Protocol 2003-1024

Pharmacogenetics Emotional Reactivity and Smoking

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A. Specific Aims

The specific aims of this proposal are to evaluate the differences in emotional reactivity (peak startle response to affective stimuli) during a cessation attempt among smokers treated with bupropion, nortriptyline or placebo and to determine if these differences are moderated by genotype. We will also assess the relationship between emotional reactivity and days to relapse, following the quit date.

This proposal will evaluate the following hypotheses:

1. The emotional reactivity of smokers during quitting will be significantly higher among those that receive placebo vs. either bupropion or nortriptyline therapy for smoking cessation. Emotional reactivity will be assessed by measuring the peak eye blink (EMG) response to an acoustic startle probe delivered during the presentation of emotionally valent stimuli (positive, negative, neutral, smoking related pictures).
2. The emotional reactivity of smokers during cessation will be moderated by genotype. Our initial hypothesis will focus on the DRD2:
   2.1. We hypothesize that emotional reactivity will be lower for those carrying the DRD2 A1 allele and using bupropion vs. A1 smokers using either nortriptyline or placebo. A1 nortriptyline users are expected to have lower reactivity than A1 placebo users[PCP1]. Homozygous A2s are expected to respond similarly to both drugs with higher levels of emotional reactivity being observed for placebo vs. either bupropion or nortriptyline.
3. Higher levels of emotional reactivity of smokers at baseline and during cessation will be inversely related to abstinence at the 3 and 6-month follow-up periods.

Although our initial genetic hypotheses are focused on the DRD2, we will also use emotional reactivity assessments to characterize the phenotypes of several other biomarkers that have potential importance for neuroregulatory function and response to pharmacotherapy for smoking cessation. These include the DRD4, SERT (serotonin transporter), DAT (dopamine transporter) and NET (norepinephrine transporter). Several exploratory hypotheses are proposed, for example:

1. We hypothesize that those carrying the long form of the DRD4 (6 repeat or longer) will experience less reactivity during either drug therapy than those homozygous for the short form.
2. We hypothesize that smokers who are heterozygous (S/L) or homozygous (L/L) for the long form of the SERT gene are likely to experience less emotional reactivity in response to nortriptyline therapy vs. either bupropion or placebo.
3. Polymorphisms of the NET gene have not been extensively characterized in humans, and its relationship to antidepressant therapy has not yet been explored. We will evaluate the relationship of one of the currently known NET polymorphisms (1287G/A) and emotional reactivity during pharmacotherapy. As a working hypothesis we expect that that smokers carrying the GG allele of the 1287G/A variant will show reduced emotional reactivity during nortriptyline therapy vs. bupropion or placebo, in comparison to their GA or AA counterparts, who are not expected to differ in response to the antidepressants.

Overview

It is estimated that cigarette smoking is responsible for over 40% of premature deaths and disability in the US. The adverse health risks of smoking increase significantly with duration and amount smoked per day, and it is precisely these heavier and more nicotine dependent smokers that are most refractory to treatment [1]. Although significant advances have occurred in smoking cessation therapy, approximately 26% of men and 22% of women continue to smoke. Smoking has been characterized as a chronic relapsing disorder. Over 40% of smokers make a serious cessation attempt each year but less than 3% of all smokers successfully quit[2]. One of the most fundamental aspects of nicotine dependence involves its neuroregulatory function on mood. The experience of negative affect, in particular, is a significant contributor to the risk of relapse and constitutes a major factor of the nicotine withdrawal syndrome. Recent advances in smoking cessation have focused on

the use of antidepressants for the treatment of nicotine dependence (i.e., bupropion and nortriptyline). While the efficacy of these treatments has been established in previous studies, we know little about how they work. The impact of these and other pharmacological treatments on the public health may be significantly improved by gaining a better understanding of the underlying psychobiological and genetic mechanisms associated with the modulation of mood during cessation (nicotine withdrawal), and the interaction between these mechanisms and treatment. This research is focused on understanding genetic and psychobiological differences in response to two pharmacotherapies for smoking cessation that target the neurotransmitters, dopamine, norepinephrine, and serotonin with differing degrees of specificity.

The specific aims of this project are to assess the effects of bupropion and nortriptyline vs. a placebo control, on changes in emotional reactivity during smoking cessation, and to determine if these effects are moderated by genotype. We will also determine whether emotional reactivity predicts abstinence and time to relapse. We will use a standardized laboratory assessment procedure, known as the acoustic startle probe, to evaluate the differences in emotional reactivity. The startle reflex (eye blink) is an orienting response that follows an unexpected auditory stimulus (startle probe). It is thought to reflect immediate changes in cortical and subcortical activity related to motivation and emotion, such as approach or defensive behavior. Negative emotional cues, such as slides of upsetting events delivered prior to the probe, activate defensive neuroregulatory pathways and increase blink response magnitude (eye muscle EMG), while positive cues inhibit the response, activating appetitive or approach pathways. Unlike retrospective self-report measures of affect, startle occurs in real time proximity to the emotional cue, is not subject to recall bias, and provides information about momentary and subtle changes in motivational predisposition and affect. This startle-affect relationship provides an ideal paradigm for studying genetic differences between smokers and the disruptive effects of smoking cessation on emotional reactivity and mood.

Our initial genetic analysis will focus on the A1/A2 polymorphism of the DRD2 receptor gene. Data from our laboratory suggest that smokers carrying the A1 allele vs. those with only the A2 are both less likely to quit smoking and experience less consistent reduction in negative affect when given venlafaxine, (a modest serotonin/low norepinephrine/very low dopamine/ reuptake inhibitor [3]). Our preliminary studies also suggests that reactivity is substantially elevated for A1 vs. A2 smokers prior to quitting and between A1 smokers attempting to quit vs. A1 controls, or A2 trying to quit and their controls. Moreover, among A1 smokers destined to relapse, emotional reactivity to negative cues is substantially higher at baseline, and sharply increases in the first few days of withdrawal. A1 smokers who never quit appear to have significantly lower levels of reactivity at baseline to positive and smoking related pictures. These results indicate that this genotype may be associated with differences in emotional processing before smokers attempt to quit and during the cessation attempt.

In this study, 354 smokers will be randomly assigned to receive bupropion, nortriptyline, or placebo. All three conditions will include a behavioral counseling component, consistent with previous research examining the efficacy of these pharmacotherapies. After a baseline startle assessment, all participants will complete startle assessments at 2,5 and 28 days post-quit. Each assessment will involve a series of startle probe trials consisting of the presentation of an acoustic stimulus (startle probe), preceded by positive, negative or neutral emotional cues.

With the research proposed in this application, we hope to understand more fully how pharmacotherapies for smoking cessation affect emotional reactivity during cessation and what role genetics may play in conferring an advantage to one treatment vs. another. For example, functional differences in dopaminergic, serotonergic or noradrenergic responsivity, which are at least partly genetically controlled, could influence the balance between reduction in positive mood or exaggeration of negative affect during a quit attempt. Relapse in smokers attempting to quit is often attributed to the level of negative affect, and an examination of the startle reflex during quitting may provide important information on emotional reactivity and predisposition to relapse. We propose using startle based assessments of emotional reactivity to characterize the endophenotype of smokers carrying candidate genes, and subsequently assess their response to drugs that have different degrees of specificity for neuromodulators thought to be associated with the candidate gene. Ultimately this knowledge may contribute to the development of more effective interventions, especially in the area of pharmacogenetics. [PCP2][PMC3]Targeting specific groups of individuals with specific treatments may improve effectiveness of pharmacologically based treatments.

B. Background and Significance

Neurotransmitters Genes and Smoking

Dopamine (DA) and the Dopamine Transporter (DAT)

Dopaminergic neurotransmission in the mesolimbic system, and particularly in the nucleus accumbens, is a recognized and critical target of many drugs of abuse, including alcohol, cocaine, and stimulants [4-6]. Dopamine neurotransmission is involved in the experience of pleasure/reward [4] and is the principal neurotransmitter involved in the initial assignment of incentive salience to environmental stimuli, which later governs motivated action (i.e., movement towards or away from the stimuli)[7]. Recent animal studies have implicated dopaminergic transmission in the reinforcing effects of nicotine on behavior [8, 9]. Nicotine can stimulate dopamine [10, 11] and inhibit its re-uptake [12, 13] , and the activation and desensitization of midbrain neurons by nicotine may play an important role in the development of tolerance [14]. Inherited variants of genes coding for proteins involved in dopaminergic transmission could contribute to genetic differences in drug use, including smoking, by regulating dopamine synthesis, metabolism and expression of the receptors. The most widely studied of these variants has been the DRD2 receptor gene.

The human D_2 dopamine receptor (DRD2) gene is located on chromosome 11q and several polymorphisms have been described. These include the uncommon *Taq*IA restriction fragment length polymorphism (RFLP) located on the 3' flanking region of the DRD2 gene, and the 5' *Taq*IB. Studies suggest that these alleles are located on a part of the chromosome believed to have regulatory influence on the dopamine receptor [15]. For this review, references to the A1 genotype correspond to individuals having either the A1/A1 or the A1/A2 genotype; and the A2 refers to the A2/A2 homozygote. The A1 allele has been associated with a decrease in D_2 receptor density [16, 17] and the D_2 receptor has been implicated as a prime target of psychotropic drugs [18]. Studies with chronic cocaine users, for example, show that significant reductions in D_2 receptor availability persist after cocaine withdrawal and are associated with dysphoric mood [19, 20]. D_2 receptor occupancy has also been associated with the intensity of the "high" produced by psychostimulants [21]. Moreover, stimulation of the D_2 receptors with bromocriptine (a dopamine agonist) decreases smoking, whereas blocking the D_2 receptors with haloperidol (a dopamine antagonist) increases smoking behavior [22, 23].

Significant differences have been noted in a general population sample for the frequency of A1 allele between current (OR =2.15) and former smokers (OR =1.17) in comparison to non-smokers[24]. Among smokers trying to quit, the frequency of A1 allele is significantly higher than non-smoker controls (48.7% vs. 25.9%, p<. 00001), inversely related to duration of previous quitting and age of initiation; and positively related to cigarettes smoked per day [25]. We have noted similar findings for the B1 allele in a study of lung cancer patients and non-patient controls, with the B1 being more common among the non-patient smokers. Both [pmc4]A1s & B1s also initiated smoking at an earlier age and made fewer attempts to quit [26]. However, in a small sample family linkage study [27] and in a separate study of German heavy smokers [28] no significant association between smoking and the DRD2 was noted. More recently, in an unpublished report Lerman[29] found weak main effects for the DRD2 (p =.1) on1 year abstinence rates but no interaction between genotype and treatment (bupropion or placebo). This study is yet incomplete and further analysis is required before conclusions can be reached. However, the general approach to studying gene drug interactions in smoking cessation is relevant to the current proposal and is discussed in more detail below (see discussion of endophenotypes).

Lerman and her colleagues [30] investigated the interaction of the dopamine transporter gene (DAT), SCL6A3-9 and the DRD2 A1 on smoking behavior. The SLC6A3 gene regulates synaptic dopamine by coding for a re-uptake protein called DAT [31]. It has a 3' variable number tandem repeat (VNTR; 40 bp) polymorphism [32]. Smokers with the SLCA63-9 allele started smoking later and quit for a longer time than those without it. These effects were not modified by DRD2 status and the SLCA63-9 was not associated with Fagerstrom dependence scores. However, the SLC6A3-9 repeat was more frequent among Caucasian nonsmokers (62%) who also had the A2 allele but not among those who had the A1 allele (46%). Similar but non-significant trends were noted for African American smokers. It appears that the "protective" effect of the SLC63A-9 for smoking (greater frequency among nonsmokers vs. smokers) is eliminated by the presence of the A1 allele. Similar findings have been reported by Sabol et al. [33], who found the odds of smoking cessation (i.e., being a former

vs. current smoker) was higher for those having the SLC63A-9 allele (51.9% vs. 42%). Smoking rates were also higher for participants carrying the A1 allele, and more people quit who had SLC6A3-9 and did not have the DRD2 A1 allele, although these effects were not statistically significant. However, the relationship between smoking and the DAT was not replicated in a large Australian study using a non-clinical sample [34] or in a US community based sample[35].

Studies have also associated the DRD4 receptor (a member of the D2 family of receptors) with smoking. The data suggest that the long form of the gene is associated with a blunted response to dopamine [36] and higher scores on the Tridimensional Personality Questionnaire [37] "Novelty-Seeking" subscale [38, 39], among smokers [33]. The long form of the gene (S/L or L/L) is comprised of 6-8 repeats, of which 7 is most common while the short form (S/S) consists of 2-5 repeats with 4 being the most frequent. A study by Shields, et al., [40] found that the long allele was more frequent among African American vs. Caucasian smokers. African Americans with this allele were more likely to be smokers, are less likely to quit, started smoking at an earlier age, and smoked more often within 30 min of waking than those with the short form.

Like the DRD2 A1, the DR4 L allele may confer some impairment in dopaminergic responsivity. Data from at least one study suggests this could increase craving when such smokers are confronted with smoking related cues [41]. Smokers in this study with the DRD4 long allele also decreased positive affect during exposure to smoking cues, in comparison to those with the short allele. The presence of smoking cues in the environment has been positively related to relapse [42]. This study is note worthy because like those described in our preliminary studies, it is among the first that attempts to define the phenotype of the candidate gene in terms that have relevance to the treatment of nicotine dependence (i.e., affect, response to smoking cues).

Serotonin (5-HT) and the Serotonin Transporter Gene (SERT)

Serotonin is involved in the regulation of many brain functions, including sleep, cognition, sensory perception, motor activity, temperature regulation, nociception, mood, appetite, sexual behavior, and hormone secretion. There appears to be an important interplay that is not completely understood, between the effects of nicotine and nicotine withdrawal on serotonergic mechanisms, on the one hand, and serotonin's role in modulating affect and dopaminergic reward systems on the other. Nicotine may stimulate serotonin (5-HT) release at high doses [43, 44] and its withdrawal may decrease 5-HT in limbic and forebrain structures, possibly by increasing the inhibitory influence of 5 HT1A autoreceptors within the raphe nuclei [45]. These structures are important in the regulation of emotion and are themselves activated by nicotine administration in humans [46]. Increased serotonin has also been associated with increased striatal dopamine concentrations [47]. Loss of serotonergic stimulation could lead to an increase in emotional reactivity during nicotine withdrawal. In fact, drugs that increase serotonin by inhibiting the action of 5-HT1A receptors, block the increased startle response in animals undergoing nicotine withdrawal [48]. Inhibiting the reuptake of serotonin also rapidly reverses the elevation in brain-stimulation reward thresholds observed in rats undergoing nicotine withdrawal [49]. Balfour [50] has suggested, however, that nicotine may ultimately decrease 5-HT in the hippocampus (possibly by increasing 5-HT1A receptors), an action that could be interpreted as depressogenic. Thus, continued smoking may be necessary to offset the negative affect associated with reduced serotonergic activity. Interestingly, Cheeta [51] has asserted that reducing serotonergic activity during nicotine withdrawal may also reverse the increase in "anxiety" observed in animals during a social interaction test. The implication for human studies is unclear. However, perhaps context (i.e., anxiety or fear inducing) can modulate the relationship between nicotine and serotonin. Acute administration studies suggest that serotonergic activity may have an inhibitory influence on the reinforcing properties of nicotine, either by reducing dopaminergic activity [52-55] or blocking its rewarding effects [56]. Reductions in VTA firing rate [57, 58] and reduced self-administration of a variety of drugs of abuse [59-61] have been shown following administration of selective serotonin reuptake inhibitors. This would suggest that drugs that inhibit the reuptake of serotonin could modulate a smokers affect during cessation (their antidepressant effect) and perhaps reduce the reinforcing properties of smoking should they relapse. However, the selective serotonin reuptake inhibitors have not generally been effective for smoking cessation (see preliminary studies and [62-64]) although there is some evidence to suggest that they may reduce craving and improve cessation rates among those who have some depressive symptoms [65].

Serotonin transporters (SERTs) are principally responsible for the deactivation of serotonin and are high affinity targets for antidepressant medications [66]. Altered SERT expression has long been suspected to

contribute to anxiety and affective disorders [67] and serotonin reuptake inhibitors have been effectively used in their treatment. The connection between smoking, depression and negative affect is extensive and discussed in a later section of this review. Suffice it to say however, that the modulatory role of serotonin on mood, particularly negative affect, makes the serotonin transporter gene a plausible candidate for influencing emotional reactivity due to nicotine and nicotine withdrawal. A single gene, 5-HTT (SERT), encodes SERTs in the CNS. Its gene product modulates the magnitude and duration of serotonergic signaling by controlling the uptake of serotonin from the synaptic junction [68, 69]. The 5-HTT is located on chromosome 17q12 and contains two polymorphic sites: a 5'-flanking promoter region (5-HTT gene-linked polymorphic region or 5-HTTLPR) and a variable nucleotide tandem repeat (VNTR) of 17bp repeats in intron two. The 5-HTTLPR contains two allelic variants: either a long variant (L allele) due to a 44-bp insertion or a short variant (S allele) due to a deletion in the promoter region. These variants are believed to regulate HTT gene transcription. The S allele is believed to be a less efficient promoter than the L allele. The S allele reduces the transcriptional efficiency of the HTT promoter, therefore decreasing serotonin transporter expression and serotonin uptake [70].

Current studies have assessed the relationship between smoking behavior and S allele or S/S genotype and the data has yielded conflicting results. In a Japanese population, males with the S/S genotype were less likely to be smokers and more likely to stop than those carrying the long form of the allele (S/L & L/L combined)[71]. However, no association between the 3 different variants (S/S, S/L, L/L) and smoking behavior (e.g., quitting history, smoking rate, age on initiation) was found among either Caucasian or African American smokers [72]. However, in a mixed racial sample, the personality trait neuroticism was positively correlated with current smoking status and measures of nicotine dependence (FTND) among smokers carrying the S allele (S/S and S/L combined) but not among L/L homozygotes [73, 74]. We found the S/S genotype in bladder cancer patients was less prevalent in current smokers (12.3%) than in never smokers (37.5%) and former smokers (39.6%), and was associated with lower FTQ scores, fewer years of smoking and more quitting activity, compared to the S/L and L/L genotypes [75].

Norepinephrine (NA) and Norepinephrine Transporter (NET)

The locus coeruleus (LC) is the major noradrenergic nucleus in the brain and its neurons are thought to regulate attention, vigilance, sympathetic nervous system activity, and to be implicated in the actions of stress, opiates and antidepressants[76]. Nicotine increases the firing rate and stimulates release of NA from the LC [77]. A recent study suggested that the effects of long term smoking on the LC and on enzymes involved in the production of NA (tyrosine hydroxylase) are similar to the morphological changes seen in the brains of individuals who suffered from major depression [78] (i.e. down regulation of α_2-adrenoceptors). Noradrenergic projections to the hypothalamus and limbic system (e.g., amygdala and hippocampus) have received considerable attention in the study of depressive disorders[79]. The original hypothesis concerning the role of NA in the regulation of mood implicated hypofunction of the noradrenergic system in development of depression. Indeed the principal action of the tricyclic antidepressants (including nortriptyline) is inhibition of NA reuptake by high affinity targeting of the norepinephrine transporters[66] thereby substantially increasing extracellular levels of norepinephrine. However, the therapeutic effect of these drugs is thought to result not from enhanced NA transmission but from some gradually developing neuroadaptations to enhanced NA [79]. While conflicting studies exist, a recent review[80] concludes that these drugs may achieve their therapeutic effect at least in some individuals by attenuating NA transmission, possibly by decreasing sensitivity and down regulating beta-adrenoreceptor in the presence of the higher levels of NA.

Just as there may be subtypes of depression that may differentially respond to different drugs, there may be important differences in how pharmacological treatments for nicotine dependence affect emotional reactivity during nicotine withdrawal. It is plausible that these differences could be related to genes that regulate NA activity, chiefly through controlling norepinephrine reuptake. The human NET (SLC6A2) gene is located on the long arm of chromosome 16 (16q12.2) and its coding regions consist of 14 exons spanning 45 kilobases (kb). The NET shares a similar general structure with the DAT and the SERT, with an overall identity in amino acid sequences of 66% and 48% with the DAT and SERT, respectively [81]. Thirteen DNA sequence polymorphisms have been described in the coding region of the NET gene [82], including five intron variants, three silent mutations and five variants predicting amino acid substitutions in the transporter protein (missense substitutions) [83].

The most compelling evidence for the involvement of the NET in drug dependence comes from a series of elegant animal studies by Xu and colleagues [84]. Using mice that completely lacked the NET gene (NET-/-) they showed that these animals had enhanced extracellular levels of NA levels and performed better (vigor in forced swim test) than untreated wild types mice in behavioral tests of "despair," and the same as wild types treated with antidepressants. Importantly, the increased NA appeared associated with enhanced sensitization and reward salience to cocaine and increased sensitivity of D2/D3 receptors. However, it also down regulated dopamine receptors, suggesting it plays a role in dopamine homeostasis. While there have been no studies with nicotine and the NET, many animal studies have shown similarities between nicotine and psychostimulants suggesting that increased NA could play a role in nicotine reinforcement, perhaps by increasing its reward value and exacerbating the effects of nicotine withdrawal.

There have been no association studies relating variations in the NET to smoking behavior. Little population data exists regarding the frequency of these polymorphisms although in one study at least 7 of the 13 appear to have rare alleles [81] which would make them unlikely candidates for any relationship with smoking behavior. Recently, the G1287A/G NET polymorphism has received some attention in the study of major depression although findings to date have been negative [82, 85]. However, the GG allele vs. the AA or AG alleles of this gene has been associated with enhanced norepinephrine turnover suggesting reduced transport and higher levels of NA for these individuals. The G1287A/G polymorphism is located in a non-coding region of the gene and while it may not directly influence NET activity, like other similar genes it may be in linkage disequilibrium (close to) with a functional polymorphism that does [86]. It is, however, noteworthy that this is the first study to demonstrate a link between a NET polymorphism and NA. We have selected the G1287A/G polymorphism for exploratory analysis of the relationship of the NET to emotional reactivity, hypothesizing that the GG smokers may be more likely to show reduced emotional reactivity during nortriptyline therapy vs. bupropion or placebo, than their GA or AA counterparts. Although we have chosen the G1287A/G polymorphism for our initial hypothesis we will assay the remaining polymorphisms of the NET for additional exploratory analysis based on having sufficient frequency and power to detect differences if they exist. The cost for the NET assay will add only a fraction (less than $10/person) to our genotyping budget and may provide clues regarding the relationship of the NET to emotional reactivity.

Focusing on Endophenotypes

In conclusion, the majority of evidence linking smoking behavior to specific genetic polymorphisms has relied on gene association studies comparing smokers to former smokers or non-smokers. Not surprisingly, these studies have yielded mixed results because they rely on making gross distinctions in a behavior (smoking or not), as opposed to studying more specific aspects of the target behavior (i.e., craving negative mood). This is not a problem unique to smoking. Other areas of investigation relating polymorphisms to complex behaviors, such as major depression, bipolar and other affective disorders have also provided conflicting results when the phenotype is cast in all or nothing terms (see Blakely for review [66]). Yet it would be hard to fathom that genes that influence neurotransmitter function, particularly dopamine, have no bearing on motivation, reward, the experience of pleasure or the modulation of mood associated with nicotine dependence, given the complex interactions between nicotine and these biogenic amines. Part of the problem may be that we are looking in the wrong place. Rather than focus on the presence or absence of a globally defined characteristic (e.g., smoking or not) we might do better by focusing on specific behavioral phenotypes, or endophenotypes, that can be tested for their relationship to genetic factors. Modulation of the startle response by affective cues is one such endophenotype. It provides a continuous measure of emotional reactivity, associated with drive and motivation (e.g. appetitive-approach, escape-reward) and is much more likely to detect genetic differences among smokers.

The negative findings for association between the DRD2 and DAT following bupropion treatment in the recent unpublished report by Lerman [29] deserve further comment in this regard because of their potential relevance to the current proposal. This study, like all of the others in this area focused, on relapse. Although relapse is considered the ultimate outcome measure for evaluating smoking cessation treatments, like the smoker/nonsmoker distinction discussed above, it is a relatively gross measure (e.g. dichotomous outcome) that is insensitive to the subtle processes and mechanisms that ultimately lead to treatment failure (i.e., negative affect). Relapse is typically measured at time points quite distal to these events. Thus, it will fail to

discriminate smokers on important characteristics revealed early in the cessation process, or even before quitting is attempted. For example, while treatment outcome studies have suggested a limited role for the DRD4 [40] in smoking cessation, the study by Hutchinson [87] clearly shows its relevance to craving in response to smoking cues, a factor that could ultimately affect a smoker's ability to sustain abstinence. A similar argument may be made for the DRD2. For example, while no differences in long term relapse to bupropion treatment were found for the DRD2 in the Lerman report [29], important differences have been observed in the early phase of treatment in terms of both cessation and negative affect reduction [88]. Moreover, in another report, Lerman and colleagues [89] have shown that a reduction in negative affect between the pre and 1 week post quit periods mediates the beneficial effects of bupropion on 8-week post-treatment abstinence rates, in the same sample used in the unpublished report mentioned above. However, an analysis of the negative affect data for a genotype by drug interactions has not yet been reported. Our studies (see preliminary studies) suggest that the impact of the DRD2 (and likely other candidate genes in the DA pathway) on smoking cessation is most pronounced early in the cessation process (i.e., the first few weeks). This is a time when negative affect may have its maximal effect of treatment success [90]. We have also shown that pharmacological treatments (venlafaxine) differentially attenuate negative affect by genotype (DRD2), and this too happens early in the cessation process. Moreover, we have also shown in our preliminary studies that the startle response provides a highly sensitive measure of emotional reactivity before and during the first week of smoking cessation that is directly associated with genetic factors (DRD2). These associations have direct relevance for understanding the genetic contribution to negative affect during cessation, a factor that has major implications for quitting success. Therefore, we would argue that a focus on relapse alone is unlikely to shed much light on the relationship between genetics and mood during cessation, nor might it tell us much about whether certain pharmacological treatments (i.e. bupropion or nortriptyline) differentially modify mood during the early stage of quitting. Refining our measure of the endophenotype associated with candidate genes by using startle assessments of emotional reactivity is likely to be a more fruitful approach.

Rationale for Studying Smoking and Mood

Negative affect is related to smoking and poor treatment outcome among smokers trying to quit. Research on the relationship between negative affect and smoking cessation has included evaluation of the effects of a past history of diagnosable major depression, which may serve as a marker for vulnerability to future depressed mood, and evaluation of the effects of pre and post-cessation negative affect (self reported ratings of dysphoria, depression, tension, etc). A positive history of major depression [91] has been associated with an increased prevalence of smoking [92-95], nicotine dependence [92], greater nicotine withdrawal severity, and both increased depressive symptoms during nicotine withdrawal [96-98] and risk of a subsequent major depressive episode [99]. History of major depression has also been associated with a reduced likelihood of quitting, [93, 100-103] particularly among those whose symptoms rise after quitting [104], although the connection between relapse and depression history has not been uniform[105-108]. Several studies have also suggested that precessation negative affect may predict relapse [108-111] although these effects may diminish when accounting for past depression [90]. [PC5]Negative affect following a quit attempt has been related to treatment failure and relapse across studies using a variety of treatment modalities (e.g., behavioral, nicotine replacement therapy (NRT), bupropion) [90, 112]. Indeed, the presence of negative affect following cessation has been found to characterize over 50% of all smoking lapses, with 19% of all lapses occurring under conditions of extreme negative mood [42]. Postcessation negative affect profoundly influences both the trajectory and duration of nicotine withdrawal [113] and the expectation that nicotine will produce desirable emotional consequences, particularly negative affect reduction, is an important predictor of cessation relapse and withdrawal severity [114]. The subsequent loss of such means to manipulate mood when one abstains from smoking, increases negative affect and raises the likelihood of relapse.

An improved understanding of the effects of smoking cessation on mood appears to be a critical step toward the development of new behavioral and pharmacological treatments for nicotine dependence. Previous investigations of smoking and mood have relied heavily on patient self-report, such as ratings of stress during regular smoking [115], or studies of the mood correlates of smokers trying to quit (e.g. [42, 116]). These studies have yielded important findings regarding the relationship between normal smoking behavior, relapse and negative mood. However, traditional means of self-report require extensive cognitive processing and may not be

sensitive to some of the subtle yet important changes in affect during cessation. Moreover the assessment of mood by retrospective self-report in clinical studies is far less sensitive to the changes in affect associated with smoking behavior than information collected while smoking is taking place or during extended periods of abstinence (ecological momentary assessment, [42, 117]). The startle response, on the other hand, is immediate and sensitive to ambient emotional cues, and could prove to be a reliable indicator of the effects of nicotine withdrawal on a smoker's affect.

Relationship of the Startle Response to Affect

The startle (eye blink) response is an orienting reflex, which occurs during the presentation of unexpected auditory stimulus (probe). Its strength is measured by the electromyographic (EMG) changes in the orbicularis oculi region of the eye. It is well established that the magnitude of the blink (EMG) response to the startling acoustic probe varies according to valence of emotional cues presented during the startle assessment. For example, blinks are enhanced when subjects view unpleasant rather than pleasant pictures (e.g., [118, 119]) and are reduced during viewing of positive as opposed to negative or neutral emotional cues [120]. Moreover, the startle response to both positive and negative affect cues is maintained after accounting for any habituation-related decrement in blink magnitude [121]. The robustness of this affect-startle effect has been demonstrated across a variety of populations including college students, incarcerated prisoners [122], anxious and phobic patients [123, 124], and 8-month-old infants [125]. An exaggerated startle response to negative emotional cues has been associated with induction of negative moods using verbal scripts among normals [126], early phases of post-traumatic stress disorder [127], exposure to fear relevant stimuli among phobic patients [124] and those with social anxiety [128].

This interplay between the strength of the startle response and foreground emotional cues is thought to reflect the influence of affective perception on the activation of underlying mechanisms governing motivation [129, 130]. Negative affect normally activates the defensive system and motivates avoidance or escape. Positive affect stimulates the appetitive system and motivates approach and exploratory behavior. Thus, an independently evoked defensive reflex, such as the startle response, provides a measure of emotional reactivity of the organism to its environment. Reactivity is augmented when the organism perceives an unpleasant stimulus since the aversive/defensive motivational system is engaged. However, this same reflex will be reduced in amplitude when the organism is processing positive emotional cues, which activates the appetitive or approach motivational system [129, 130].

These dynamic characteristics of the startle response have important implications for the study of drug motivation and nicotine dependence, since the engagement of the aversive emotional system would appear to be an obvious consequence of negative affect experienced during nicotine withdrawal. Improving our understanding of factors that modulate this response, including pharmacological and genetic ones, can have important implications for the treatment of nicotine dependence. For example, we may show that bupropion or nortriptyline has differential effects on startle magnitude in the presence of negative emotional cues, and these effects are modulated by genotype. If successful, future studies could use this approach to determine which candidate drugs or behavioral procedures provide an optimum level of affect modulation during nicotine withdrawal, and for which participants. Intervention procedures could be easily compared in the laboratory before launching larger field investigations to test their efficacy and a detailed profile of the differential effectiveness of certain treatments could be ascertained However, before any of these potential treatment-matching scenarios can be realized, basic laboratory work as proposed in the current study must be carried out.

Antidepressants used for Smoking Cessation

Bupropion Bupropion (Amfebutamone) is an antidepressant that is chemically dissimilar to tricyclic, tetracyclic, selective serotonin re-uptake inhibitors (SSRIs), or other antidepressants. The sustained-release (SR) form of bupropion was approved as non-nicotine based pharmacotherapy for smoking cessation in 1997. Bupropion's mechanism of action is thought to be mediated through both dopaminergic (DA) and noradrenergic (NA) systems. Findings from in vitro experiments suggest that bupropion is a modest inhibitor of DA uptake and weak inhibitor of NA uptake [131]. In vitro, bupropion's potency score for NA and DA reuptake inhibition (lower scores=more potency) is 1400 IC_{50} and 570 IC_{50}, in comparison to 3.4 IC_{50} and 3500 IC_{50} for

nortriptyline, respectively [132]. Nortriptyline is one of the most potent NA reuptake inhibitors. Bupropion produces a dose-dependent increase in dopamine concentrations in the nucleus accumbens in the mesolimbic system of rats [133]. Terry and Katz [134] also found that rats trained to bar press in response to bupropion injection would exhibit the same behavior when they were injected with other dopamine reuptake blockers. In all, 9 of 10 tested dopamine uptake inhibitors fully substituted for bupropion. None of the serotonin or norepinephrine uptake inhibitors tested achieved either full or partial substitution. Thus, it has been suggested that bupropion's mechanism of action in smoking cessation is mediated at least in part, by the stimulatory effects it has on the mesolimbic dopamine system [135]. Other researchers suggested that bupropion may also act as a nicotine antagonist , which might reduce the reinforcing properties of nicotine. For instance, Slemmer and her colleagues[136] reported that bupropion selectively blocks nicotine's antinociceptive, motor, hypothermic, and convulsive effects in rats. It also blocks the activation of a number of nicotinic acetylcholine receptors (nAChRs) that include [BS6]$\alpha_3\beta_2$, $\alpha_4\beta_2$, and α_7.

Interestingly, studies examining bupropion's antidepressant effects suggest it is more effective in suppressing the firing rate of norepinephrine than dopamine neurons at locus coeruleus in rats [137, 138]. A decrease in neuronal firing rate is an indication of high level of synaptic norepinephrine concentrations possibly caused by a blockade of neurotransmitter reuptake. Thus, it is suggested that bupropion's antidepressant effects may be mediated through noradrenergic rather than dopaminergic pathways.

A number of studies have demonstrated that bupropion SR is an effective treatment for smoking cessation [139-142]. Abstinence rates in one of the first clinical trials [140] averaged 19.0% vs. 44.2% at the end of treatment and 12.4%, vs. 23.1% at the end of one year, for the placebo and 300mg bupropion, respectively. A similar cessation advantage for either bupropion alone or bupropion combined with a nicotine patch was noted in a subsequent trial [141]. Extended use of bupropion has also been shown to prevent relapse [139]. With respect to mood and withdrawal symptoms measured during drug treatment, withdrawal symptoms were generally higher in the placebo vs. active drug group in these studies, but no differences in depressive symptoms, as measured by the Beck Depression Inventory (BDI), have been observed. In contrast, negative affect reduction as measured by the PANAS (Positive and Negative Affect Scale [143]), was found to be a significant mediator of bupropion's efficacy [89]. The PANAS is robust measure of negative affect in smoking cessation trials [90], while few studies support such a role for the BDI. The BDI is a better measure of treatment outcome for depression and these clinical trials exclude individuals who meet criteria for major depression so, it is unlikely this measure would provide much information.

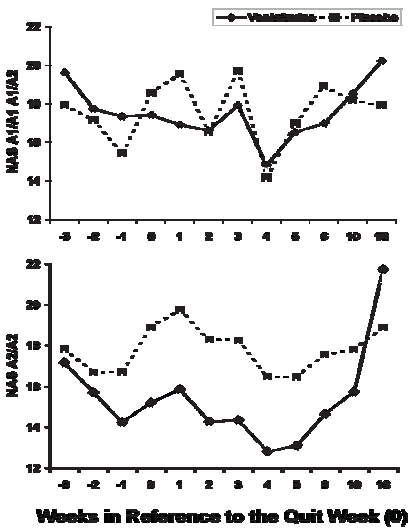
Nortriptyline Nortriptyline is a tricyclic antidepressant that has highly specific effects on NA reuptake inhibition [132]. It is10 times more potent than venlafaxine (a drug used in our previous studies) in this regard and about 7 times less effective at serotonin reuptake inhibition. In contrast to bupropion, it is almost 50 times more effective at NA reuptake inhibition but 6 times less effective at dopamine reuptake inhibition (in vitro) [132]. In the most recent Clinical Practice Guidelines, nortriptyline has been recommended as an effective treatment for smoking cessation [144]. Prochazka [145] showed that nortriptyline produced significantly higher sustained abstinence rates over a 6 month period compared to placebo (14% versus 3%, respectively). Participants who received nortriptyline also experienced less severe withdrawal symptoms including craving, anger/irritability, anxiety, concentration difficulty, restlessness, impatience, and insomnia. Hall and colleagues [146] have also shown that independent of depression history, participants who received nortriptyline achieved a significantly higher sustained (12 months) abstinence rate than those received placebo (24% versus 12%, respectively).

C. Preliminary Studies

We recently completed a study evaluating the relationship between cessation treatment outcome and the DRD2 A1 allele. Participants were 134 smokers that took part in a larger clinical trial evaluating the effects of an antidepressant medication (venlafaxine or placebo) plus standard care (brief counseling and nicotine replacement). Venlafaxine is an antidepressant that inhibits the reuptake of serotonin and to some extent norepinephrine. We hypothesized an overall poor response rate for smokers carrying the A1 allele, which might be improved with the addition of the drug to the treatment regimen, particularly for those with high levels of negative affect. The results from this trial showed significant overall treatment effects at the end of the 18-week drug intervention (37% vs. 25%) but no interaction with depression history or genotype was observed.

Abstinence at the one-year follow-up, while higher in the active drug group, did not significantly differ from the control [147]. However, we did find a main effect for genotype on abstinence. Smokers carrying the DRD2 A1 allele (A1/A1/A2) quit significantly less often than the homozygous A2s ($F_{(1,131)}$=4.14, p=.04; OR=1.82, 95% CI=1.02, 3.32)[148]. This effect was most pronounced during the early weeks of quitting. Moreover, as shown in **Figure 1** we noted a significant pharmacogenetic effect of the drug on negative mood while quitting. Smokers absent the A1 allele (A2/A2) responded to the drug with a substantial reduction in negative affect during the entire post-quitting period for which it was available ($F_{(1,130)}$=6.71, p=.01). These effects were reversed when the drug was withdrawn. In contrast, smokers carrying the A1 allele, showed no significant reduction in negative mood during drug therapy [88]. These results are contrary to what we expected ,but if the A1 allele is implicated in reduced DA transmission, the results may be due to the relatively weak DA and NA reuptake properties of venlafaxine. While efficacious in the treatment of depression, in comparison to other antidepressants, venlafaxine is almost 5 times less effective than bupropion at DA reuptake, 10 times less effective at NA reuptake inhibition than nortriptyline and is among the least potent inhibitors of serotonin reuptake of all the SSRIs [132]. We [PCP7]conclude that genotype is important for predicting abstinence but venlafaxine, and likely other drugs in this class (SSRI) are unlikely to be effective for the control of negative affect during cessation, for the A1 smokers. The current study will ascertain whether bupropion (or nortriptyline) can differentially modify emotional reactivity of A1 or A2 smokers tying to quit, and whether or not this ultimately leads to a better treatment outcome[PCP8].

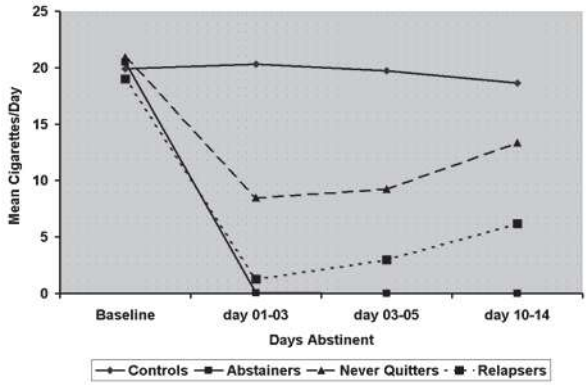
Figure 1 Negative Affect by Genotype and Treatment



During the last two years we were part of a Lung Cancer SPORE (P50CA70907) that provided the support for preliminary data described below. The goal of this project was to characterize the endophenotype associated with potential genetic markers of nicotine dependence (e.g., DRD2 A1 and others), using psychophysiological measures of emotional reactivity (startle) and measures of negative affect during a cessation attempt. The methodology is similar to that proposed for the current proposal. Smokers, with an expressed interest in quitting were recruited from the general community and randomly assigned to Quit and Control conditions. All participants were exposed to four laboratory startle assessment sessions: (1) baseline, (2) 1-1.5, (3) 3-5, and (4) 10-14 days post-quit in the Quit group and at the corresponding points in time for the Control group. Each session involved the presentation of slides from the International Affective Picture System[149], of positive, negative and neutral valence, plus slides of smoking cues. Three of four slides within each category were paired with an acoustic probe resulting in the startle response (see methods section). Smokers in the Quit group were asked to quit smoking following their baseline assessment while those in the Control Group were asked to smoke normally this time. Smokers in the Quit group received a program of behavioral counseling to facilitate abstinence. Controls were treated following their last laboratory session[PCP9].

Results[PCP10]. The startle and physiological data for 96 [PCP11](57-Treatment and 39-Controls: Note randomization is done on approximately a 2:1 ratio) of 150 planned participants have been processed and were available for analysis at the time of this writing. In the Quit group, 28 (50%) were abstinent on the quit date and at every session thereafter, while 16 (30%) relapsed some time after the quit date and 13 (20%) failed to abstain on their quit date and smoked more than one cigarette at each of the post quit assessments. Genotyping [PCP12]information (DRD2) is currently available on 62 participants (34-Treatment and 28-Controls). The proportion of participants carrying the A1 allele (A1/A1 or A1/A2) did not differ by group (χ^2.<1, p=>.05), gender (χ^2 <1 p>.05), race (χ^2=3.1, p=0.08), or abstinence status (χ^2=3.89, p=0.28).

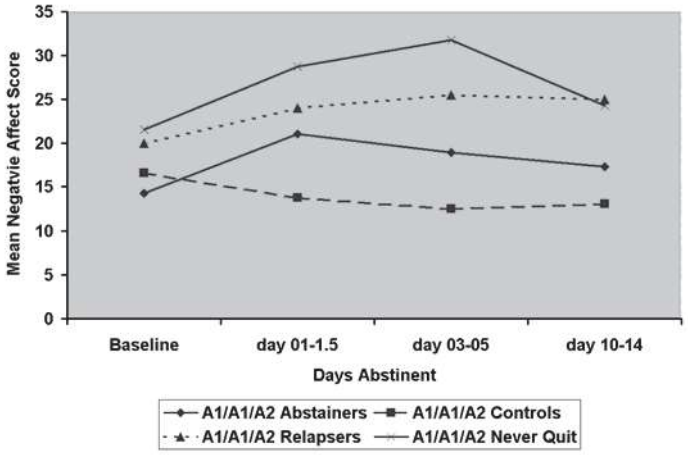
Figure 2 Cigarettes/Day by Abstinence Status

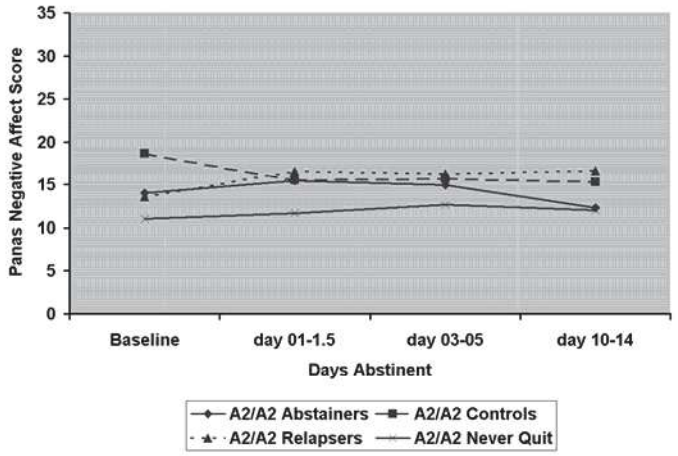


Cigarette Consumption in Relation to Negative Affect

During Cessation Our results showed that daily cigarette consumption did not differ between the Quit (22.5/day) and Control (21.4/day) participants or between A2 (21/day) and A1 (18/day) participants at baseline. However, as shown in **Figure 2,** after the quit date *all smokers* in the Quit group, regardless of Abstinence status (Relapsers, Abstainers and Never Quitters) or Genotype significantly reduced their cigarette consumption ($F_{(9,92)}$=28.14, p=.0001- for the Time by Abstinence status interaction). Over the two-week post-quit period, Abstainers dropped to zero cigarettes/day; both Relapsers and Never Quitters averaged well below their baseline; and Controls showed no significant reduction in smoking. At the same time, all smokers in the Quit group, independent of abstinence status, experienced a significant increase in PANAS negative affect scores ($F_{(9,91)}$=3.99, p=.0003- for the Time by Abstinence status interaction), reflecting the adverse effects of nicotine withdrawal. Given these facts, some analyses described below include all smokers in the Quit group as well as breakdown by Abstinence status. This practice is often followed in clinical trials that examine the effects of a particular treatment on the post quitting nicotine withdrawal or negative affect for all smokers exposed to the intervention.

Figure 3 Negative Affect Scores by the DRD2 Genotype & Abstinence Status





Smoking Negative Affect and Genotype A significant group (Quit vs. Controls) by Genotype interaction was observed for PANAS negative affect scores ($F_{(1,58)}$=10.57, p=.0001), with A1 Quit participants being the highest. Baseline differences using an a priori contrast (F-test for simple effects) showed no differences by Group or Genotype. However, comparisons at subsequent time points showed negative affect of A1s in the Quit group exceeded that of controls while the same was not true of the A2s. Analysis by Abstinence status (Abstainers, Relapsers, Never Quitters and Controls) also showed a significant Abstinence X Genotype interaction ($F_{(3,54)}$=6.63, p=0007) for

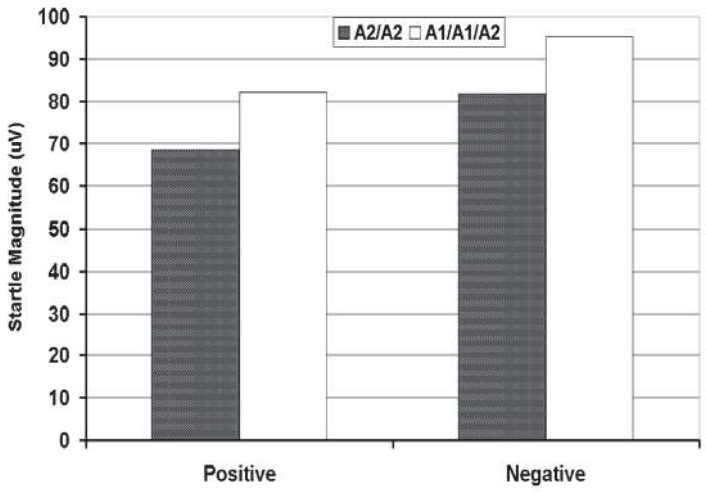
PANAS negative affect. Contrasts at each of the sessions revealed that among the A2s, negative affect did not differ by abstinence status, at any time point. However, as shown in Figure 3, among the A1s, Never Quitters and Relapsers experienced the highest level of negative affect followed by Abstainers and then Controls. We repeated this series of analyses controlling for the following variables: hours continuously abstinent at the time each post quit assessment; number of cigarettes smoked in between sessions; race; and gender. No differences in the results were noted.

Enhancement of Startle to Negative Stimuli at Baseline. To evaluate the hypothesis that startle responses to negative stimuli would be enhanced compared to neutral and positive stimuli, we first examined the baseline startle responses of all participants. This analysis may be regarded as a validity check for our procedures ensuring that we can replicate the basic valence modulation effect on startle. We used mixed model linear regression with startle amplitude from the baseline session as the dependent variable and valence as the independent variable. The model included the value of the startle response to neutral stimuli as a covariate. In this way, we could compare the relative magnitude of startle responses to negative and positive stimuli, adjusted for the response to neutral cues. A significant effect due to valence was found in the expected direction ($F_{(1,93)}=44.40$, $p<.0001$), with responses to negative stimuli (Lsmean=92.66 uV, SE=2.44) being significantly higher than responses to positive stimuli (Lsmean=77.23 uV, SE=2.21). No significant differences were noted for main effects due to Group (Quit vs. Controls), gender or race. Thus, all smokers (regardless of group) responded similarly to negative cues at baseline.

Modulation of Baseline Startle by Genotype When we examined baseline startle responding to positive and negative stimuli (adjusted for neutral responses), significant differences ($F_{(1,58)} = 8.52$, $p=0.0005$) were noted by Genotype (A1s vs. A2s). As shown in Figure 4, smokers carrying the A1 allele showed a significantly higher response to both negative and positive stimuli than those with the A2 allele. We also evaluated responding to smoking cues (pictures of cigarettes, people smoking, ash tray, etc.) at baseline and found that overall reactivity to smoking cues was similar in magnitude to that of positive pictures for both groups, although higher responses were observed for the A1s. Interactions with Group (Quit/Control) were not observed. Genotype main effects remained significant when controlling for gender or race. The [pmc13]results suggest that the startle response is a useful tool for detecting baseline (smoking and non deprived) differences in emotional reactivity that at least in part, are genetically mediated. Moreover, taken together with the PANAS data shown in Figure 3, our results suggest that the A1's enhanced emotional reactivity to both negative and positive stimuli seen at baseline, may be assessing a dimension of pre-quitting emotional reactivity not apparent on the baseline PANAS. It may also presage the increased negative affect observed during the first week of quitting for all the A1s but not the A2s in the Quit group.

Modulation of Startle as a function of Nicotine Withdrawal and Genotype In this series of analyses, startle responding served as the dependent variable, with Valence (negative, positive, cigarette) and Time (baseline, post-cessation days 1-1.5, 3-5 and 10-14 and corresponding time points for the controls) as within subjects factors. Abstinence status (Abstainer, Relapser, Never Quitter and Controls) and Genotype (A1/A1/A2 vs. A2/A2) were the between subjects factors. Responses to neutral stimuli served as the covariate. A significant interaction was observed for the Valence X Time X Abstinence X Genotype component ($F_{(27,54)}=3.39$, $p=.003$). This interaction, while seemingly complex, produced some very interesting and robust findings. To parse the interaction, we first used a series of contrasts to evaluate the differences within the Genotype by Abstinence status combination, at each session. Separate analyses were carried out for each Valence. Given our space limitations we present graphical images for the results involving only negative and

Figure 4. Baseline Startle by Genotype

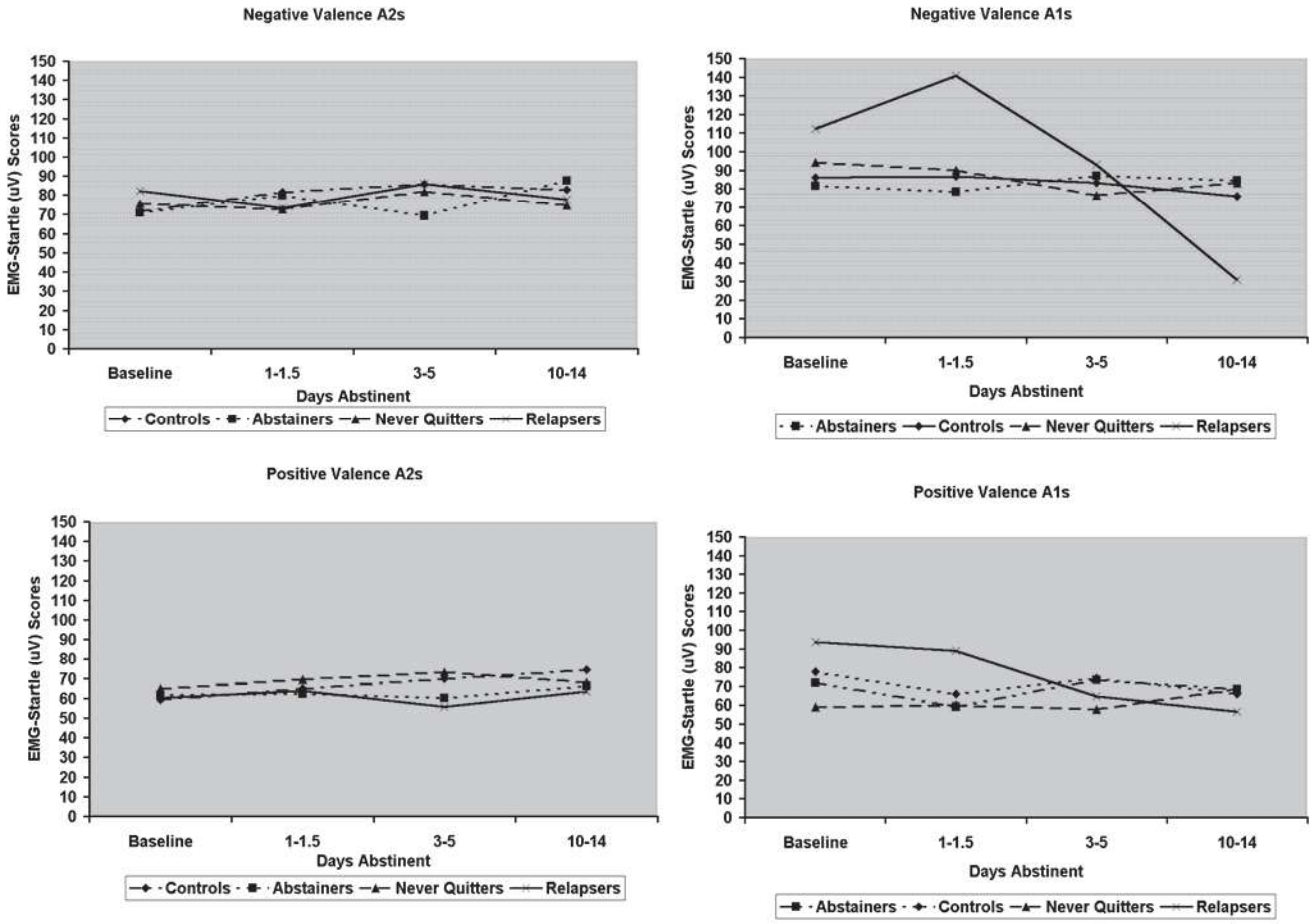


positive stimuli, as they are of primary interest to our study. It should be noted that the main effects of Genotype and Valence were maintained within this analysis, with overall responding of the A1s being greater than the A2s, and startle to negative pictures exceeding those to positive and cigarette pictures.

As shown in Figure 5, the results for negative valence show that A2 Abstainers, Relapsers and Never Quitters, and Controls, differed neither at baseline nor at any subsequent point in time. However, A1 Relapsers were higher than all other A1 or A2 groups, including controls, at baseline and remained that way until the final assessment, at which time their reactivity rebounded below their initial baseline level. Moreover, A1 Relapsers reacted sharply to negative stimuli during the first 24-36 hours of withdrawal, increasing startle even further above the A1 Controls, Abstainers or Never Quitters. No such increase was observed for the A2s. The response of the A1 Relapsers to positive stimuli at baseline, while well below their response to negative cues, was also significantly above all other A1 or A2 groups. The response of the A1 Relapsers to positive cues did not rise during the 1-1.5 day period following quitting, as it did for negative cues. However, their response to positive cues dropped below their baseline by the end of the two-week post-cessation period. A1 Never Quitters showed the lowest baseline response to positive stimuli of all the groups, and fluctuated little during the post quit assessments, with the exception of a slight rise in reactivity at the 10-14 day post-quit mark. A1 and A2s also differed in their reaction to cigarette pictures. For the A2s, reactivity to cigarette pictures was similar in magnitude to positive stimuli across all post-quit sessions. However, the reactivity of all A1s to cigarette pictures was significantly below their responding to positive cues, at the post-quit assessments.

Given the clear baseline differences between A1 and A2 smokers, we also evaluated startle responding during cessation, using the baseline level of responding as a covariate in the models described above. The results were similar to our earlier analyses: an interaction was observed between Abstinence status, Genotype, Time and Valence ($F_{(18,54)}$=2.75, p=.002). A significant increase in startle responding to negative stimuli for the A1 Relapsers was noted at the 1-1.5 day mark, followed by a dramatic decline at two weeks post-quit, consistent with our earlier findings.

Figure 5 Startle Responses for A1 and A2 Smokers & Controls during Cessation



Our analysis of this data will continue but these interim results suggest several interesting findings. First, it appears that smokers carrying the A1 allele are predisposed to higher levels of emotional reactivity at baseline (normal smoking), suggesting a greater activation of defensive motivational systems than the A2s. Moreover, these differences appeared to be enhanced by a cessation attempt, as evidenced by higher levels of reactivity of all A1s in the Quit group, regardless of abstinence status. A1 smokers who are destined to become relapsers are more reactive than all other groups, even at baseline, and significantly increase reactivity to negative cues during first 1-1.5 days of abstinence. This is often regarded as a vulnerable time for relapse and startle reactivity to negative stimuli may provide a way to differentiate those who are most vulnerable to relapse in the early phase of quitting. The rise in reactivity is likely a response to acute nicotine deprivation, and it would appear to reverse by day 3-5, and 10-14, as the Relapsers return to smoking. The reason for the rebound below baseline at 10-14 is unclear but it could signal significant suppression of emotional reactivity as they return to smoking, consistent with the acute effects of nicotine observed in our other studies[150]. In addition, relapse (smoking) not only suppresses defensive activation to negative cues, but increases approach motivation to positive cues, as evidenced by the reduction in reactivity of the Relapsers to positive stimuli. Cessation may also differentially affect approach motivation associated with smoking cues. Among the A1s, startles are lower to smoking cues vs. other positive stimuli, possibly signaling an increase in approach motivation for smoking cues, during cessation. The same is not true of the A2s. While further work is needed to clarify these results, such differences may be related to an increased saliency of the reinforcer (e.g., cigarettes), which for the A1s may be evidence of a narrow reinforcement gradient (i.e., drug related stimuli have greater motivational significance than other positive events).

We have also completed some preliminary analyses involving some of the other genotypes, noting Group (Quit vs. Control) by genotype interactions for the SERT and DRD2 B1, similar to what we found for the A1. These analyses were not complete at the time of this writing but the results for the B1 are very similar to what we observed for the A1. In addition, an interesting finding was observe for the SERT. Smokers carrying the S/S form of the SERT show a significant decline in startle following abstinence, whereas those with the S/L or L/L polymorphism show a significant elevation. This meaning of this finding is unclear but suggests that like the A1s, S/L or S/L smokers are more likely to react to nicotine withdrawal with enhanced activation of avoidance pathways, but emotional reactivity of S/S smokers is reduced. In one of our recent studies [75] we have found evidence of reduced nicotine dependence among S/S smokers. Individuals with this genotype are thought to have higher levels serotonin than those with S/L or S/S genotypes and this might reduce their dependence on nicotine (e.g., they may have less need to use nicotine for affect regulation). We did not find evidence in this preliminary analysis of higher levels of negative affect (PANAS) for Treatment S/S-S/L smokers, but this remains a question to be addressed as we accrue more participants.

D[PCP14]. Research Design and Method

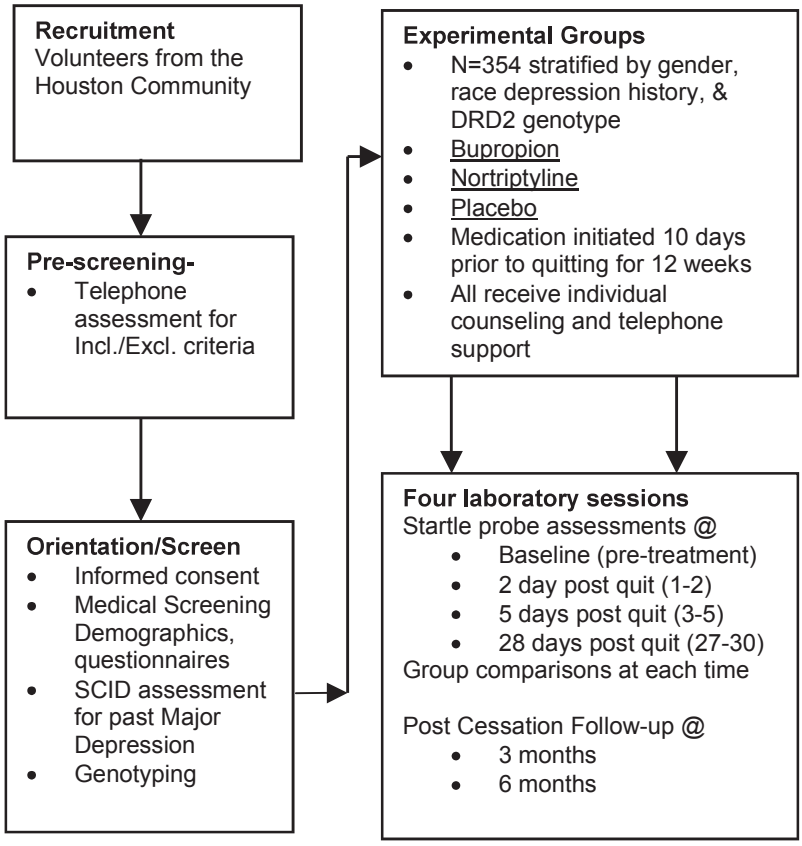
Design Summary

The main objectives of this study are to assess the effects of bupropion and nortriptyline on smokers' emotional reactivity during a quit attempt, as assessed by startle responding to positive and negative emotional cues, and smoking related stimuli; whether this emotional reactivity predicts time to relapse; and, if genetic differences in influence the magnitude of these effects. The present study will use a 3x4x4 repeated measures design, with Group (Bupropion/Nortriptyline/ Placebo) serving as the between groups factor, and Days (days post-quit/laboratory assessments) and stimulus valence (positive, negative, neutral, cigarette) serving as within subject factors. The sample will be stratified for race, gender, previous history of major depression, and the DRD2A1/A2 genotype, since the DRDD2 marker is of primary interest. As shown Figure 6, 354 smokers will be exposed to four laboratory assessment sessions over a period of 38 days. Medication will be administered in a blinded fashion. Participants will complete a baseline laboratory session before any treatment begins (while still smoking). Immediately after their baseline laboratory session participants will be randomized into one of the three treatments and begin taking the appropriate medication. After ten days of medication, all groups will quit smoking for the duration of the study. Participants in all groups will also receive a series of in-person smoking cessation counseling sessions, two prior to quitting and five after the quit date, plus a series of telephone counseling calls at selected intervals in between the in-person counseling sessions. After completion of all laboratory and counseling sessions, all participants will return for follow up assessments at 3

months (10 days after medication ends) and 6 months post quit. In addition, telephone assessments of abstinence status will be carried out 2 and 4 months post-quit. A study time line is provided on page 20.

Subject Recruitment. Participants who want to quit smoking will be recruited from Houston metropolitan area using newspaper, radio and TV public service announcements, feature articles in the MD Anderson newsletter, and posted flyers. They will be offered monetary compensation for their time and for maintaining their abstinence. We expect to have no difficulty recruiting the needed number of smokers. The psychophysiology laboratory is a resource of the MDACC Tobacco Research and Treatment Program (TRTP). The TRTP is directed by the PI and has a high degree of community visibility. We have had considerable success recruiting participants for our clinical trials research.

Figure 6 Design Summary and Study Flow



Population Description The population of the greater Houston community from which the sample will be drawn (Harris and its 6 adjacent counties) is estimated at 4,636,908 people. The ethnic distribution has been reported as 73.5% Caucasian; 18.4% Hispanic or Latino (of any race); 12.6% African-American; 3.2% Asian; and .5% Native American, [151]. Although the literature on the relationship of the DRD2 A1 allele to alcoholism [152], cocaine [153], other substance abuse [15], and smoking [24, 154] has been largely limited to Non-Hispanic Caucasians and some studies with African Americans [40 155], we will recruit without regard to race or ethnicity, as we did in our preliminary studies. As described in the Inclusion Enrollment Report (see Table 6) we have had reasonable success recruiting from ethnic minority populations.

Table 1 Inclusion Exclusion Criteria

Inclusion Criteria:

Age: 18-60 years old

Ethnicity: All

Smoking: >10 cigarettes per day & expired CO > 10ppm.

English speaking & have a telephone

Other: provide informed consent & agree to all assessments & study procedures

Exclusion Criteria:

Current psychotropic medication or current (w/i 6mo.) psychiatric disorder (PRIME-MD).

Involvement in any smoking cessation activities

Contraindications for bupropion (e.g., history of seizure disorder) or nortriptyline (e.g., alcohol dependence)

Uncontrolled Medical Illness

Pre-Screening Telephone Assessment All smokers will be prescreened by telephone for basic eligibility requirements (see Table 1). An initial description of the study design will be provided and data will be obtained on age, smoking history, other tobacco use, medical history, medication use, and pregnancy/lactation status. Participants will also be administered a version of the PRIME-MD, modified for use over the telephone (see **Appendix 3**). The PRIME-MD screens for the five major mental disorders (DSM-IV) commonly encountered in the general population (mood, anxiety, somatoform, alcohol, and eating disorders). The PRIME-MD consists of a 25-item patient self-report questionnaire, which is administered first, and is followed by a more detailed interview, if the participant endorses a sufficient number of positive responses on the questionnaire. Follow-up items

are used to determine if DSM-IV criteria are met for these current psychiatric disorders and adequate reliability and validity have been demonstrated [156]. Our experience administering this instrument and a subsequent SCID (Structured Clinical Interview for DSM-III-R, [157] in another study [158], suggests that the phone based PRIME-MD successfully eliminates virtually all smokers with current psychiatric disorders[PMC15]. Administering the telephone screening takes 10-15 minutes and places a minimal assessment burden on potential participants.
Orientation/Screening Visit All subjects who remain eligible after pre-screening will be scheduled for subsequent in-clinic screening visit. Following an initial orientation and explanation of the study, interested participants will be asked to provide informed consent and complete additional assessments as shown in **Table 2.**

Table 2 Study Time Line and Procedures

STUDY TIME FRAME																				
Study Day	0	3	10	17	20	21	22	23	24		25	32	39	46	48	78	94	104	134	202
Days in Reference to the Quit Day (0)	-20	-17	-10	-3	0	1	2[a]	3	4		5[b]	12	19	26	28[c]	58	74	84	114	182
Months Post Quit															1	2*		3	4*	6
Weeks of Medication			1														12			
TYPE OF CONTACT																				
Phone Pre-Screen Inclusion Exclusion Criteria & Prime MD	X																			
Orientation/ Screening		X																		
Laboratory (startle) session			X				X				X				X					
In Person Counseling Session			X	X			X				X	X	X		X					
Telephone support					X	X		X	X					X						
ASSESSMENTS																				
Informed consent		X																		
Demographics		X																		
Smoking History		X																		
Medical History		X																		
EKG		X																		
Concomitant Meds		X	X	X			X				X	X	X		X			X		
SCID		X																		
Genotyping		X																		
Saliva Cotinine		X					X									M		X	M	X
Expired CO		X	X	X			X				X	X	X		X			X		X
Abstinence/ SSQ		X	X	X	X	X	X	X	X		X	X	X	X	X	X		X	X	X
WSWS		X	X	X			X				X	X	X		X			X		X
PANAS		X	X	X			X				X	X	X		X			X		X
CES-D		X	X	X			X				X	X	X		X			X		X
Medication Schedule/Events																				
Dispense Medication			X	X			X				X	X	X		X					
Pill Count			X	X			X				X	X	X		X			X		
Adverse Events/Medication check				X			X				X	X	X		X	X		X	X	
Medication Level				X							X									

a- Range of 24-36 hrs; **b-** Range of 3-5 days**; c**- Range of 26-30 days***** - Phone Interview only; **M**- mail cotinine requested for abstainers only;

Medical screening for potential contraindications for either bupropion or nortriptyline use will then be conducted by our study physician, Dr. Walter Baile. These conditions include the presence of a seizure disorder, a history or current diagnosis of eating disorders (bulimia or anorexia), recent myocardial infarction, hypersensitivity to tricyclic antidepressants or bupropion, concomitant use of monoamine oxidase, tricyclic antidepressants, or other bupropion products. Standard blood chemistries may be ordered as medically appropriate to evaluate a participant's health status. Furthermore, to identify cardiovascular or other exclusionary conditions for nortriptyline use, all participants will receive a 12-lead electrocardiogram before the initiation of medications.

History of Depression

All eligible smokers will also be evaluated for current and history of Major Depression using the Affective disorders portion of the SCID [156, 157] administered at their orientation visit. Eligible participants who meet criteria for past major depression (>6 months previous) will be categorized as history positive. The current depression section of SCID will also provide a cross check on our initial screen for current depression serving to exclude those initially classified as not currently depressed but who meet diagnostic criteria for current depression, at the interview. It should be noted that this happens relatively rarely, occurring in only 2 of 165 participants in a recent clinical trial. Reliability and validity of the SCID has been adequately demonstrated in both non-patient [157] and substance abusing populations [159].

As shown in Table 2 participants will be asked to complete additional assessments throughout the course of the study. Brief descriptions of these assessments are provided below and copies of individual instruments have been provided in **Appendix 3**.

The Demographic, Health and Smoking Health Questionnaires

These instruments expand on the data obtained during the pre-screening, providing more detailed information on demographics, health/medication history, alcohol, caffeine, and other drug use, for use in the medical screening. Information on smoking history (e.g., years smoked, previous quit attempts, relapse, current smoking rate, and other nicotine/tobacco use) is also obtained. These questionnaires have been used in our previous and current cessation studies to provide descriptive data for the study population (e.g., [160, 161]).

The Fagerstrom Test for Nicotine Dependence (FTND)

The FTND is a 6 item questionnaire that measures nicotine dependence by assessing various components of smoking behavior such as daily intake, difficulty in refraining from smoking, time to first cigarette, etc.[162, 163]. In some studies, the scale has been found to correlate with cotinine level [164, 165] and to predict smoking treatment outcome [166]. It was modified from the most commonly used nicotine dependence measure, the Fagerstrom Tolerance Questionnaire [167].

The Wisconsin Smoking Withdrawal Scale (WSWS) The WSWS [168] will be used to assess withdrawal symptoms. We will use the Anger, Anxiety, Concentration, and Sadness subscales of WSWS, and the Craving subscale to ascertain the effects of quitting on mood and urges to smoke, respectively. The WSWS has scale coefficient alphas between 0.75 and 0.93 and the mood and craving scales demonstrate increases as a function of nicotine abstinence as well as prediction of treatment outcome. Items from the Minnesota Withdrawal [169] are included in the scale for comparability to earlier studies using that measure.

The Positive and Negative Affect Scale (PANAS)

The PANAS [143] is comprised of two 10-item mood scales: Positive Affect (PA) and Negative Affect (NA). Participants rate different feelings and emotions on a scale of 1-5. Various time instructions (e.g., today, past few days, past week, general, etc.) have been used with acceptably high alpha reliability ranging from .86 to .90 for PA and .84 to .87 for NA. Post-cessation PANAS negative affect is a robust predictor of relapse [90].

The Center for Epidemiologic Studies Depression Scale (CES-D)

The CES-D is a 20-item self-report measure developed to [PMC16]assess depressive symptoms in community (nonclinical) populations [170] and in recent studies of smoking cessation [110]. This scale consists of four factors: depressed affect, enervation, lack of positive affect and interpersonal problems.

Smoking Status Questionnaire (SSQ) & Expired CO

The SSQ is a 16-item interview administered questionnaire that will be used to assesses smoking behavior and abstinence throughout the course of the study. Abstinence will be verified by an expired CO reading of < 8ppm at each in-person measurement occasion. Using a time-line follow back procedure all smoking in between assessments will also be recorded.

Nicotine/Cotinine

Cotinine is the first metabolite of nicotine and has a half-life of about 20 hrs; nicotine has a half-life of approximately 2 hours [171]. Saliva nicotine levels will be assessed immediately following smoking in the baseline laboratory assessment. This will allow us to quantify available nicotine, immediately before the startle probe assessment. Cotinine data also obtained at this time will provide information regarding the participant's tobacco exposure within the previous 24 hours. Baseline cotinine values will be used in the descriptive analysis of smoker characteristics, along with other variables from the smoking history questionnaire. Cotinine values (<25ng/ml) from subsequent assessments will be used as a crosscheck on abstinence requirements as described below (see Abstinence compliance following startle assessments page 25). Dr. Helen Van Vunakis of Brandeis University has agreed to analyze the samples for nicotine and cotinine using the HPLC method of Hariharan & VanNoord [172]. This assay provides sensitivity to <1ng/ml of nicotine and cotinine. Dr. Van Vunakis' laboratory does the cotinine and nicotine assays for our current clinical studies. We anticipate no problems in transporting samples or obtaining valid results.

Adverse Event Monitoring & Concomitant Medication

At each of post-baseline laboratory and in-person counseling session, participants will be assessed for side effects and concomitant medications using standard FDA guidelines recommended for these two procedures. Adverse events will be reviewed by our study physician who will recommend changes in dosage or other clinical intervention as appropriate for the event. Adverse event monitoring will continue up to 30 days after medication is completed, or longer if on going events are observed.

Genotyping Eligible volunteers will be asked to provide a blood sample during the orientation visit for conducting genetic analyses. Genotyping assays are described in the **Appendix 4** and summarized below. Genomic DNA will be extracted from each coded blood sample for polymerase chain reaction (PCR) analyses using standard phenol extraction methods.

DRD2

Genotyping for the DRD2 TaqI A and TaqI B polymorphisms will follow the procedures of [26]. Smokers will be categorized into three DRD2 *Taq*IA genotypes: the predominant homozygote (A2/A2), the heterozygote (A1/A2), and the rare homozygote (A1/A1). Smokers will also be categorized into three DRD2 TaqIB genotypes: the predominant homozygote (B2B2), the heterozygote (B1B2), and the rare homozygote (B1/B1).

DRD4

For the DRD4 dopamine receptor gene, genotyping will be performed as described by [173]. Smokers will be classified as having long genotypes (L/L or S/L (i.e., homozygous or heterozygous for an allele $\geq$ 6 repeats) or short genotypes (S/S (both alleles < 6 repeats))[40].

DAT

Genotyping procedures for the dopamine transporter gene (SLC6A3) will be similar to that of [32].The SLC6A3 genotype will be classified as described by [30], according to the presence or absence of the 9 allele (9/9 or 9/* vs. */*, where * refers to alleles other than 9). We will also analyze DAT data by categorizing smokers with and with out the 10 allele as described by Vandenbergh and colleagues.[35]

SERT

Genotyping for the serotonin transporter (5-HTTLPR) will be carried out according to the methods of [174]. Two variants of the 5-HTTLPR have been described: the long (L) variant (528 bp) and the short (S) variant (484 bp).

NET

Several variants of the norepinephrine transporter (NET) gene have been identified. One of these polymorphisms, an exonic silent RFLP (1287G/A) in the NET gene, will be analyzed as previously described[86]. For all assays, 20% of the samples will be repeated for quality control. Additionally, all gels will be read independently by two investigators.

Treatment Intervention

Counseling Component

An individual behavioral counseling intervention will be provided to all participants to facilitate their cessation and continued abstinence over the course of the laboratory assessments. Behavioral counseling is a recommended standard of care to be used in conjunction with pharmacotherapy [175]. The counseling intervention is adapted from our Life-Cheq Smoking Cessation Treatment Manual [176] (see **Appendices 1 and 2**), developed for use in our other studies and used in our preliminary studies. Our individual counseling sessions are shorter in duration but comparable in scope to the group counseling provided in one of the original trials of nortriptyline (12 weekly group counseling sessions, 90 minutes each) [146], albeit longer than that used in the original trial of bupropion (7 weekly individual counseling sessions, 1 call, 10-15 minutes each) [140]. We feel this is appropriate balance given the fact that this is the only intervention received by the placebo group. Studies have shown that difficulty maintaining abstinence in the first two weeks of cessation is frequently associated with relapse [177, 178]. However, behavioral counseling reduces this risk substantially. Counseling will not eliminate withdrawal symptoms (as documented in our preliminary studies) but will provide basic coping skills and support for maintaining abstinence. As shown Table 2, seven individual counseling sessions and 5 telephone support calls will be provided over the 38 days of the program. Each contact will last approximately 30 minutes. To facilitate compliance with the initial stages of abstinence, two treatment sessions will be provided before the quit-date to prepare the smoker for cessation. Further treatment sessions will be scheduled immediately after each laboratory session and at 2-7 day intervals between laboratory visits. A telephone counseling session will also be provided on the quit date and at selected intervals in between the in-person counseling sessions.

Bupropion

Smokers in the Bupropion Group will receive a 12-week regimen of bupropion (150 mg q AM for three days; 150 mg b.i.d. thereafter). Bupropion has been used effectively for smoking cessation in regimes ranging from 7-12 weeks [140-142]. Our procedures will closely resemble those used in earlier trials. Participants will begin bupropion on the day they attend their baseline laboratory session (10 days before the target quit day), but after the session is completed. Bupropion will be tapered to 150 mg/day one week before the conclusion of the regimen.

Nortriptyline

Smokers assigned to the Nortriptyline Group will receive a 12-week regimen of nortriptyline. Medication administration procedures will be similar to those used by Hall et al. [146] in one of the first randomized clinical trials using nortriptyline for smoking cessation. Ten days before their target quit day and following the baseline startle assessment, smokers in this group will receive 25 mg/day of nortriptyline for three days. Medication dose will then be increased to 50mg (25 mg bid) for four days. Serum levels will be assessed at the end of the first week of medication (3 days before quitting) and the dosage will be increased to a maximum of 50 mg bid if plasma concentrations do not reach the recommended therapeutic range (between 50 and 150 ng/mL). Blood samples will be drawn again at week 4 (5 days after quitting) of the medication and dosage will be adjusted again and checked the following week if necessary. The modal dose in the Hall study [146] was 100mg/day. Participants will remain on the adjusted dose until the eleventh week of medication when the dosage will be tapered to 50 mg/day.

Placebo

Participants in the placebo group will receive a total of 12 weeks of placebo plus the behavioral counseling described above. All other assessment procedures will be identical to the other groups.

Medication Blinding

Medications will be supplied by the Drug Product Services Laboratory in the Department of Clinical Pharmacy, University of California, San Francisco (see letter of support section I). This pharmacy made the nortriptyline and bupropion placebo preparations used in Dr. Sharon Hall's nortriptyline and bupropion research studies [146, 179] and has agreed to provide our study with similar preparations. To keep all medications similar in appearance, active and placebo formulations will be enclosed in unmarked sealed and brushed capsules and shipped to MD Anderson Department of Experimental Therapeutics Research Pharmacy for patient distribution. The MD Anderson pharmacy will implement the randomization schedule as developed by the study statistician. Because nortriptyline levels can only be detected in the nortriptyline group, only the study

physician (and his staff) will be un-blinded as to treatment condition, as they will be responsible for the dose titration and medication checks. Our study physician will not be involved in the counseling or startle assessments and he or a member of his staff will conduct the adverse reports assessments. All other members of the research team will remain blind to treatment condition.

To maintain the integrity of the blind among the participants themselves and the other research staff, about 10% of all participants in the placebo and bupropion groups will also undergo the same schedule of blood analysis as the nortriptyline group. We feel this is an appropriate compromise to maintain a blinded research protocol and at the same time, minimize the risk of unnecessary blood draws among the bupropion and placebo groups. The code for who will be selected for the blood draw will be imbedded in the randomization schedule. Medication will be un-blinded in the event of drug related a serious adverse event,

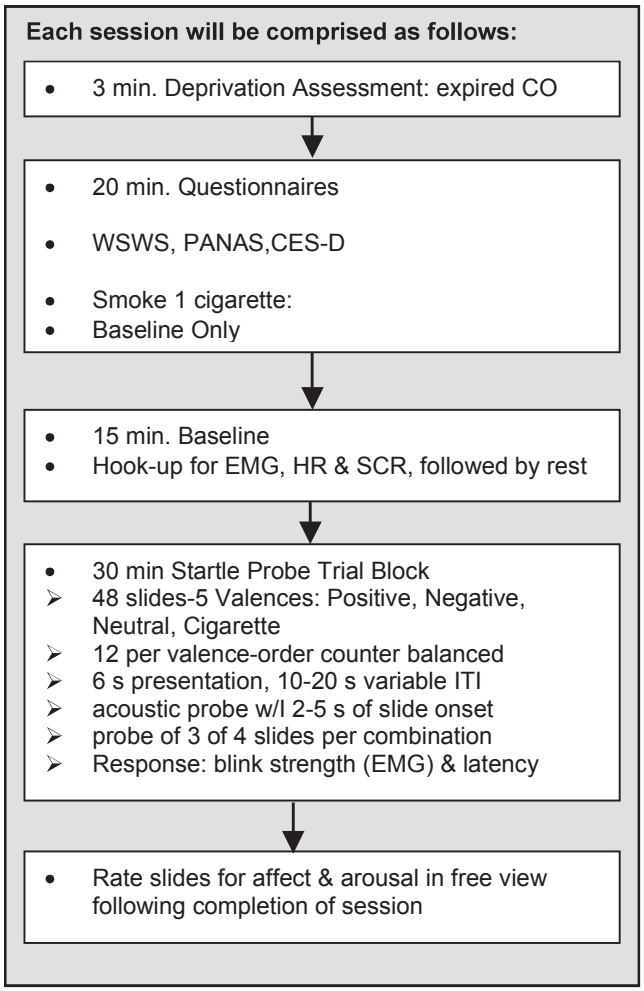
Abstinence Compliance and Startle Assessments

As shown in **Table 2**, compliance with abstinence requirements will be checked at the beginning of each laboratory session, all telephone counseling sessions, and each in-person counseling session that does not coincide with a laboratory startle assessment. Abstinence at each startle assessment will be defined in two ways. The first will involve stringent criteria of no smoking (not even a puff) from the target quit date to the day of each post-quit startle assessment plus a corresponding expired CO of <8ppm. Less stringent criteria of abstinence will also be applied to evaluate the effects of the quit attempt among all smokers, even those who do not meet the stringent criteria of abstinence as defined above. The less stringent criteria will be as follows: (1) Abstinence prior to the first post-quit startle probe assessment (2 days pos-quit) be defined as a self-report on the SSQ of being abstinent on their quit date, and smoking no cigarettes at least 24 hours prior to the assessment plus a current CO <8ppm. (2) For the time period encompassing the 5 and 28 days post-quit startle assessments, abstinence will defined by the following conditions (a) abstinence on the quit date; b) abstinence at the first post-quit assessment; c) self-report of abstinence at the 5 and 28 day post-quit laboratory assessments with corresponding CO levels <8ppm, and (c) having smoked no more than 1 cigarette, between the 2 and 5 day assessments, and no more than 2 cigarettes in the 4 weeks between the 5 and 28 day post quit laboratory assessments. Our data will be analyzed using both criteria to evaluate abstinence. We took a similar approach in our preliminary studies and saw no differences in the startle comparisons between abstainers defined by either the more or less stringent criteria, although both were different from controls. Participants who are not abstinent on the quit day or prior to the 2nd laboratory session (by the less stringent standard) will not undergo the 2nd laboratory assessment procedure at their originally scheduled time. Within the context of their scheduled counseling, these smokers will be provided with support and given an additional opportunity to abstain from smoking for a 2 day period as called for in the first startle assessment. Participants who fail a second time or who relapse after their 2nd laboratory session (using the less stringent criteria described above) will continued to be counseled to quit and will be evaluated on the same schedule of laboratory assessments as the abstainers. Our preliminary data clearly show that these, “never quitters”, or “relapsers”, both significantly reduce their smoking and experience a substantial increase in negative affect and startle reactivity as a function of nicotine withdrawal. Thus even though some smokers may not quit entirely, they experience considerable distress and do not return to baseline levels of smoking, at least for the period in which the behavioral counseling is provided. In our preliminary studies and in the current study the counseling is coincident with the startle assessments and is designed to encourage continuous quitting activity even if they initially fail. Hence, as described in our preliminary studies we will analyze our startle data using models that both separate and combine relapsers and never quitters. This analytic strategy will maximize the information gained form this experiment.

Based on our preliminary data about 25%-50% of the participants may fail to achieve abstinence/maintain abstinence after their quit date, using only the behavioral counseling provided. Our preliminary studies are not yet complete and our abstinence rates have ranged been as high as 75% at other times when we have undertaken interim analysis. To enhance abstinence rates in the present study, we have both increased the counseling frequency, in comparison to our previous study, and provided a monetary bonus for abstinence. With these adjustments, we feel it is reasonable to expect 60%-75% of those in the placebo group (counseling alone) will be abstinent at the 28-day post-quit assessment. Based on the initial clinical trials with both drugs, we expect 75%-80% of those in both drug groups to have achieved abstinence by day 28 post-quit. Our power calculations for interactions involving abstinence status, treatment and genotype have

taken this range of abstinence into account (see page 29). We have also outlined a strategy for comparing all participants in the drug treatment groups to placebo, similar to those we conducted between the Quit and Control groups in our preliminary studies. This approach is outlined in our data analysis section, along with our plan to evaluate differences in abstinence status between and within groups.

Figure 7. Laboratory Protocol



<u>Abstinence compliance following startle assessments (28 days post quit).</u>

As shown in Table 2, we will assess abstinence and days to relapse using a telephone SSQ interview administered at 2 and 4 months post-and at the 3 and 6 month post-quit follow-up points. Participants will have completed the medication regime 10 days prior to the 3-month follow-up. Abstinence from the phone interviews will be verified using return mail cotinine, At the 3 and 6 months follow-up, abstinence will be verified by expired CO (<8ppm) obtained in the clinic. Cotinine will also be assessed at follow-up to provide a measure of tobacco exposure among those who fail to quit. For of these assessments, relapse will be defined in two ways: 1) using the traditional 7-day point prevalence criteria at each assessment (no smoking not even a puff in the 7 days prior to the assessment) and 2) using the recently developed guidelines from the SRNT committee on measurement of abstinence [180], which defines relapse as having smoked for 7 consecutive days, or, a puff a week for 2 consecutive weeks, beginning 2 weeks after the target quit date.

We recognize that the telephone interviews described for the 2 and 4 months pos-quit time points may seem redundant with the 3 and 6 months follow-ups, that are more typical of a clinical trial. However, this study is not exclusively a treatment outcome comparison in which abstinence rates at 6 months are the only focus. A primary dependent variable in this study is the *number of days* to relapse in relation to startle responding and we feel it is appropriate to assess abstinence status more often in order to better capture this data. Participants who report abstinence in these telephone assessments will be asked to provide a saliva cotinine sample using a mail kit developed for a current clinical trial. Participants will also be paid $5 for each saliva sample provided. The procedure of having participants provide saliva samples by mail has worked successfully in previous studies conducted by our group [181]. We have had few problems using the mail-in cotinine procedure in our current trial. Experience in our current trial, suggests that participants become very familiar with the salivette collection procedure since it is used in prior clinic visits. The kits contain a Teflon strip to stimulate saliva production, a cotton dental roll which is placed in the mouth for 30-45 seconds (until well saturated), a salivette collection tube to retain the dental roll after use, and a self-addressed bio-container envelope with pre-paid postage for over-night mail.

Laboratory Startle Assessment Sessions

The protocol for the individual laboratory startle assessments will be similar for all four sessions, and is described in **Figure 7.** Each laboratory session will begin at approximately the same time of day for each participant (10-11am)**.** Participants will be asked to limit their intake of coffee (or equivalent) to no more than 1 cup prior to 8:00 am on the day of each laboratory session. Smoking for all groups will be unrestricted prior to

the first (baseline) laboratory assessment session. The first session will be used to assess normal startle responses to affective stimuli prior to any nicotine deprivation or pharmacological treatment. Subsequent sessions will be used to assess the effects of treatment modality (nortriptyline, bupropion, placebo) and genotype on startle responses to affective stimuli during the 28 days following the quit attempt. The target days for these assessments are 2 days post quit (range 1-3 days); 5 days post (range 3-5) and 28 days post-quit (range 26-30). Ranges are provided as in our preliminary studies to accommodate scheduling changes resulting from missed visits or a repeat visit 2 (see abstinence criteria). Moreover, we have extended our final session from 10-14 days in our preliminary study to 28 days in the current proposal to more readily capture medication effects on startle responding about midway through drug treatment and towards the end of the 30-day withdrawal period.

At the beginning of each laboratory session, participants will be asked to complete the WSWS, CES-D, and PANAS, as described above. At the baseline session all participants will be instructed to smoke one cigarette after completing these instruments, to ensure similar conditions of non-deprivation preceding the startle probe trials. This is similar to the procedure used by Tiffany and colleagues [182] and is identical to the baseline session described in our preliminary study.

Within each of the four sessions, smokers will be exposed to 48 startle probe trials. Each trial will involve the presentation of a picture with either positive, negative, neutral emotional content, or a smoking cue, while an acoustic stimulus is administered. Each startle probe trial will involve the assessment of eye-blink magnitude.

Startle Probe Trial Composition: Slides (stimulus materials) & Instrumentation

After completing the questionnaires (20 minutes), EMG electrodes will be attached around the participant's left orbicularis oculi region. A Biopac photoelectric sensor (plethysmograph) will also be placed over the participant's left index finger to monitor concurrent changes in heart rate and two mini-electrodes will be placed adjacently on the left hypothenar eminence to measure skin conductance. As shown in **Figure 7**, participants will be asked to rest quietly, while seated in a comfortable recliner, for a 15-minute baseline period. The subject will be told that a series of slides will be presented and that each slide should be viewed the entire time it is on the screen. In addition, the subject will be told that occasional noises may be heard over the headphones and can be ignored. Then one trial block consisting of 48 slides will be presented using procedures similar to those listed in the preliminary studies. Each slide will be presented for 6s, followed by a randomly determined interslide interval, 10 to 20 seconds in duration. The valence slides were selected from the International Affective Picture System (IAPS) (Center for the Study of Emotion and Attention, 1995) and are the same as used in our preliminary studies. These slides have been used in many previous startle probe experiments and have been standardized for valence and arousal (e.g., [120, 124, 183]). The smoking cue slides (lit cigarette, ash tray, people smoking, etc.) were developed in our laboratory for our preliminary study [184] and will also be used here. The slides will be equally divided among the three valence categories and the smoking cues. Although the same slides will used in each session, they will be counterbalanced across sessions so that subjects will see pictures from each category equally often in each position. Counterbalancing for slide order will be arranged so that, across subjects, each slide will be associated with a startle probe equally often. Using a computer controlled visual analog scale (electronic slider), participants will be asked to rate their current mood (e.g., interested, distressed, nervous, alert, tired, upset, etc.) and craving (none-very strong) following one out every fourth slide within each valence.

Startle probes and picture stimuli will be controlled and digitally presented with E-Prime software (Psychology Tools, Inc.). Picture stimuli will be projected onto a 3 x 5 foot white screen with an image size of 20 x 30 inches. Participants will sit approximately six feet from the viewing screen. The acoustic startle stimulus will consist of a 50-ms presentation of white noise with instantaneous rise time. The noise burst will be presented over matched (left and right) Telephonics TDH-49 headphones at an intensity of 100 dB. A startle probe will be presented during 32 of the 48 slides at a random time of 2-5 seconds after slide onset, such that a probe will be presented during 8 of the 12 slides within each valence condition. To enhance unpredictability of the startle presentation, startle probes will also be presented during 12 randomly selected inter-slide intervals (ITIs). Responses to startle probes given during the ITIs will be used as a control measure to assess startle responding in absence of emotional stimuli.

Physiological Recording and Data Reduction

For eye blink recordings, the electromyogram (EMG) from the orbicularis oculi region under the left eye will be amplified with a bioamplifier (Biopac, Inc.) using a bandpass filter of 90-1K Hz and contour-following integrator with a time constant of 125 ms. To enhance recording sensitivity during the blink response, the EMG signal will be digitized at 1000 Hz from 50 ms before until 250 ms after acoustic startle probes. The Biopac MP100 data acquisition software permits real time scoring of each blink for peak and latency. Trials with clear movement artifact or excessive baseline activity will be rejected and trials with no blink will be scored as 0 magnitude.

As a concurrent measure of autonomic arousal, we will monitor heart rate and skin conductance using Biopac MP100 physiological monitoring system and data acquisition software. Previous studies suggest that heart rate deceleration is marked during negative picture viewing [120, 185]. In contrast, skin conductance responses increase with arousal level of the stimuli but are enhanced for both positive and negative events, in comparison to neutral slides [120]. Heart rate data will be recorded continuously throughout the session and edited to correct for any missed or double triggered intervals. It will then be transformed to averages for every half second, and the values during slides will be expressed as change scores deviated from a 1-s pre-slide baseline. The average of seconds 2-4 (6.5-s values) will be computed for a summary score to use in subsequent descriptive analysis. Skin conductance responses (SCRs) will be scored as the largest response between 0.9 and 4 s after slide onset [186]. A log transformation (log[SCR + 1]) will be performed to normalize the distribution. The skin conductance amplifier will be calibrated prior to each session to record a range of 0-40 μS. All raw data, analog signals and scoring transformations will be stored directly on a 900Hz computer interfaced with the physiological recording instruments. Real time display of EMG (startle probe) activity, HR, and SC will be implemented during the session using the corresponding software.

At the end of the fourth and final session, all 48 slides will be repeated in a free-viewing procedure. The subject will be asked to view each slide for as long as desired (to a maximum of 30 s), terminating with a button press. Viewing time will be recorded to the nearest millisecond. The subject will then rate the slide on affective dimensions (positive, negative) and arousal (low, high) using an interactive computer display. These ratings, along with HR and SCR measures will be used as a cross-validation of the slide's intended effect on emotional valence (negative/positive) and arousal (high).

Participant Compensation

Participants will be paid $300 for attending all laboratory sessions. Payments will be structured as follows: $60 per completed laboratory session ($240 total), $20 bonus for remaining abstinent at each of the 3 post-baseline sessions[PCP17]. In addition, following all laboratory sessions, participants will be paid $5 per assessment for providing saliva samples to verify their abstinence.

Data Analysis

Prior to inferential procedures, extensive descriptive analyses will be conducted of the startle magnitude scores. Standard descriptive statistics, including means, standard deviations, ranges, etc., will be computed together with ninety-five percent confidence intervals. Graphical methods, including boxplots and histograms, will also be employed to closely examine the distributions of the scores. If required, potential normalizing transformations will be explored. Bivariate associations between the scores and selected demographic variables including age, ethnicity, gender, initial cotinine, FTND score, depression history, baseline smoking level and other initial assessments will be evaluated Pearson's product moment correlation coefficients and ANOVA. Descriptive analyses of the distributions of the WSWS, CES-D, PANAS scores and other psychological assessments between groups and over the laboratory sessions will also be conducted. In addition, we will conduct descriptive and graphical analyses of the affective ratings for each slide obtained at the end all sessions. The end of study ratings from the free viewing situation will be plotted against startle magnitude as a check of the intended manipulation of valence. As described by Cuthbert, et al. [120], startle magnitude will also be plotted against skin conductance levels to verify the arousal level (high) of the selected slides. Skin conductance has been shown to reliably track subjective arousal independent of valence [120]. Similar descriptives will also be provided for the in-session mood and cravings ratings within each valence.

Statistical Approach

Table 3 Estimated Genotype & Allele Frequency

Genotypes	Allele	Controls Freq. (%)	Smokers Freq. (%)
DRD2A	A1A1 or A1A2	25.9[a]	42.6[g]
	A2A2	74.1	57.4
DRD2B	B1B1or B1B2	13.2[b]	41.6[g]
	B2B2	86.8	58.4
DRD4	SS	79[c]	76[i]
	SL or LL	21	24
DAT	9/9 or 9/10	55.8[d]	46.7[d]
	10/10	44.2	53.3
SERT	SS or SL	70.3[e]	59.6[g]
	LL	29.7	40.4
NET 1287G/A	GG	44[f]	unknown
	GA	44	
	AA	12	

a=[154] b=[190] c= [191]d= [30] e=[192] f=[85] g=[147] i=[72]

Our primary analytic strategy involves the use a mixed model approach to examine the effects of the dependent variables (e.g., startle magnitude, time to rela9pse) across assessments. The mixed model approach provides a generalization to the classic linear regression model, using likelihood functions instead of least squares to estimate effects.[187] The mixed model approach is ideally suited for analysis of repeated measures data in that it allows for more specific estimation of the correlation structure of the residuals, and more efficiently handles unbalanced designs and missing data, without excluding participants or imputing values.[188, 189]. Fit statistics (e.g., Akaike's Information Criterion) will be evaluated for all models to ascertain the best fit of the correlation structure of the dataset. We use a computer program, PROC MIXED, (SAS Institute Inc, Cary, NC) to estimate and test the models. This procedure was used in our preliminary studies.

Power

Analysis of the startle responses will be conducted using mixed model regression. The study design involves both between subject and within subject fixed effects and correlations due to repeated measures on the participants. Within subject fixed effects include Days (baseline, post quit days 2,5, 28) and slide valence (positive, negative, neutral, cigarette). Between subjects effects include Treatment (Placebo, Bupropion, Nortriptyline) and Genotype (e.g., A1/A1 or A1/A2 and A2/A2 for our main hypothesis, and others as described for secondary hypotheses). Because of the nonstandard design, power calculations were conducted using simulations in SAS (PROC MIXED). To simplify calculations, we assume that the response to the slides at each level of valence within session will be averaged. The repeated measures on each subject are associated with correlations between observations from the same subject across sessions, and between observations from the same subject within session. We estimated these correlations using data from our previous work. For the primary hypothesis described below, we will fit three models. The first (Model 1) will restrict analyses to positive, negative and cigarette, stimuli and use the score to the neutral stimuli as a covariate, the second (Model 2) will use data from all valences together, and the third (Model 3) will use all data but examine post-quit scores controlling for baseline scores. The within subject-between-session and within subject-within session correlations varied for each model in our preliminary work. For Model 1, we estimated the within subject-between session and within subject-within session correlations to be .03 and .45, respectively; for the Model 2 we estimated them to be .7 and .9, respectively, and for Model3, we estimated them to be .6 and .9, respectively.

Table 4 Effect Sizes & Corresponding Differences in Startle Magnitude (μVolts) For Comparisons Genotype Main Effects Varying Prevalence Rates of the At Risk Allele

Prevalence of "at risk" allele	Model 1	Model 2	Model 3
.5	.15 (3)	.29 (20)	.29 (16)
.4	.16 (3)	.30 (20)	.30 (16)
.3	.17 (4)	.32 (22)	.31 (17)
.2	.20 (4)	.36 (25)	.35 (19)

We estimated power based on 354 subjects and a Type I error rate of 0.05. Power calculations were repeated for each of the three models. For Hypothesis 1, we estimated power for the group contrast between placebo and either bupropion or nortriptyline. A sample size of 354 subjects provides 80% power to detect effect sizes of .17, .32 and .30 standard deviations between these groups for Models 1, 2 and 3, respectively. Given the variability estimates in the raw startle magnitude scores we found by applying the three models to our previous work, these effect sizes translate into group differences in startle magnitude of 4, 22 and 16

µvolts, for Models 1,2 and 3, respectively. These values are well within the range observed in our preliminary studies where the overall difference between treatment and controls averaged 27 µvolts. This range also provides adequate power for detecting interactions with valence for any of the Models, as the range of differences between positive and negative valence between groups, is above 16 µvolts. For Hypothesis 2, we estimated power for the genotype comparisons across a range of possible prevalence for the "at risk" allele, among smokers. The expected allele frequency among smokers is shown in **Table 3.** Data from controls are provided for comparison but except in the case of the NET, are not considered in the power calculations. Power analyses for genotype effects are summarized in **Table 4**. Effect sizes are shown in standard deviation units. The corresponding difference in raw scores (µvolts) is shown in parentheses. Our major hypothesis involving genotype center on the DRD2, for which the A1 is the "at risk allele. As shown in **Table 3,** the expected frequency in our sample of smokers is above 40%. Effect sizes at this frequency and above vary from .15-.30, which corresponds to differences of 3-16 µvolts (see **Table 4),** for the three respective models**.** In our preliminary studies, the average difference in startle magnitude across all valences between A1 and A2 smokers who quit during the study was 26 µvolts, so we will have adequate power for these comparisons.

As described in the proposal, we will also examine other genotypes in exploratory analysis. For purposes of these comparisons, the "at risk" allele can be considered the GG allele of the NET, the L form of the DRD4 and the SERT, and the DAT 10/10. Data on the GG genotype of the NET are unknown for smokers so we used the control data from Zill [85]. For all but the DRD4 the effect sizes and corresponding startle magnitudes are similar to the A1. For the DRD4 L allele the frequency is estimated at .24, corresponding to an effect size of .2-.35, and startle differences between 4-25 µvolts, across the three models. If the differences in startle magnitude are similar to those of the A1, we will still have adequate power to detect them, although this might be at the upper range for Model 2.

For the moderation of emotional reactivity during cessation by genotype, we again provide estimated power for the three models in **Table 5**. The effect estimates represent the difference in emotional reactivity between placebo and bupropion or nortriptyline between each of the two genotypes (e.g. A1 vs. A2). Our preliminary studies indicate that the difference between A1 participants in Quit group and A1 Controls (adjusting for baseline) ranged from 25-60µvolts over the two week of cessation period. Corresponding differences for A2s ranged from 25-2µvolts, and a significant interaction between genotype and Group (Quit vs. Control) was observed (i.e., A1 Quitters showed an elevated startle during cessation. Based on this estimate we will have adequate power for detecting 2 way interactions with genotype, for all Models for our primary hypothesis (DRD2), and for our exploratory analysis of other genotypes, if similar results are observed. Our analytic strategy also calls for exploratory analysis of possible interactions between genotype abstinence status (Abstainers Relapsers Never Quitters), and treatment conditions. We expect all smokers in treatment to experience significant startle elevation as a consequence of abstinence or the reduced smoking observed among the Relapsers or Never Quitters, at least through the first week following cessation. If we estimate an overall abstinence rate at the end of treatment of 60%, and an at risk allele frequency of .43 (for the A1), that is the equivalent of a .26 "at risk" allele rate in **Table 5**. Therefore we should be able to detect differences in startle magnitude of 8-43µvolts, depending on the Model, for interactions between genotype, abstinence status and treatment. This range of startle activity also provides us with adequate power to detect interactions with valence, should they occur, as seen in our previous studies. Differences between A1 Relapsers, Never Quitters and Abstainers and A1 Controls across valences ranged from 7 to 40µvolts, across assessments. For A2s the range was more narrow (4-22µvolts). Thus, we should have we should have adequate power to detect interactions with valence, genotype, abstinence and treatment, should they occur.

Table 5 Effect Sizes & Corresponding Differences in Startle Magnitude (µVolts) For Comparisons Genotype Interactions at Varying Prevalence Rates of the At Risk Allele

Prevalence of "at risk" allele	Model 1	Model 2	Model 3
.5	.32 (7)	.57 (39)	.57 (31)
.4	.34 (7)	.60 (41)	.59 (32)
.3	.37 (8)	.63 (43)	.62 (34)
.2	.42 (9)	.72 (49)	.70 (39)

Power for hypothesis 3 was estimated by dichotomizing emotional reactivity into two groups (high versus low), in order to make use of available standard power formulas. Our actual dependent measure will be a continuous measure of startle, which will offer a much finer gradation of the incremental differences in

abstinence attributable to startle activity. Nevertheless, these calculations should provide a conservative estimate of power. We used data from Hall [146] to estimate abstinence rates for nortriptyline vs. placebo. Her data provides comparability to our study as she used an extensive behavioral counseling in both her placebo and nortriptyline treatment groups. The abstinence rates at the end of treatment, 3 months and 6 months follow-up for the nortriptyline group were 64%, 54% and 42%, and for the placebo group were 42%, 26% and 26%, yielding an average abstinence rate of 53%, 41% and 34% at the 3 respective time points. The bupropion/placebo abstinence rates of Hurt [140] are somewhat lower than observed for nortriptyline, but were not used in these calculations because the behavioral treatment provided in that study was not as extensive as provided here. We believe our counseling intervention would increase rates similar to the nortriptyline group. However, the current study is not specifically powered to detect absolute differences in the abstinence rates between the two drug groups. The literature suggests that the rates for the two drug treated groups should be very similar to each other, assuming a similar level of behavioral counseling in both. However, we do expect to find differences in how smokers respond to each treatment in terms of emotional reactivity and genotype (Hypothesis 1 and 2) and that emotional reactivity predicts abstinence, independent of treatment (Hypothesis 3). If we assume an overall abstinence rate across all three groups at 6 months of approximately 40%, then 150 subjects per group (dichotomizing into high versus low) provides 80% power to detect differences in 6 month abstinence rates of 48% versus 32%, between high and low startle groups. If the overall abstinence rate is 30%, then we have 80% power to detect differences in the 6 months relapse rates of 37% versus 21%, and for overall abstinence of 20%, we have 80% power to detect differences of 26% versus 12%. These estimates are well within expected scenarios in the smoking cessation treatment literature. Thus, we have adequate power to differentiate startle responses across a range of abstinence rates.

Primary Hypotheses

1. The emotional reactivity of smokers during nicotine withdrawal will be significantly higher among those that receive placebo vs. either bupropion or nortriptyline therapy for smoking cessation. Emotional reactivity will be assessed using the eye blink response to an acoustic startle probe and by evaluating responses to emotionally valent stimuli (positive, negative, cigarette) relative to neutral visual slides.
    1.1. Mixed model repeated measure regression analyses will be used to evaluate this hypothesis, regressing peak startle magnitude, on Group (Bupropion, Nortriptyline, Placebo with Days (e.g., baseline, post-quit days 2, 5, 28) as the repeated measures factor. Three types of models will be evaluated. Model 1 will be restricted to startle responses to positive negative and cigarette stimuli and will include valence (positive/negative/cigarette) as a factor. This model will also include the corresponding value of the startle response to neutral stimuli as a covariate. In this way, we can compare the relative magnitude of startle responses to each valence adjusted for the response to neutral cues. The main effect of treatment Group is of primary interest in this model and will be evaluated first, followed by Days, and the Group X Day interaction. Other interactions including Group X Valence and Group X Valence X Days will also be explored. To assess Group differences (if any) at the beginning of the study, an a priori contrast on the baseline values only will also be carried out for this model, evaluating the main effect of Group and the Group by Valence interaction. In Model 2, startle will not be restricted to positive, negative or cigarette valences but will include all valences. The main effect of Group will be evaluated first, followed by Days, each of the two way interaction terms, and the Group X Day X Valence interaction. An a priori contrast will also be carried out as in Model 1 on the baseline values, to assess Group differences (if any) at the beginning of the study. The third model will involve only the post-quit assessments. This model is similar to Model 2, but will include the corresponding baseline value for each stimulus valence as a covariate. In this way, post cessation startle values may be expressed as a function of the corresponding pre cessation baseline values. As described in our preliminary studies, we believe this analysis can be quite informative, as it focuses on post cessation startle in relation to responses under nondeprived (baseline) conditions.
2. The emotional reactivity of smokers during cessation will be moderated by genotype. Our initial hypotheses focuses on the DRD2:
    2.1. We hypothesize that emotional reactivity will be lower for those carrying the DRD2 A1 and using bupropion vs. A1 smokers using either nortriptyline or placebo. A1 nortriptyline users are expected to

have lower reactivity than A1 placebo users[PCP18]. Homozygous A2s are expected to respond similarly to both drugs with higher levels of emotional reactivity being observed for placebo vs. either bupropion or nortriptyline.

This hypothesis will be evaluated using models similar to those described for hypothesis 1, but with the addition of Genotype (A1/A2 or A1/A1 vs. A2/A2) as a term in each model. We will specifically evaluate the main effect of Genotype first, followed by the Genotype X Group interaction (the term of interest). Other interactions involving Genotype with Days and Valence will be explored as described for Models 2 and 3 above. Planned contrasts involving the difference in startle magnitude between each Group condition, within Genotype, will also be carried out.

3. Higher levels of emotional reactivity of smokers at baseline and or during cessation will be inversely related to abstinence at the 3 and 6 month follow-ups.

3.1. Two types of analysis will be carried out. Our primary focus will be on predicting days to relapse. For this analysis, startle responses will serve as the independent variable with days to relapse as the dependent measure. The primary analysis will focus on the average startle response from the baseline assessment. In secondary models, average response to each valence will be included as predictors to determine the relative predictive strength of the different valences. Startle response from each of the post-quit assessments will also be examined as time dependent variables to determine if startle responses from the other assessments predicts relapse. Each model will also be evaluated controlling for or not controlling for treatment Group. For this analysis we will use Cox proportional hazards regression. We are also interested in predicting abstinence (yes/no) at the 3 and 6 month follow-up. Therefore we will also use the GLIMMIX Macro from SAS, which is an adaptation of the PROC Mixed procedure for hypothesis 1 and 2 for dichotomous out come variables (abstinence) to test whether startle responses are related to abstinence, modeling baseline, valence and time dependent characteristics of the model in the manner proposed above. Interactions between genotype (DRD2 A1 vs. A2) and startle in the prediction of abstinence, will also be explored using in the separate and combined valence models.

Covariates will be included in all models described above to correct for any baseline differences and help improve power. The covariates to be used include ethnicity, age, gender, smoking history, and depression history. Interactions between ethnicity or gender and genotype will be explored. If we find statistically significant interactions, separate models for each ethnicity or gender will also be tested.

The contribution of the WSWS, CES-D, PANAS scores and in-session ratings of craving and mood, to the observed startle effects in the primary hypotheses will be explored by fitting these variables as covariates in the above models. In addition we will examine the relationship between these measures, startle values for each valence, and with in sessions affect ratings using correlational procedures. Changes in these measures over time will also be evaluated using the same Mixed Model approach described above with Group and Genotype serving as between groups factors and Days as the repeated measure. Finally, the primary analyses of the startle magnitude described above will be repeated on the changes in skin conductance response and heart rate to determine the pattern of autonomic arousal displayed for baseline and withdrawal conditions.

Secondary Analyses Involving Other Markers

Although our initial genetic hypotheses are focused on the DRD2, we will also use assessments of emotional reactivity to characterize the phenotypes of several other biomarkers that have potential importance for neuroregulatory function and response to pharmacotherapy for smoking cessation. These include: the DRD4, SERT (serotonin transporter), DAT (dopamine transporter) and the NET (norepinephrine transporter). Several exploratory hypothesis are proposed.

We will test the hypotheses that those carrying the long form of the DRD4 (6 repeat or longer) will experience less reactivity during either drug therapy than those homozygous for the short form.

We will test the hypotheses that smokers who are heterozygous (S/L) or homozygous (L/L) for the long form of the SERT gene are likely to experience less emotional reactivity in response to nortriptyline therapy vs. either bupropion or placebo.

Polymorphisms of the NET gene have not been extensively characterized in humans and its relationship to antidepressant therapy have not yet been explored. We will evaluate whether or not one the

currently known polymorphisms (1287G/A) is associated with differences emotional reactivity during pharmacotherapy. As a working hypothesis we expect that smokers carrying the GG allele of the 1287G/A variant would be more likely to show reduced emotional reactivity during to nortriptyline therapy vs. bupropion or placebo, in comparison to their GA or AA counterparts, who are not expected to differ in response to the antidepressants.

Each of these genetic markers will be included in the descriptive analysis described above. We will test our exploratory hypothesis by repeating the mixed models analysis described above for our main hypothesis and test for main effects and interactions as we described for the DRD2.

E. Human Subjects Research

Protection of Human Subjects

1. Risks to the Subjects

Human Subjects Involvement and Characteristics: Subjects recruited for this study (N=354) will be current smokers from the Houston metropolitan community. Inclusion criteria are presented in Table 1**.** All smokers meeting these qualifications will be accepted into the study. Given the nature of the study design it will be necessary to eliminate subjects who do not speak English or have a telephone.

Sources of Materials: Participants will be providing physiological data in the form of heart rate, skin conductance, and facial EMG. Participants will also provide several saliva samples for cotinine analysis and a serum blood sample for the use in genetic analyses. Questionnaire data will be obtained that assess previous smoking and health history, mood, attitudes about quitting, coping behavior, and perceived stress. All data will be collected specifically for research purposes and will be coded to maintain confidentiality. The investigations into the genetics of nicotine dependence are just beginning. At present there is no specific disease “risk” information that would require automatic disclosure of the genetic profile to the participants. However, at the conclusion of the laboratory sessions we will share genetic profile information with all interested participants and provide a lay interpretation of the existing literature, with appropriate caveats.

Potential Risks: As with most antidepressant medications, bupropion and nortriptyline carry risks of side effects. The typical side effects are not usually serious in nature (e.g., dry mouth, anxiety) and often abate within a few days to weeks after starting medication or once the medication is withdrawn. A detailed description of these effects, are provided below. We will follow the Data and Safety Monitoring procedures for reporting and review of adverse events, approved by MD Anderson for this trial. Participants may also experience tobacco withdrawal effects (e.g., increased irritability, difficulty concentrating, etc.) during smoking cessation. None of these effects typically result in serious adverse health consequences. There are no known risks associated with being subjected to startle probes and picture stimuli. Physiological monitoring carries the minor risk of skin irritation from adhesives, however this reaction is rare and easily treated. It is unlikely that completing questionnaires would lead to any potential risks for participants. In sum, it is highly unlikely that any legal, social, or psychological problems will result from this research.

2. Adequacy of Protection Against Risks

Recruitment and Informed Consent: Participants will be recruited from the Houston community sample using: (1) mail, public service announcements, media interviews, MD Anderson Internet access, newspaper advertisements, MD Anderson Conquest Magazine; (2) through the MDACC community liaison and outreach offices, sending advertisements and mailers to all affiliated providers on the mailing list. Consent will be obtained at the onset of the orientation/baseline interview. Participants will be provided with a detailed description of the study, information on risks, and on their right to withdraw from the study.

Protection Against Risks: Bupropion is an FDA approved medication for smoking cessation and its administration will follow approved guidelines. Nortriptyline is recommended as a second line medication in the Clinical Practice Guidelines for Smoking Cessation. Our procedures closely follow those used in previous clinical trials involving these medications. Our study physician will identify participants who have contraindications for bupropion or nortriptyline use and will monitor participants for adverse reactions while they are on medication.

Given the non-invasive, minimal risk nature of the proposed research, we anticipate that the types of adverse experiences that may occur, if any, will focus on concerns about medication side effects, phlebotomy, the discomfort of nicotine withdrawal, possible distress associated and with sensitive issues arising during data collection. We have taken several steps to minimize these risks, as described below.

Adverse Experiences Associated with Nicotine Abstinence/Withdrawal
Participants may experience nicotine abstinence/withdrawal effects. These effects may include irritability, difficulty concentrating, insomnia, anxiety, dysphoria, and increased hunger. None of these effects result in serious adverse health consequences.

Adverse Experiences Associated with Bupropion Use
Comprehensive screening will be conducted to ensure that all participants with contraindications for bupropion use (e.g., current or prior diagnosis of seizure disorder, current or prior diagnosis of bulimia or anorexia nervosa, concomitant use of monoamine oxidase inhibitors or other bupropion products, and hypersensitivity to bupropion) are excluded from participation. The most commonly adverse experiences associated with bupropion use are dry mouth and insomnia. Other frequent adverse experiences may include asthenia, headache, dyspepsia, flatulence, vomiting, agitation, irritability, sweating, and urinary frequency. Adverse effects and concomitant medications will be assessed at each of the post-baseline laboratory and counseling session. Participants' blood pressure will also be measured at each of laboratory assessment. The study physician will monitor participants' complaints of adverse events and, when necessary, adjust the dosage or discontinue medication. Adverse experiences and medication assessments will continue until completion of the regimen (day 84 of the program).

Adverse Experiences Associated with Nortriptyline Use
Comprehensive screening will be conducted to ensure that all participants with contraindications for nortriptyline use (e.g., recent myocardial infarction, concomitant use of monoamine oxidase inhibitors, and hypersensitivity to nortriptyline) are excluded from participation. The commonly adverse experiences associated with nortriptyline are often antimuscarinic in nature. They include dry mouth, constipation, urinary retention, blurred vision, increased intra-ocular pressure, and hyperthemia. Other adverse effects may include drowsiness and headache. In addition to monitoring the adverse effects, concomitant medications, and blood pressure at each of the post-baseline laboratory session, blood samples will be collected at weeks one and four of medication use to ensure the participants are receiving the therapeutic dose. The study physician will monitor participants' complaints of adverse events and, when necessary, adjust the dosage or discontinue medication. Adverse experiences and medication assessments will continue until completion of the regimen (day 84 of the program).

Adverse Experiences Associated with Blood Collection
Syncope, hematoma, and infection are among the common adverse experiences associated with phlebotomy. To minimize participants' exposure to these adverse effects, trained phlebotomists will be employed to handle all blood collection procedures.

The Tobacco Research & Treatment Program (TRTP) clinic is located adjacent to the Department of Clinical Cancer Prevention, which has trained medical personnel on staff that will be available to assist the study physician and other personnel in managing medically related study issues. Confidentiality will be protected by identifying all subjects by ID numbers in all data files except those controlled by the data

manger study physician and the PI. Genetic analyses are provided by sample number coded on each collection container and can not be connected to individual participant names by the laboratory conducting the assays. Only the PI and data manager will have access to the master file linking genetic and other data to participant names. All information will be reported in aggregate form and individual participants will not be identified in any public reports or documents. We expect these procedures to be highly effective for protecting participant confidentiality.

3. Potential Benefits of the Proposed Research to the Subjects and Others

A primary benefit to participants in the proposed study is smoking cessation. All participants will receive an empirically validated treatment for smoking cessation and we anticipate that many of them will continue to be non-smokers after the completion of the study. Smoking cessation is important in cancer prevention, reducing medical costs, and increased well-being for both the participants and society in general.

4. Importance of the Knowledge to be Gained

The development of a new methodology for studying one of the most important factors in cessation relapse (i.e., negative affect) has the potential to significantly increase the efficacy of future treatments for smoking cessation. Differentiating the most salient aspects of smoking and nicotine withdrawal among certain groups of smokers, such as those carrying the A1 allele, may provide a basis for treatment matching, and the development of new interventions that specifically target the primary reinforcing properties of the drug for those individuals. Given the significant benefits that would accrue with increased effectiveness in smoking cessation, these potential benefits far outweigh the relatively minor risks associated with the proposed research.

Inclusion of Women

Women participants will be included in this research and will comprise approximately 50% of the population sample. In our previous research we have encountered no difficulty in the recruitment of women participants.

Inclusion of Minorities

The population of the greater Houston community from which the sample will be drawn (Harris and its 6 adjacent counties) is estimated at 4,636,908 people. The ethnic distribution has been reported as 73.5% Caucasian; 18.4% Hispanic or Latino (of any race); 12.6% African-American; 3.2% Asian; and .5% Native American, [151]. Although the literature on the relationship of the DRD2 A1 allele to alcoholism [152], cocaine [153], other substance abuse [15], and smoking [24, 154] has been largely limited to Non-Hispanic Caucasians and some studies with African Americans [40] [30], we will recruit without regard to race or ethnicity. As described in the preliminary studies section, we have had good success recruiting from ethnic minority populations, especially African Americans and have been able to find comparable distributions among those smokers with and without the DRD2 A1 allele. Our success with Hispanic smokers has been more modest although it must be noted that smoking rates are lower in the Hispanic and Latino community in comparison to the non-Hispanic community. We expect to attract minority smokers to the proposed study using direct public service advertisements targeted for minority smokers on Houston radio stations and newspapers supporting a large minority audience. Houston has two television stations, and several radio stations and newspapers that serve the Hispanic Community. The office of Public Affairs at MD Anderson has also agreed to assist us by arranging for our participation in institution wide Cancer Prevention outreach programs directed at the Hispanic Community. Such events are sponsored several times a year in areas of the community with high concentrations proportions of minority Houstonians. We will focus additional recruitment effort on these venues to increase our recruitment of Hispanic smokers. Such efforts will be in addition to the normal interviews, advertisements, and news releases conducted on our behalf by the Office of Public Affairs at MDACC. Recruitment and enrollment for the proposed study is scheduled to begin within 3-5 months of funding.

Table 6 Inclusion Enrollment Report

Study Title: Genetics Mood & Nicotine Withdrawal -Previous study for the current application

Total Enrollment: 150-completed Target **Protocol Number:** BS98-293

Grant Number: P50CA70907

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race				
Ethnic Category	**Sex/Gender**			
	Females	**Males**	**Unknown or Not Reported**	**Total**
Hispanic or Latino	6	5	0	11
Not Hispanic or Latino	66	66	0	132
Unknown (Individuals not reporting ethnicity)	0	0	0	0
Ethnic Category: Total of All Subjects*	72	71	0	143
Racial Categories				
American Indian/Alaska Native	1	0	0	1
Asian	1	4	0	5
Native Hawaiian or Other Pacific Islander	0	0	0	0
Black or African American	28	28	0	56
White	37	30	0	67
More than one race	2	6	0	8
Unknown or not reported	3	3	0	6
Racial Categories: Total of All Subjects*	72	71	0	143 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)				
Racial Categories	**Females**	**Males**	**Unknown or Not Reported**	**Total**
American Indian or Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0
Black or African American	0	0	0	0
White	3	1	0	4
More Than One Race	0	1	0	1
Unknown or not reported	3	3	0	6

Table 6 Inclusion Enrollment Report

Study Title: Genetics Mood & Nicotine Withdrawal -Previous study for the current application

Total Enrollment: 150-completed Target **Protocol Number:** BS98-293

Grant Number: P50CA70907

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race				
Ethnic Category	Sex/Gender			
	Females	Males	Unknown or Not Reported	Total
Racial Categories: Total of Hispanics or Latinos	6	5	0	11
* Includes all pilots & those with unusable data				

Table 7 Targeted/Planned Enrollment Table

Study Title: Pharmacogenetics and Smoking Therapy

Total Planned Enrollment: 354

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	29	28	57
Not Hispanic or Latino	148	149	237
Ethnic Category Total of All Subjects*	177	177	354
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	20	20	40
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	57	57	114
White	120	120	240
Racial Categories: Total of All Subjects *	177	177	354

Inclusion of Children

We will exclude smokers under the age of 18 because our hypotheses regarding interactions between startle response and nicotine withdrawal are not necessarily applicable to adolescents. There are likely to be significant differences between adults and adolescent in numerous domains including physiological (e.g., physiological responses to nicotine may be different in adolescents), and psychological (e.g., developmental processes may affect mood self-reports). Therefore, the study of the emotional processes related to smoking behavior among adolescent smokers requires a separate focus on those factors that are relevant for this population. In addition, the research protocol outlined in the application requires participants to smoke a baseline cigarette. Fulfilling this protocol with participants under the age of 18 is a violation of Texas state law, that is, it is unlawful for us to furnish tobacco to minors (SS 161.082) and it is unlawful for minors to possess or consume tobacco products (SS 161.252). Finally, the proposed medications listed in this application are not approved for use in children.

Data Quality and Integrity

Because of the ongoing monitoring of the project, study investigators and staff are responsible for ensuring that data quality assurance procedures are developed and maintained. Several procedures will be used to maintain the integrity of the data. All databases will be stored in a centralized location on one of the departmental servers, which is backed up daily, with access limited to specific users at the discretion of the PI. The PI will

assure that audits of selected subsets of data are performed and that appropriate safeguards of participant privacy are maintained. Privacy safeguards will include appropriate password protection and physical security for all computer systems.

Additional quality assurance procedures include a data collection protocol documented in a protocol manual; a two-stage editing procedure for survey data collection consisting of the initial review of the data collection form by a project member immediately following data collection, and a second review by a project member who will record any significant deviations from the protocol; and regular meetings between the study statistician, the PI, data managers, and other project staff to review problems and solutions, and discuss concerns. Data entry systems, whether via a CATI system, scannable forms, or hand entry with verification, specifically provide field checks, range checks for continuous variables and valid value checks for categorical variables; checks for legitimate dates and times and logical consistency. A specific audit trail system that identifies the date, time, and individual making changes on the database will be part of the data-entry system. During data collection, we will issue reports weekly, or even following any new data entry, depending on the needs of the project. Queries and reports will be provided to the study statistician and the PI. Preliminary analyses will be initiated shortly after data collection begins to allow monitoring of data quality.

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G. Consortium/Contractual Arrangements

We have entered into a subcontract with Hamon Center For Therapeutic Oncology Research, at UT Southwestern Medical Center in Dallas, TX., to provide the genetic analysis described in this proposal. Dr. Gail Tomlinson is the PI of the sub contract. She will oversee all aspects of processing the blood samples into DNA, their cataloguing and storage, and subsequent genotyping analyses for genes relevant to nicotine addiction as described in the proposal. Dr. Tomlinson has over 20 years experience leading research into genetic abnormalities in lung and breast cancer and other cancers including the conduct of multiple clinical trials and translational research. All of this is well documented in papers in the peer-reviewed literature and her biosketch. She has provided all the analysis to date for our previous trial (approximately 200 samples) and current clinical trial on scheduled smoking (approximately 1000 samples). Dr. Tomlinson's laboratory set up all of the protocols for accessioning large number of blood specimens and preparing DNA for other genetic epidemiology studies, as well. Her lab has established all of the PCR based genotyping studies for the DRD2, DRD4, SERT, CYP2A6, DAT receptor alleles and will set up all the assays for genotyping for other relevant genes (such as the norepinephrine transporter gene). Her lab is establishing high-throughput methods for these genetic analyses. Her lab, and the Hamon Center For Therapeutic Oncology Research, at UT Southwestern Medical Center in Dallas, TX is fully equipped, modern new facility, for the conduct of molecular biology and molecular genetic research including having two ABI 377 sequencers/genotypers. Dr Tomlinson has at her disposal, two of our other collaborators, Dr. Eric Nestler, Professor and Chairman, Department of Psychiatry, The University of Texas Southwestern Medical Center at Dallas, and Dr. John Minna, Professor and Director, Hamon Center For herapeutic Oncology Research, at UT Southwestern Medical Center in Dallas, TX. Drs. Tomlinson, Cinciripini , Nestler and Minna are linked via email and the Internet and the Hamon Center has a large file server for exchanging information.

H. Consultants

Terry Blumenthal, Ph.D.

Terry D. Blumenthal is a Professor of Psychology at Wake Forest University, Winston-Salem, NC, where he has been teaching and conducting human psychophysiological research for 15 years. Dr. Blumenthal received his B. Sc. from the University of Alberta, Edmonton, Canada, and his M. S. and Ph. D. from the University of Florida. He taught psychology courses and conducted research for three years at Hamilton College, Clinton, NY, before joining the faculty at Wake Forest University. In addition to his position in the Department of Psychology, he is Adjunct Assistant Professor in the Neuroscience Doctoral Program, and a member of the Center for Investigative Neuroscience, at Wake Forest University School of Medicine (formerly Bowman Gray School of Medicine). Dr. Blumenthal is regarded as an expert in the use of the startle response as a tool in research dealing with topics in many areas of psychology, including psychophysiology, psychopharmacology, social, developmental, personality, cognitive, and research methodology. He has published dozens of papers and presented over 100 peer-reviewed papers and posters in the area of human psychophysiology in the past 20 years. Dr. Blumenthal has also served as a consultant to the Department of Defense Polygraph Institute, the Brain Resource Company (Australia), Imperception Inc. (Los Angeles, CA), the Australian Research Council, the Swiss Institute of Technology, and research labs in several countries.

Dr Blumenthal has served as a consultant to our previous startle projects and has provided invaluable technical expertise on physiological recording methodology and startle scoring methodology. He will have a similar role on the current project, providing technical expertise and assisting in certain aspects of data interpretation (artifact checking/rejection etc). Dr Blumenthal will work with the PI, review the laboratory protocol, and cross check our data scoring algorithms throughout the course of the project.

Eric J. Nestler, M.D., Ph.D.

Dr Nestler is the Lou and Ellen McGinley Distinguished Professor and Chairman, Department of Psychiatry, The University of Texas Southwestern Medical Center at Dallas. Dr. Nestler received his B.A. in 1976, Ph.D. in 1982, and M.D. in 1983, all from Yale University. After completing residency training in psychiatry at McLean Hospital and Yale in 1987, he joined the Yale faculty where he became the Elizabeth Mears and House Jameson Professor of Psychiatry and Neurobiology. From 1992 to 2000, Dr. Nestler served as Director of the Abraham Ribicoff Research Facilities and of the Division of Molecular Psychiatry. In 2000, Dr. Nestler moved to The University of Texas Southwestern Medical Center at Dallas, where he is the Lou and Ellen McGinley Distinguished Professor and Chairman of the Department of Psychiatry. Dr. Nestler is the recipient of numerous awards and honors, including the Pfizer Scholars Award (1987), Sloan Research Fellowship (1987), McKnight Scholar Award (1989), Efron Award of the American College of Neuropsychopharmacology (1994), and Pasarow Foundation Award for Neuropsychiatric Research (1998). He serves on the Board of Scientific Counselors of the National Institute on Drug Abuse, and on the Scientific Advisory Boards of the National Alliance for Research in Schizophrenia and Depression and of the National Alliance for Autism Research. He is currently a member of the National Advisory Mental Health Council. Dr. Nestler was elected to the Institute of Medicine in 1998. The goal of Dr. Nestler's research is to better understand the ways in which the brain responds to repeated perturbations under normal and pathological conditions. A major focus of the research is drug addiction: to identify molecular changes that drugs of abuse produce in the brain to cause addiction, and to characterize the genetic and environmental factors that determine individual differences in the ability of the drugs to produce these changes. This work is based on the view that a greater knowledge of the neurobiological basis of drug addiction will lead to more effective treatments and preventive measures.

Dr. Nestler will provide intellectual and technical expertise to the project. He will assist the PI and Dr. Tomlinson in interpreting and the genetic analysis and will provide advise for the choice of additional genetic markers related to drug dependence that could be incorporated into the study as new scientific discoveries become available. At the onset of the study and periodically thereafter, he will meet with the PI and his staff, to review the assessment and genetic analysis protocols and discuss updates or changes that may be incorporated into the current design.

John Minna, M.D.

John D. Minna, M.D. Is Professor of Internal Medicine and Pharmacology and Director of the Hamon Center for Therapeutic Oncology Research and the Moncrief Center for Cancer Genetics at the University of Texas Southwestern Medical Center, Dallas, TX. His research has focused on the molecular pathogenesis of lung cancer and its translation into the clinic. As part of this he has been studying the role of nicotine receptors in lung cancer pathogenesis, and polymorphic markers leading to inter-individual variations in nicotine addiction. He is Principal Investigator on a Lung Cancer SPORE (Special Program of Research Excellence Grant), which is a joint endeavor with M. D. Anderson Cancer Center.