4 VNTR risk allele demonstrate an increased craving response for alcohol, using a controlled laboratory design. Participants will be asked to report their craving for alcohol at different levels of intoxication during intravenous alcohol administration.

   - Hypothesis 1: We expect participants with the risk allele to report higher levels of craving for alcohol after receiving small doses of intravenous alcohol as compared to individuals without it.

2. Specific Aim 2: To examine personality factors that may mediate/moderate the relationship between the DRD4 receptor gene and craving for alcohol. It has been suggested that personality factors such as novelty seeking may mediate/moderate the relationship between the DRD4 receptor gene and the risk for alcohol addiction (Bau et al., 2001). We will collect self-report measures of novelty seeking, sensation seeking, and impulsivity from every participant. In addition, self-report data will be corroborated by neurocognitive tests measuring the constructs of impulse control (stop-signal task) and sensitivity to reward (door-opening task).

   - Hypothesis 2: Consistent with the current literature, we expect individuals with the risk allele to score lower on measures of impulse control and higher on measures of impulsivity/sensation seeking compared to individuals without the risk allele. We will then test these personality factors as possible moderators/mediators of the relationship between the 7 repeat allele of the DRD4 VNTR and elevated craving for alcohol.

3. Specific Aim 3: To examine the relationship between the GABA alpha 6 Pro385Ser SNP and the risk factor expressed by one's sensitivity to the effects of alcohol (phenotype). Level of response to alcohol represents a robust risk factor for alcohol addiction (Schuckit, 1996; Schuckit et al., 1997). Our study involves measuring participants' response to alcohol at different points in the ascending and descending arms of their Blood Alcohol Concentration (BAC) using standard self-report measures of intoxication quality and intensity.

   - Hypothesis 3: Based on prior research (Schuckit et al., 1999), we expect individuals with the Pro385/Ser genotype of the GABA alpha 6 receptor gene to demonstrate lower sensitivity to the physiological and subjective effects of alcohol during the experimental procedure.

2. BACKGROUND

Recent research in the field of alcohol addiction has focused on the identification of risk factors for the development of problem drinking. Some of the most empirically supported risk factors include family history of alcoholism (Kendler et al., 1997), level of response to alcohol (Schuckit, 1996; Schuckit, Tipp, Smith, Wiesbeck, & Kalmijn, 1997), personality traits such as novelty seeking, impulsivity, and sensation seeking (Finn, Mazas, Justus, & Steinmetz, 2002; Finn, Earleywine, and Pihl, 1992; Earleywine and Finn, 1991) and elevated craving for alcohol (Sinha & O'Malley, 1999). More recently, researchers have attempted to use their
knowledge of trait markers for alcoholism in order to identify genetic correlates of these traits. In a review of genetic markers of alcohol abuse, Ferguson and Goldert (1997) define trait markers as "risk markers for the development of alcoholism, which are useful in identifying the individuals at risk, whether or not a drop of alcohol has ever passed their lips."

Despite the evidence for a strong genetic component to problem drinking, it is unlikely that any one specific gene is responsible for the transmission of alcoholism (Pickens, 1991; Shuckit, 1998). Therefore, researchers have proposed a different approach to the identification of genetic mechanisms underlying alcohol use disorders (Shuckit, 1999). This approach consists of identifying specific behavioral phenotypes related to the disorder of interest and using the proposed phenotype to search for potential genotypes that underlie the behavioral marker. The ideal phenotype is narrowly defined, readily identifiable, related to the clinical manifestation of the disorder, and associated with an underlying biological mechanism (Hutchison, McGeeary, Smollen, Bryan, & Swift, 2002). The intermediate phenotype approach is a promising research strategy and has become increasingly popular in the field of addictions.

**Craving for alcohol and the DRD4 dopamine receptor**

The dopaminergic system has been repeatedly associated with maladaptive use of substances such as alcohol (Koob & Bloom, 1988). Alcohol has been shown to stimulate the release of dopamine in the brain (Fadda, Argiolas, Mellis, Serra, & Gessa, 1980). Research has suggested that the effects of dopamine in addictive behavior are driven by the dopamine-mediated reinforcement in the mesolimbic reward pathway (Koob & Bloom, 1988). The use of craving as a potential phenotype for alcohol use disorders fits this conceptualization of the dopaminergic system as central to the development of addictions. Craving has been defined as a strong desire or urge to consume alcohol (for reviews see Bohn, 1995). Craving and loss of control over drinking have been associated with the activation of the mesolimbic and mesocortical dopamine pathways in the brain following alcohol consumption. In addition, craving for alcohol is part of the current definition of alcohol dependence adopted by the International Classification of Diseases (ICD 10). Hence, craving for alcohol represents a great candidate trait marker (phenotype) for the identification of individuals at risk and for the search of genetic correlates of alcohol use disorders.

A recent study by Hutchison and colleagues (2002) using craving as a phenotype, has linked increased craving response following cue exposure to the VNTR polymorphism of the D4 dopamine receptor gene. Specifically, they have found that individuals who were homozygous or heterozygous for the 7 (or longer) repeat allele, classified as DRD4 L (long), showed significantly higher craving for alcohol in the laboratory as compared to DRD4 S (short) participants. Other studies have reported similar findings linking alcohol dependence to the chromosome 11p, which is in close proximity with the DRD4 receptor gene (Long et al., 1998). A number of studies however, have failed to demonstrate an association between the D4 dopamine receptor gene and alcohol addiction (Sander, Harms, Dufeu, Kuhn, Rommelspacher, & Schmidt, 1997; Soyka, Preuss, Koller, Zill, & Bondy, 2002; Bau, Roman, Almeida, & Hutz, 1999). A possible explanation for the contradictory findings is the use of different trait markers (e.g., sensation seeking; alcohol-seeking behavior) in different investigations. The proposed study will provide a conceptual replication of these intriguing findings. In addition, this proposal includes additional parameters (i.e., personality factors) that may explicate this association between the DRD4 VNTR polymorphism and alcohol craving.

**DRD4 and personality traits**

Certain personality traits have been frequently associated with alcoholism and researchers have postulated that these traits constitute risk factors for the development of problem drinking and addictive behavior in general. These traits include novelty seeking (Sander, Harms, Dufeu, Rommelspacher, & Schmidt, 1997), sensation seeking (Finn, Earleywine, & Phil, 1992; Earleywine & Finn, 1991), and impulsivity (Finn, Mazdaz, Justus, & Steinmetz, 2002). These personality traits in turn, have recently been linked to sensitivity to reward, mediated by the dopaminergic system. Specifically, some studies have demonstrated an association between the DRD4 VNTR polymorphism and characteristics like impulsivity and sensation seeking, such that individuals with the D4 L allele report higher levels of sensation seeking and impulsivity compared to D4 S individuals (Noble, Ozkaragoz, Ritchie, Ahang, Belin, & Sparkes, 1998), and lower levels of harm-avoidance.
(Bau, Roman, Almeida, & Hutz, 1999). Other studies however, have found no association between the D4 VNTR polymorphism and sensation seeking (Soyka, Preuss, Koller, Zill, & Bondy, 2002). The inconsistency in the findings may be due to measurement differences (self-reports scales), inadequate sample size, or methodological differences.

The association between DRD4 and personality characteristics is not specific to alcohol addiction. A number of studies have shown that the D4 VNTR polymorphism correlates highly with childhood disorders, particularly ADHD and Conduct Disorder (Muglia, Jain, Macciardi, & Kennedy, 2000; Holmes et al., 2002, Faraone et al., 1999). A recent meta-analysis of the D4 and ADHD has found consistent support for their association (Faraone, Doyle, Mick, Biederman, 2001). ADHD and conduct disorder in turn, have both been associated with higher indices of substance use (Kuperman et al., 2001; Young et al., 2002). Furthermore, ADHD, CD and substance use disorders seem to share as common features low levels of impulse control and high sensitivity to reward (i.e., inability to wait for a larger reward). In addition, a recent study by Bau and colleagues (2001) has reported a significant interaction between a measure of sensation seeking and the DRD4 genotype in predicting alcohol seeking behavior. Taken together, these findings suggest that the effects of the D4 dopamine receptor gene on alcohol use disorders may be mediated/moderated by issues of impulse control, which encompass impulsivity and sensation seeking. The current proposal will address both the mediating and moderating hypotheses by incorporating personality/cognitive traits associated to the DRD4 VNTR polymorphism in the model that predicts craving for alcohol. In addition, our multi-method approach to the measurement of these personality/cognitive traits will increase our power to detect individual differences.

GABA alpha 6 and level of response to alcohol

Gamma-aminobutyric acid (GABA) plays a major role as a neurotransmitter in the central nervous system. Research has shown that sons of alcoholic parents demonstrate an increase in plasma GABA concentrations following alcohol consumption compared to controls (Moss, Yao, Burns, Maddock, & Tarter, 1990). Follow up studies have confirmed that alcohol affects GABA receptors (Korpi et al., 1992). Additional research has linked the polymorphism of the GABA alpha 6 subunit gene to benzodiazepine sensitivity, which has clear implications for conditions such as alcoholism (Iwata, Cowley, Radel, Roy-Byrne, & Goldman, 1999). Schuckit and colleagues (1999) have recently reported an association between GABA alpha 6 Pro385Ser SNP and sensitivity to alcohol. Specifically, they have found that individuals with the Pro/Ser genotype were more likely to demonstrate a low level of response to alcohol compared to participants with the Pro/Pro genotype. Furthermore, using a longitudinal design, they have found that individuals with the Pro/Ser genotype were significantly more likely to develop alcohol dependence than Pro/Pro individuals (Shuckit, Mazzanti, Smith, Ahmed, Radel, Iwata, & Goldman, 1999). In conjunction, these findings suggest an association between the polymorphism of the GABA alpha 6 receptor gene and one's level of response to alcohol as well as to the development of alcohol dependence during one's lifetime. The proposed investigation will expand the current findings by utilizing a larger sample, which will allow us to integrate individuals with moderate sensitivity to alcohol in our analysis. Furthermore, we will measure sensitivity to the effects of alcohol using both the standard measure of subjective response (SHAS) and an additional measure that breaks down the effects of alcohol into sedation and stimulation (Biphasic Alcohol Effects Scale).

Summary of Rationale

This proposal has reviewed evidence suggesting that the VNTR polymorphism of the D4 dopamine receptor gene is associated with craving for alcohol following cue exposure (Hutchison et al., 2002) sensation seeking (Nobel et al., 1998), and reward sensitivity (Bau et al., 1999). Craving for alcohol, sensation seeking, and sensitivity to reward are in turn risk factors for the development of addictions. Hence, these findings suggest that the DRD4 VNTR polymorphism may represent a genetic risk factor for the development substance use disorders. These effects however, may be moderate/mediated by the association between the candidate polymorphism and certain personality traits (i.e., sensation seeking). In addition, we have reviewed preliminary findings linking the Pro385Ser SNP and one's sensitivity to the effects of alcohol (Schuckit et al., 1999). In conclusion, the literature on genetic correlates of behavioral markers of risk for alcohol use disorders has advanced considerably. However, a number of methodological concerns limit the interpretation of the
research findings. For example, the study by Hutchison et al., showing higher craving response in individuals with DRD4 L allele did not take into account personality and environmental factors (e.g., family history of alcoholism) that may interact with the underlying genetic liability and lead to the expression of the craving phenotype. Likewise, the investigation on the effects of the Pro385Ser substitution used an all male sample pre-selected on their level of response to alcohol (high vs. low). It has yet to be tested whether these findings will remain significant in a sample that includes females and individuals with moderate levels of sensitivity to alcohol.

**Significance and Innovation of the Proposed Research**

A number of candidate gene studies have been limited by the lack of an association between a genetic component and a specific behavioral marker or outcome. This proposal has reviewed studies suggesting an association between specific phenotypes (i.e., craving for alcohol; level of response to alcohol) and genetic markers (i.e., D4 dopamine receptor; GABA alpha 6 receptor). These studies represent advancement in the field since they use a theoretical approach to the search for genetic correlates of behaviors. These studies however, are limited by a number of methodological issues. The proposed research will address some of these limitations and will expand the generalizability of the findings by incorporating a multi-method assessment approach in conjunction with an alcohol administration method (i.e., infusion paradigm) that allows for testing under steady levels of desired alcohol intoxication. Each behavioral construct will be measured using multiple assessment methods that include self-report, behavioral, and physiological measures. Another advantage of our design is the intravenous alcohol administration, which will insure that participants are being tested at steady levels of intoxication while not being exposed to alcohol cues, which is critical to the concept of craving. Finally, the proposed research design will allow for a conceptual replication of prior findings as well as an expansion of existing research through the use of additional parameters such as personality (e.g., sensation seeking, impulsivity) and environmental factors (e.g., alcohol consumption) as well as an adequate sample size and a more refined research methodology.

**Preliminary Studies.**

Our laboratory has conducted a number of projects that combine the use of behavioral measures to genetic variables. Dr. Hutchison has recently completed an investigation designed to examine the effects of the DRD4 VNTR polymorphism on craving for alcohol following cue exposure in the laboratory (Hutchison et al., 2002). Specifically, this research has shown that individuals with the DRD4 L genotype reported significantly greater craving for alcohol after consuming alcoholic beverages but not placebo. The same was not true for individuals with the DRD4 S genotype (see figure 2). In addition, Dr. Hutchison is currently analyzing the effects of the Pro385Ser SNP on sedation and stimulation following alcohol consumption. Consistent with results reported by Schuckit et al. (1999), individuals with the Pro/Ser genotype demonstrated less sedation and stimulation after drinking as compared to individuals with the Pro/Pro genotype (figure 1). These results are part of an upcoming symposium presentation at the Research Society for Alcoholism (RSA) 2003.

Our laboratory has also conducted initial DNA screening and pilot runs of the experimental design proposed here. At present time 38 participants have completed the screening session and have provided DNA samples (saliva samples). The results of our DNA analyses for the two candidate genes are presented in table 1. These results suggest that we are able to successfully recruit participants with the desired genotypes.

**Table 1. Genotypes for screened participants.**

<table>
<thead>
<tr>
<th>DRD 4 VNTR Polymorphism</th>
<th>GABA α 6 Polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long (≥7 repeats)</td>
<td>Short (&lt; 7 repeats)</td>
</tr>
<tr>
<td>n = 18</td>
<td>n = 20</td>
</tr>
<tr>
<td>Total number of samples analyzed = 38</td>
<td>Total number of samples analyzed = 16</td>
</tr>
</tbody>
</table>
In addition, the alcohol infusion administration has been tested in our laboratory in order to access its feasibility and the amount of risk involved. The results of several pilot runs were very encouraging and have shown that the intravenous alcohol administration is both feasible from a medical standpoint and safe to research participants. Lastly, the current research proposal has been approved by the Human Subjects Committee and has been funded by the General Clinical Research Center at the University of Colorado, Boulder. The GCRC will cover all medical expenses and will provide the medical staff necessary to perform the screening physical exam and the intravenous alcohol administration.

3. RESEARCH DESIGN AND METHODS

Overview. The primary aim of this study is to investigate the association between 2 specific behavioral markers (craving for alcohol and sensitivity to alcohol) and 2 underlying genotypes (DRD4 VNTR polymorphism and GABA Pro385Ser SNP). In addition, we will investigate possible moderators in the relationship between the DRD4 gene and increased craving for alcohol. We have identified a number of a priori, theory-based candidate mediating variables, including sensation seeking, impulse-control, and sensitivity to reward.

Participating college students (n=80) will complete a series of baseline questionnaires and provide a DNA sample (saliva samples) during the initial screening session. DNA material will then be analyzed and eligible participants will be invited to come back for a medical screening visit to ensure the safety of the intravenous alcohol administration. Participants who pass the medical screening will be invited to the experimental session. We will compare 40 participants with the DRD4 L against 40 individuals with the DRD4 short allele according to their urge to drink (craving) during the experimental session. In addition, a matched-control design will allow us to compare 40 individuals with the GABA alpha 6 Pro/Ser genotype versus 40 individuals with the Pro/Pro genotype on their level of response to alcohol. The experimental session will
consist of an intravenous alcohol administration followed by measures of craving response and sensitivity to alcohol performed at baseline, and at the following points in the ascending arm of the blood alcohol concentration: .02, .04, and .06.

Sample size determination. Sample size was selected to permit analysis of the primary research questions at an alpha of .05 and power level of .80. Calculation of effect size, required sample, and power were performed according to Cohen (1988). To test hypotheses 1 and 2 regarding the effects of the DRD4 receptor gene on craving for alcohol we have based our power analysis on the effect sizes reported in Hutchison et al. (2002) which suggests a moderate to large effect of the DRD4 gene on urge to drink (d = .64). We have decided to use a conservative estimate of effect size (d = .60). Thus, 80 participants (40 in each cell) will provide sufficient power (Cohen, 1988; Cohen, 1992). Concerning hypothesis 3, we have based our power analysis on the effect sizes reported by Schukit et al. (1999), where the GABA alpha 6 polymorphism was found to have a large effect on participant's level of response, d = .86. A sample size of 80 participants will grant us a power of .99 to detect group differences in level of response as a function of the GABA alpha 6 genotype (Cohen, 1992; http://www.sst.ucla.edu/calculators).

Study Participants. See section 5.

Screening Procedure. Persons who express interest in the study will be contacted by telephone for a phone screening session, during which they will be provided detailed information about the study. Participants will be made aware of the physiological measures, the intravenous alcohol administration, and any potential risks or discomforts associated with the study. Participants will then be asked if they are still interested in participating, in which case a few eligibility questions will be asked (according to the eligibility criteria in section 5). If criteria are met based on screening questions, participants will be invited to come to the laboratory where upon their agreement a sample of DNA will be collected (following the procedures described below) and a series of questionnaires will be filled out. Participants will be compensated $10 for providing a DNA sample and for completing the questionnaires. Finally, upon results of DNA analyses (DRD4 and GABA alpha 6 analyses will take place) participants will be called back and invited to a medical screening visit.

DNA Collection, Storage, and Analysis. DNA will be collected following published procedures (see Freeman et al., 1997; Walker et al., 1999). Subjects swab their cheeks with three cotton swabs, followed by a rinse of the mouth with tap water. The swabs and water are placed in a sterile 50 ml polypropylene tube. The DNA samples will be used to identify the genetic factors of interest in this proposal. DNA will be essayed for candidate polymorphisms using previously reported methods (Hutchison, McGearv et al., 2002; Shuckit et al., 1999).

Table 2. Timeline for Screening Session (duration: 40-45 minutes; at the Colorado Alcohol and Smoking Lab)

<table>
<thead>
<tr>
<th>Time</th>
<th>Task</th>
<th>Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00 AM</td>
<td>DNA collection and questionnaires</td>
<td>AEI; SSSV; TPQ-NS; IMPS; AUQ; Urge Form; RAPI; SHAS; POMS.</td>
</tr>
</tbody>
</table>

Medical Screening Visit. Eligible participants will be invited to come to the General Clinical Research Center (GCRC) at the University of Colorado at Boulder for a medical screening visit. The purpose of the visit is to ensure the safety of the infusion procedure and to review eligibility for the study (i.e., pregnancy and drug testing). Participants will have their liver enzymes tested to insure the absence of liver problems, which would suggest their ineligibility for the procedure. Likewise, participants whose drug test come out positive for substances other than marijuana will be excluded from the study.
Table 3. Timeline for Medical Visit (duration: 40-45 minutes; at the GCRC)

<table>
<thead>
<tr>
<th>Time</th>
<th>Task</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00 AM</td>
<td>Medical Screening Visit</td>
<td>CBC; coag; liver profile; glucose; pregnancy test; urine drug screen.</td>
</tr>
</tbody>
</table>

**Experimental Session.** The experimental session will take place at the General Clinical Research Center (GCRC) since the alcohol administration will be done intravenously, as a means of controlling for blood alcohol concentration (BAC). Upon arrival at the GCRC, participants will be provided with a detailed consent form and will be breathalyzed to ensure that they have not been drinking prior to the session. Only participants with blood alcohol concentration of zero will be allowed to participate in the study. Female participants will be given a urine pregnancy screen to insure that they are not pregnant, in which case they will be excluded from the study. Weight will be measured to determine the appropriate amount of alcohol required to reach a peak BAC of .06. Gender will also be considered in this calculation. Participants will then complete the baseline assessments which include a neurocognitive battery and a few self-report measures.

After baseline measures, an intravenous catheter will be placed in a forearm vein and kept open using a D5W infusion. This procedure will be performed by the medical staff at the General Clinical Research Center (see expanded procedure explanation below). Participants will then be brought to a BAC of .020 and will perform physiological (heart rate and blood pressure) and subjective tests of craving and response to alcohol. BAC will be raised to .040 and the same tests will be administered. Finally, the participant will reach a BAC of .060 (equivalent to 2-3 drinks) and will complete the last set of tests. At this time, the catheter will be removed and subjects will be given refreshments. We expect the intravenous procedure to start at 1:45 pm and to last for approximately 2 hours. We will keep participants under observation until their blood alcohol level has come down to .02, at which time they may leave with a designated driver, by bus or by taxi. Participants will be asked not to drive for 3 hours following the testing session, and the experimenter will review the risks of residual impairment before participants leave GCRC. Lastly, participants will be compensated $50 for completing the experimental session.

Table 4. Timeline for Experimental Session (duration: 3 hours; at the GCRC)

<table>
<thead>
<tr>
<th>Time</th>
<th>Task</th>
<th>Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:00 PM</td>
<td>Baseline – Neurocognitive Tests</td>
<td>Stop Signal Test; Door-Opening Test</td>
</tr>
<tr>
<td>1:15 PM</td>
<td>Baseline Assessment</td>
<td>Heart Rate; Blood Pressure; Craving (AUQ; Urge Form); Response (SHAS; BAES); POMS</td>
</tr>
<tr>
<td>1:45 PM</td>
<td>Intravenous Procedure</td>
<td>Heart Rate; Blood Pressure; Craving (AUQ; Urge Form); Response (SHAS; BAES); POMS</td>
</tr>
<tr>
<td>2:30 PM</td>
<td>Blood Alcohol Concentration: .020</td>
<td>Heart Rate; Blood Pressure; Craving (AUQ; Urge Form); Response (SHAS; BAES); POMS</td>
</tr>
<tr>
<td>3:00 PM</td>
<td>Blood Alcohol Concentration: .040</td>
<td>Heart Rate; Blood Pressure; Craving (AUQ; Urge Form); Response (SHAS; BAES); POMS</td>
</tr>
<tr>
<td>3:30 PM</td>
<td>Blood Alcohol Concentration: .060</td>
<td>Heart Rate; Blood Pressure; Craving (AUQ; Urge Form); Response (SHAS; BAES); POMS</td>
</tr>
<tr>
<td>4:00 PM</td>
<td>Debriefing and Refreshments</td>
<td></td>
</tr>
</tbody>
</table>

**The Intravenous Alcohol Administration.** The intravenous alcohol administration is an essential component to the success of this investigation since control over blood alcohol levels is a critical feature of most alcohol challenge paradigms. As described above, an intravenous catheter will be placed in a forearm vein and will be kept open using a D5W (5% alcohol concentration) infusion. A two-step procedure will insure that participants
reach the desired level of blood alcohol concentration (BAC) at a safe and comfortable pace:

A) Using a 5% alcohol solution our protocol will follow a previously tested and published nomogram (Subramanian, 2002) utilizing the following calculated infusion rates: .166 ml/min/kg for males and .126ml/min/kg for females. These infusion rates are expected to bring participants’ BAC up by .02 in approximately 20 to 40 minutes. Participants will be tested at 3 different levels of intoxication: BAC = .02; .04 and .06. The handling and administration of the alcohol solution will be performed by the GCRC medical staff following standard infusion procedures. Breath alcohol concentrations will be measured every 10 minutes using a Breathalyzer. Previous studies have demonstrated that breath measures of BAC are as reliable (and less invasive) as blood tests (Davidson, Camara, and Swift, 1997).

B) We will also utilize an alcohol clamping technique. This procedure will enable us to maintain a constant breath alcohol concentration for prolonged periods of time, allowing testing to take place. Alcohol clamping consists of adjusting the infusion rate based on real time breath alcohol measures (Ramchandant et al., 1999; Subramanian et al., 2002; O’Connor et al., 1998).

Measures. The following measures will be given to each participant to examine the main hypotheses in this proposal. A number of demographic and alcohol use measures will be included in order to collect descriptive information on our sample (including an assessment of family history of alcoholism).

Aim 1: Measures of Craving for Alcohol.
- **Alcohol Urge Questionnaire (AUQ).** An 8-item scale where subjects rate their craving for alcohol at the present moment. Participants are asked to use a 7-point likert scale expressing agreement with statements such as "I crave a drink right now".
- **Urge Form.** A 4-item scale measuring craving for alcohol at the present moment. Participants are asked to rate their urge to drink alcohol in a 10-point continuum. Questions include items such as “How strong is your urge to drink alcohol right now?”

Aim 2: Personality and Cognitive Measures Related to DRD4 and Alcohol Problems.
- **Impulsivity and Sensation Seeking Scale (IMPSS).** 19 items measuring impulsivity and sensation seeking, personality factors that may predict responses to alcohol and drugs (Zuckerman, 1996).
- **Sensation Seeking Scale (SSS-V).** Consists of several subscales (Thrill Seeking, Experience Seeking, Disinhibition, and Boredom Susceptibility) which measure traits that appear to be related to greater alcohol and drug-induced levels of stimulation. This scale has been used in numerous addictions studies and has good reliability and validity (Zuckerman, et al, 1978).
- **Tri-dimensional Personality Questionnaire (TPQ-NS).** 35-item Novelty Seeking Subscale has good reported reliability and validity (Cloninger, 1987). This scale has been demonstrated to successfully predict a range of substance use measures and disorders.
- **Stop-signal test.** A 10-minute computerized measure of inhibitory control in which participants are asked to act in response to an auditory stop signal (e.g., Rieger and Gauggel, 1999).
- **Door-opening task.** A 10-minute computerized measure designed to assess sensitivity to changing reward and punishment contingencies. This measure has been shown to predict substance use problems (Rodriguez-Fornells, Lorenzo-Seva, & Andres-Pueyo, 2002).

Aim 3: Measures of Level of Response to Alcohol.
- **Subjective High Assessment Scale (SHAS).** A 13-item scale where subjects rate their perceptions of change in the overall feelings of intoxication (Shuckit et al., 1997).
- **Biphasic Alcohol Effects Scale (BAES).** The BAES will be used to collect information on changes in self-reported stimulation after alcohol administration. The BAES has demonstrated high reliability with alphas between .82 and .94 (Earleywine & Erblich, 1995; Martin et al., 1993). Across multiple consecutive daily sessions, the BAES has a reported alpha coefficient of .80 (Earleywine & Erblich, 1995).
- **Profile of Mood States (POMS).** A 40-item questionnaire used to assess changes in mood during the baseline period and at different levels of intoxication during the experimental procedure. The POMS is a reliable and valid measure of affect (Johanson & Uhlenhuth, 1980; McNair, Lorr, & Droppleman, 1971).
J. Data Analysis Plan.

**Missing data.** We do not expect much missing data since the required commitment for the study is low. In the event that a subject fails to complete the experimental session, we will recruit additional subjects to achieve the desired sample size. Moreover, if there are missing data for subjects who complete the study, we will estimate and input those values using appropriate statistical procedures.

**Pretest equivalence.** To confirm the efficacy of random assignment and in order to check for group equivalence across demographics, drinking history, and all other baseline measures we will perform t-tests on continuous items and \( \chi^2 \) tests of categorical items. Any variables on which the two groups are unequal at pretest will be covaried in all further analyses.

**Population Stratification.** Population stratification effects may increase the chances of making a type I error in a genetic case-control design like ours. To test the possible influence of population stratification in our results we will assay 20 additional highly polymorphic markers to generate an estimate of population stratification that will then be used to conservatively adjust the statistical test for association between the candidate marker and the outcome variable. If significant population stratification is detected, further analyses of the DRD4 VNTR and GABA alpha 6 will be adjusted following published procedures (Devlin & Roeder, 1999; Pritchard & Rosenberg, 1999). However, we do not expect significant population stratification since stratification has not been found in previous studies in our laboratory with similar samples and because other studies using heterogeneous samples drawn from populations in the U.S. have failed to detect significant stratification (Egan et al., 2001; Silverman et al., 2000). Lastly, population stratification normally has a very small influence unless investigations include cases and controls from nearly distinct strata, which is not the case in the proposed investigation.

**Hypothesis 1: DRD4 and Craving for Alcohol.** The effects of the DRD4 gene polymorphism on craving for alcohol will be tested using a 2 (DRD4: S vs. L) by 3 (BAC: .02, .04, .06) repeated measures ANCOVA with the average urge score at each level of intoxication as the dependent variable and baseline measures of craving as a covariate.

**Hypothesis 2: DRD4 and Personality/Cognition.** To examine moderation of craving for alcohol by candidate gene and personality variables, a series of ANCOVAs will be performed wherein the main effect of personality factors and the interaction between personality constructs and the DRD4 candidate gene will be included as additional predictors of craving response during the intravenous exposure to alcohol. Possible mediation will be tested by comparing a model that includes the mediating variable against a model that does not.

**Hypothesis 3: GABA alpha 6 and Level of Response to Alcohol.** The effects of the GABA alpha 6 gene polymorphism on level of response to alcohol will be tested using a 2 (GABA alpha 6: Pro/Pro vs. Pro/Ser) by 3 (BAC: .02, .04, .06) repeated measures ANCOVA with the average response (SHAS) score at each level of intoxication as the dependent variable and baseline measures of response as a covariate. We will treat level of response as a continuous rather than categorical predictor in order to increase statistical power and to account for individuals who show a moderate response to the effects of alcohol.

H. Project Timeline. The proposed timetable for all data collection, analysis, and manuscript preparation is 2.5 years. We expect to recruit 120-150 subjects during the screening phase and to have about 80 eligible participants. To accomplish this goal, we plan to recruit approximately 5 subjects per week during the screening phase and to run approximately 2 subjects per week during the experimental phase. Our recruitment plan is consistent with subject flow in previous studies in our lab.

<table>
<thead>
<tr>
<th>Month 1-3</th>
<th>Month 4-18</th>
<th>Month 19-23</th>
<th>Month 24-40</th>
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</thead>
<tbody>
<tr>
<td>1. Prepare testing materials</td>
<td>1. Recruitment</td>
<td>1. Recruitment finished</td>
<td>1. Final Analysis</td>
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<tr>
<td>2. Pilot experimental protocol</td>
<td>2. Screening phase (data collection &amp; DNA testing)</td>
<td>2. Preliminary Analysis</td>
<td>2. Data Write-up and submission for publication</td>
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<td>4. Experimental Sessions</td>
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