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**Acute Plasma Tryptophan Depletion and Smoking Abstinence:  
Withdrawal, Mood and Quantitative EEG Correlates  
and the Acute Smoking Response**

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## **Abstract**

Since research has implicated a role for serotonin function in smoking abstinence and in smoking behavior, the present study examined the effects of a tryptophan-depleting amino acid mixture and the subsequent smoking of a single cigarette on the nicotine withdrawal syndrome, mood and quantitative electroencephalography (EEG) in chronic smokers acutely deprived of smoking. In a double-blind, placebo-controlled, repeated measures design, eighteen male smokers were tested on two separate occasions, three days apart. On each occasion they: (a) ingested a nutritionally balanced amino acid mixture containing tryptophan or ingested a similar mixture devoid of tryptophan, and (b) sham smoked and smoked a single cigarette. Nicotine withdrawal symptoms, mood states, EEG and expired-breath carbon monoxide (CO) concentrations were measured four times: (a) immediately before smoking deprivation and ingestion of the amino acid mixtures (baseline); (b) 5 hr after smoking abstinence and ingestion of the amino acid mixtures; (c) immediately after sham smoking, and (d) after cigarette smoking. Total plasma tryptophan levels were measured two times: before and 5 hr after amino acid mixture ingestion.

A significant reduction of plasma tryptophan (71%) was achieved by the tryptophan-depletion mixture. Smoking deprivation led to clear time-dependent increases in negative mood state and nicotine withdrawal ratings and reduced EEG activation. While acute tryptophan depletion did not exacerbate this withdrawal in abstinent smokers as evidenced by the absence of change in mood and withdrawal ratings, it was associated with altered brain state arousal as indicated by the reduction in  $\alpha_2$  amplitude. While

the smoking of a cigarette reduced and reversed to baseline some of the smoking abstinence-induced effects, these effects were not influenced by the tryptophan depletion mixture. Localized and lateralized EEG effects in abstinent smokers and following tryptophan depletion were also not observed.

These findings suggest that in chronic male smokers reduced serotonin neurotransmission is not an important characteristic of acute smoking withdrawal effects. These results do not support previous research that has implicated altered serotonin function in tobacco withdrawal and in smoking behavior.



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## **Introduction**

**The harmful effects of cigarette smoking have now been well documented.**

**Cigarette smoking has been linked to numerous fatal and non-fatal diseases, including cancer, heart disease and chronic obstructive lung disease (United States Department of Health and Human Services [USDHHS], 1988). In spite of overwhelming evidence of smoking's adverse health effects, many individuals continue to smoke. Recent estimates indicate over one quarter of all adults currently smoke and while most of these reportedly want to quit most are unable to do so (Center for Disease Control [CDC], 1996).**

**Testifying to the difficulty of giving up this behavior are survey reports indicating that the permanent quit-rate each year is less than 5% (CDC, 1996).**

**Despite a great deal of basic and clinical research effort on the most effective methods for helping smokers give up this persistent habit, there is still no agreement on how to best achieve permanent smoking cessation. Thus, even after undergoing the most efficacious smoking cessation programs integrating behavioral, psychological and physiological methods, less than one-third of smokers maintain abstinence one year after treatment (Shiffman, 1993). One factor considered to be responsible for the high failure rate is the tobacco withdrawal syndrome (USDHHS, 1988). Upon cessation of smoking individuals generally experience various signs and symptoms including negative affective states, physical symptoms and an increased desire to resume smoking (Hatsukami, Hughes, Pickens, & Svikis, 1984).**

### **A. Behavioral Effects of Smoking Abstinence**

The mechanisms mediating smoking behavior have been rigorously studied in recent years. Increasing attention has been devoted to the study of smoking abstinence symptoms. Compelling evidence now demonstrates that cigarette smoking is a form of dependence to the drug nicotine. The drug dependence contributes to the maintenance of smoking behavior and to the failure of treatment interventions (USDHHS, 1988). Nicotine is a psychoactive agent that is reinforcing in both animals and humans and can produce behavioral and physiological dependence (USDHHS, 1988). The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) describes the specific indicators needed for the diagnosis of nicotine dependence (American Psychiatric Association [APA], 1994). The diagnosis is based on the presence of a minimum of three of seven indicators, which include physiological dependence, occurring at any time within a 12-month period. Physiological dependence includes the development of nicotine tolerance and/or withdrawal. Tolerance is evident when diminished responses (such as nausea and dizziness) occur with repeated use of nicotine. Withdrawal is evident when cessation of nicotine use leads to unpleasant behavioral or physiological changes.

A substantial proportion of smokers has nicotine dependence, as defined by the DSM-IV, including symptoms of withdrawal following abrupt cessation of smoking (Cottler et al., 1995; Gust, Hughes, & Pechacek, 1988). For example, data from the DSM-IV field trials revealed that of 829 nicotine dependent smokers, 64% reported experiencing both tolerance and withdrawal symptoms (Cottler et al., 1995). Indeed, smokers often report that they use nicotine to avoid or relieve unpleasant withdrawal



symptoms and attribute failure to quit or maintain abstinence to these unpleasant symptoms. Thus, the study of smoking withdrawal symptoms is important because the symptoms are a mechanism that have been offered to explain why individuals continue to smoke, experience difficulty when attempting to quit and relapse to smoking (USDHHS, 1988).

The symptoms that accompany smoking abstinence have been well documented (Hughes, Higgins, & Bickel, 1994 ). Many retrospective surveys and controlled laboratory and clinical studies have revealed that nicotine withdrawal is associated with a cluster of physiological, behavioral and subjective mood-related symptoms. Physiological changes include: a decrease in heart rate, blood pressure, respiratory rate, adrenalin, cortisol, body temperature, an increase in weight and decreased electrocortical brain wave activity as evidenced by electroencephalographic (EEG) activity (Hatsukami, Hughes, & Pickens, 1985; Hatsukami et al., 1984; Hughes & Hatsukami, 1986; Pickworth, Heischman, & Henningfield, 1995).

The behavioral effects of smoking deprivation include impaired performance in various tasks involving sensory, motor, attentional and cognitive abilities (Heischman, Taylor, & Henningfield, 1994). Additionally, increased aggression and hostility in smoking-deprived individuals has also been demonstrated (Schechter & Rand, 1974).

A number of subjective and mood-related changes also occur with smoking abstinence. Some of these have been consistently replicated in studies involving acute and chronically abstinent smokers and self-quitters (Cummings, Giovino, Jaen, & Emrich, 1985; Hatsukami et al., 1984; Hughes, 1992; Hughes & Hatsukami, 1986; Hughes, Gust,

Skoog, Keenan, & Fenwick, 1991; Shiffman & Jarvik, 1976). These include: craving, irritability, nervousness or anger, impatience, anxiety, restlessness, dysphoric or depressed mood, and concentration difficulties.

Other withdrawal-related symptoms such as drowsiness, sleep disturbances, somatic complaints (e.g., gastrointestinal discomfort and headaches) and changes in skin or body temperature, have not been consistently demonstrated and are therefore in need of further study (Hughes & Hatsukami, 1986; Soldatos, Kales, Scharf, Bixler, & Kales, 1980).

While the majority of inquiries into the effects of smoking abstinence have utilized differing indicators to define a withdrawal syndrome, the majority of studies reviewed above have produced results that correspond with the clinical definition of the Nicotine Withdrawal Syndrome as described by the DSM-IV (APA, 1994). The DSM-IV indicators of nicotine withdrawal are: (a) dysphoric or depressed mood; (b) insomnia; (c) irritability, frustration or anger; (d) anxiety; (e) difficulty concentrating; (f) restlessness; (g) decreased heart rate, and (h) increased appetite or weight gain (Appendix A). Nicotine withdrawal is indicated when a minimum of four of eight withdrawal symptoms are present. These criteria were selected on the basis of a review of the scientific literature on nicotine withdrawal which showed these symptoms to be valid and the more common and reliable effects of smoking abstinence (Hughes & Hatsukami, 1986; Hughes, Higgins, & Hatsukami, 1990; USDHHS, 1988). The DSM-IV also provides additional associated features of smoking withdrawal including laboratory findings of: (a) electrocortical slowing of the EEG; (b) decreased metabolic rate, catecholamines, cortisol levels and

orthostatic response; (c) impaired neuropsychological testing, and (d) associated behavioral features of craving and desire for sweet-tasting food (APA, 1994).

Study of the incidence and magnitude of the DSM-IV nicotine withdrawal symptoms indicates that the symptoms involved are variable. Reports indicate that most deprived smokers experience at least one or two withdrawal symptoms, 25-50% report four or more, and another one-quarter do not experience any symptoms of withdrawal (Hughes & Hatsukami, 1986; Hughes, Gust, & Pachacek, 1987).

The onset and duration of withdrawal symptoms have been examined over a range of smoking abstinence periods: 24-hours (Keenan, Hatsukami, & Anton, 1989; Parrott, Garnham, Wesnes, & Pincock, 1996), 2-weeks (Shiffman & Jarvik, 1976), 3-weeks (Cummings, Giovino, et al., 1985), 4-weeks (West, Hajeck, & Belcher, 1989), 10-weeks (Stitzer & Gross, 1988) and 26-weeks (Hughes, 1992). Overall, these analyses have shown that withdrawal symptoms seem to develop within a few hours of acute smoking deprivation, reach maximal peak intensity within one to three days, and decline to preabstinence levels in a month (Hughes, 1992; Parrott et al., 1996).

Although the duration of the symptoms vary among individuals, they generally last approximately two to four weeks (Hughes 1992). Exceptions to this finding are the urge to smoke and increased hunger and weight gain (Benowitz, 1988; Hughes, 1992). These exceptions may persist for as long as 6 months following cessation. However, relapses have been noted to occur well after withdrawal symptoms have abated (Patten & Martin, 1996). This may imply that factors other than the pharmacological removal of

nicotine, such as the loss of a reinforcer or disruption of a habitual behavior, play a role in the development of the withdrawal syndrome (Hatsukami et al., 1985).

Predictors of the severity of withdrawal in smokers have also been assessed. Studies have provided some evidence showing that the number of cigarettes smoked per day (Hall, Ginsburg, & Jones, 1986), the number of years of smoking (Cummings, Giovino et al., 1985), and nicotine (Hatsukami, et al., 1985) or cotinine (a metabolite of nicotine) (Pomerleau, Fertig, & Shanahan, 1983) levels in blood are positively correlated with withdrawal severity. These results, however, have not been consistently replicated among studies (Hughes et al., 1990; Shiffman & Jarvik, 1976).

Evidence to support the notion that withdrawal results from nicotine deprivation may be found in studies that have shown that the specific absence of nicotine produces a withdrawal syndrome. For example, studies have found that the withdrawal effects after deprivation of either smokeless tobacco products (e.g., chewing tobacco) (Hatsukami, Gust, & Keenan, 1987) or nicotine polacrilex gum (Hughes, Hatsukami, & Skoog, 1986) are comparable, although slightly less severe, to the withdrawal syndrome of cigarette smokers. Accordingly, alleviation of cigarette smoking withdrawal effects can be achieved with nicotine in the polacrilex gum (Hughes et al., 1990), the transdermal patch (Palmer, Buckley, & Faulds, 1992) or tobacco smoke (Hughes et al., 1984). Hughes and colleagues (1984), for example, studied withdrawal symptoms in a repeated measures design and alternated in the same participants periods of smoking deprivation and cigarette smoking. This design allowed for a systematic examination of smoking withdrawal-induced effects and of the consistency of these effects within the same

individual. Results showed that withdrawal effects during smoking deprivation returned to pre-abstinence levels when smoking was resumed and reappeared during a subsequent deprivation period. The withdrawal symptoms during the two deprivation periods were consistent both within and across participants. The most consistently reported symptoms of withdrawal were: bradycardia, irritability, restlessness, drowsiness, insomnia, an increase in overall withdrawal discomfort and in general mood disturbance (as defined by the Profile of Mood State Questionnaire [POMS]) (McNair, Lorr, & Droppleman, 1971).

Finally, another line of evidence in support of the nicotine withdrawal syndrome comes from studies that have altered the nicotine yield of cigarettes and have examined its effects on the development of withdrawal symptomatology. While there has been some difficulty in reproducing these results, lowering the nicotine content of cigarettes has generally been shown to induce a withdrawal syndrome (West, Jarvis, Russell, Carruthers, & Feyerabend, 1984).

In sum, an accumulation of evidence is consistent with the idea that nicotine deprivation is associated with a wide range of psychological and physiological withdrawal reactions. Furthermore, although the incidence and magnitude of these signs and symptoms varies across studies and among individuals, studies have documented a number of consistent withdrawal effects. While the aim of the previous discussion was to provide an overview of the effects of smoking deprivation, the following sections will focus on subjective mood states that accompany smoking abstinence; in particular, the focus will be on negative affective states.

## **1. Effect of Abstinence on Negative Affect**

Nicotine withdrawal is reliably linked with negative affect. Negative affect, defined as any negative mood state such as depressed mood, irritability, anger, aggression, fatigue, nervousness, and anxiety has been reported during smoking abstinence (e.g., Hughes & Hatsukami, 1986; USDHHS, 1988). Considerable research supports a link between cigarette smoking and negative affect. Smokers frequently claim that smoking helps reduce a host of unpleasant affective states and nicotine is the agent believed to be responsible. Some evidence to support this claim comes from studies that have examined the motives for smoking. For example, one study has reported that 70% of adult smokers claimed that they smoked more when “feeling worried” or “feeling blue” (Russell, Peto, & Patel, 1974) and another study found that smokers reported smoking cigarettes to “feel calmer” (Best & Hakstian, 1978).

Early studies that employed factor analysis on questionnaire data have identified a factor termed Negative Affect Reduction (Ikard, Green, & Horn, 1969; McKennell, 1970). This factor is comprised of items suggesting that smoking is used as a means for relieving distress. This dimension of negative affect regulation has since been reproduced in other studies (Best & Hakstian, 1978; Shiffman, 1993).

Studies that have examined risk factors for the initiation of smoking have shown that various psychological characteristics are related to the onset and maintenance of smoking. For instance, Kandel and Davies (1986) found that depressive mood in adolescence predicted heavy smoking as adults. Related studies indicate that smoking maintenance tends to be greater for persons with psychological distress. For example,

neuroticism, depression, and extroversion have been found to discriminate adult smokers from nonsmokers ( Anda et al., 1990; Eysenck, 1973). Prospective studies have linked the risk of smoking in adulthood (Lerner & Vicary, 1984) and in early adolescence (Selzer & Oechsli, 1985) with early childhood traits of aggression, anxiety and a neurotic personality.

Comparable findings occurred in a study by Istvan and Matarazzo (1984) who found that alcoholics were three times more likely to be smokers than non-alcoholic smokers in the general population. Correspondingly, studies that have examined the association between smoking and psychiatric disorders suggest that there is a high incidence of smoking among individuals with depression, schizophrenia, anxiety disorders, attention deficit disorder and suicide (for a review see Glassman, 1993; Khantzian, 1990).

The notion that negative affect increases the probability of smoking in smokers has been examined in the laboratory. Studies have generally found that during stressful situations the rate of smoking is increased among regular smokers (Rose, Ananda, & Jarvik, 1983). For example, several investigations have employed electric shock (Schachter, Silverstein, & Perlick, 1977) and performance anxiety (Rose et al., 1983) as a stressor and found that smoking increased during these situations relative to a nonstressful control condition.

Another line of evidence that reaffirms the strong linkage between smoking and negative affect comes from studies that have investigated the precipitants of smoking relapse. Converging data from several different types of studies support the view that

affective distress is associated with failure to maintain smoking abstinence. Retrospective self-report studies consistently find that ex-smokers often report negative affect as a major precipitant for relapse to smoking (Cummings, Jaen, & Giovino, 1985). Data obtained from clinical trials have produced results showing that high pretreatment levels of negative affect predict smoking status at post-treatment follow-up.

For instance, Pomerleau, Adkins, & Pertschuk (1978) documented that high dysphoria at pretreatment was the only withdrawal symptom associated with eventual failure to quit smoking one year later. Of the smokers who remained abstinent at one year follow-up, those who smoked more during dysphoric states were less successful in remaining abstinent than those who smoked more during non-dysphoric states (26% vs 50%). In a related study, Shiffman (1982) found that of the smokers who called a smoking cessation crises hotline, that offered support for those who feared a relapse or had relapsed, 70% of crises were triggered by dysphoric affect. Callers cited depression (21%), anger (26%), and anxiety (42%) as precipitants.

Taken together, these studies support the notion that: 1) some smokers smoke to reduce negative affect; 2) negative affect is associated with the initiation and maintenance of smoking; 3) a dysphoric state occurs on cessation of smoking, and 4) negative affect is reduced by tobacco smoking.

## 2. Abstinence, Depressed Mood and Depression

In recent years, there has been considerable interest in the role played by specific symptoms of the nicotine withdrawal syndrome in relation to unsuccessful smoking



cessation. In particular, attention has focused upon the impact of depressed mood during smoking abstinence. As indicated above, depressed mood following smoking cessation is a symptom of the DSM-IV nicotine withdrawal syndrome (APA, 1994).

Studies have evaluated the relationship of depressed mood on smoking treatment outcomes (Covey, Glassman, & Stetner, 1990). Findings indicate depressed mood to be predictive of an inability to successfully quit smoking (Covey, Glassman, & Stetner, 1990; Hughes, 1992). For instance, Hughes (1992) examined smoking status at 7, 14, 30, 90, & 180 days following smoking cessation in 630 self-quitters. He found that depressed mood was the only withdrawal symptom that was associated with a lower likelihood of cessation. Other findings, however, have failed to consistently support this link (Hughes et al., 1991; Norregard, Tonnenson, & Peterson, 1993). The heterogeneity of smokers and duration of abstinence differed among these studies and may therefore have led to the mixed findings.

Various studies involving clinical and community-based samples using questionnaires to assess symptoms of depression have consistently indicated that depressive symptoms are higher in smokers than non-smokers. In a large-scale survey, Anda and colleagues (1990) analyzed data from a nation-wide survey using the Center for Epidemiologic Studies Depression Scale (CES-D) to assess depression. Their findings showed that the incidence of current smoking increased as depression scores on the CES-D increased and smokers with high depression scores were significantly less likely to be successful in quitting smoking. Moreover, follow-up data collected nine years later,

indicated that smokers with high depressive symptom scores on the initial CES-D were 40% less likely to have quit than initially non-depressed smokers.

Several other community-based studies using the CES-D or similar questionnaires (e.g., Beck Depression Inventory) have found a similar increased prevalence of smoking in individuals with depressed mood (Covey, Glassman, & Stetner, 1990; Kendler et al., 1993). While the above studies make a strong case for the notion that smoking is related to depressive symptoms, it has been argued that the questionnaire-assessed state of depression may represent a limitation since questionnaires, such as the CES-D scale, may be sensitive to other stressful states such as generalized anxiety, low-self esteem and substance abuse disorders (Glassman, 1993). Thus, utilization of measures of depression with increased validity and sensitivity, either alone or in conjunction with behavioral and/or physiological measures, may lead to less ambiguous interpretation of the extent to which depressive symptoms are associated with smoking.

Lending further support to the notion that there exists a substantial association between smoking and depressive symptoms, several investigators by using psychiatric diagnoses rather than psychiatric symptoms showed that smoking is more common in patients diagnosed with a current major depressive disorder than nonpsychiatric controls (Borrelli et al., 1996; Hughes, Hatsukami, Mitchell, & Dahlgren, 1986). Major depressive disorder, as defined by the DSM-IV, is a mood disorder lasting at least two weeks with the essential feature of either depressed mood or loss of interest in most activities, as well as additional associated symptoms that lead to impaired functioning (APA, 1994). In an influential study, Glassman and colleagues (1988) showed that 49% of patients

diagnosed with a major depressive disorder were more likely to smoke compared with 30% of controls from the general population and that this association persisted after a number of factors such as institutionalization, gender, marital status, socioeconomic status, anxiety disorder, and alcohol or coffee use, were statistically controlled.

Epidemiological studies have also shown the frequent concurrent occurrence of smoking and major depression. A survey study that examined an epidemiological data set that contained information on both psychiatric diagnoses and smoking for 3000 individuals, showed that relative to nonsmokers, major depression was over two times more prevalent among smokers (2.9 % versus 6.6%; Glassman et al., 1990). Kendler and colleagues (1993) also found a significant association between major depression and smoking in over 1500 female twins and suggested that the two conditions are genetically-linked. Another study that evaluated a random community sample of 1200 young adults reported a stronger link between major depressive disorder and nicotine dependence than to non-dependent smoking (Breslau, 1995).

Evidence derived from smoking cessation studies has repeatedly found that not only is smoking linked to a current depression it is also linked to a historical depression as well. Multiple studies have documented the observation that non-depressed smokers with a *history* of major depressive disorder are over-represented in smoking cessation programs and twice as likely to fail in their attempt to quit smoking (Ginsberg, Hall, Reus, & Munoz, 1995; Glassman et al., 1990; Hall, Munoz, & Reus, 1991; Niaura, Goldstein, Depue et al., 1995).

For example, in a large-population based study Glassman and colleagues (1990) reported that previously-depressed smokers were significantly less likely to have succeeded in quitting smoking than those without such a history (14% versus 28%). In a study that examined smoking data derived from a clinic, Hall and associates (1991) observed a higher rate of past depression among smokers and a decreased probability of quitting. While numerous studies have documented prevalence rates ranging from 35% to 60% for a history of major depression in smoking participants (Glassman et al., 1988; Hall, Munoz, & Reus, 1990, 1991), these rates are unusually high, particularly in view of the fact that the average rate of major depression is less than 10 percent (Baldessarini, 1984).

Smoking cessation trials have produced results showing that smokers with a history of depression are less successful in quitting smoking and report more frequent and severe post-cessation withdrawal symptoms, especially depressive mood, than smokers without a history of depression (Covey & Glassman, 1990; Hall et al., 1991; Hughes, 1992). For example, one study reported that withdrawal symptoms, particularly depressed mood, was significantly increased among smokers with past depression (75%) relative to those without such a history (30%) one week after being assigned to a placebo condition of a clinical trial (Covey & Glassman, 1990). Using the Profile of Mood States (POMS; McNair et al., 1971), others have observed a similar increased prevalence of depressed mood following smoking cessation (Hall et al., 1991). Evidence from several clinic-based studies have observed that smokers with a history of depression also report more depressive symptoms at pretreatment (Niaura, Goldstein, Abrams, & Brown, 1995;

Dalack, Glassman, Rivelli, Covey, & Stetner, 1995) which, in turn, is connected to a higher probability of smoking relapse (Hughes, 1992).

Finally, a few clinical and anecdotal reports have produced findings demonstrating that within weeks of smoking cessation some individuals with a history of depression, developed a severe clinical depression that was distinct from withdrawal-related dysphoria (see Covey, Glassman, & Stetner, 1998). Moreover, the depression was resolved soon after the resumption of smoking (Glassman et al, 1990; Flanagan & Maany, 1982). Given this, Glassman (1993) has argued that “the development of major depression after quitting smoking is more than the coincidental onset of depression in at risk-individuals since the depression is effectively resolved with the resumption of smoking.”

In conclusion, these studies suggest that depression, whether it is current, historical, or subclinical (i.e., negative affect) is associated with smoking and may present as a major obstacle to quitting smoking. The findings reflect both the difficulties these individuals have in quitting as well as their general vulnerability to be smokers. Moreover, upon smoking cessation these individuals are at increased risk of developing unpleasant emotional states that range from mild dysphoria to clinical depression.

### 3. Experimental Evidence of Nicotine Effects On Mood And Depression

A few studies have empirically addressed the issue of whether smoking and nicotine genuinely relieve unpleasant dysphoric states and improve mood. Studies have confirmed that smoking reduces negative affect during exposure to various stressors.

Non-deprived smokers compared to smokers deprived for an hour or more have been shown to be less anxious (Gilbert & Spielberger, 1987; Jarvik, Caskey, Rose, Herskovic, & Sadeghpour, 1989), angry and irritated (Heimstra, 1973).

Studies that have manipulated the nicotine-yield in cigarettes and employed nicotine replacement therapies have provided evidence suggesting that nicotine is the agent responsible for the modulation of affect. For instance, Gilbert and co-workers (1989) demonstrated that negative affect was reduced in smokers smoking high versus low nicotine-yield cigarettes. Accordingly, studies comparing nicotine polacrilex gum with placebo gum in deprived smokers indicate that withdrawal symptoms are relieved by nicotine gum (Hughes et al., 1984; Hughes et al., 1991; West et al., 1984). Not all symptoms were consistently attenuated, however. Frequently relieved symptoms were irritability, anger, anxiety and impatience (Hughes et al., 1991). Less reliably alleviated were depressive mood, restlessness, annoyance and hostility (Hughes et al., 1991). These diverse findings across studies have been attributed to differences in the degree of nicotine dependence in individuals and on the test dose administered.

The effects of nicotine administered transdermally on symptoms of withdrawal have also been examined. For example, Hughes and colleagues (1990) found that nicotine administered transdermally during two weeks of smoking abstinence significantly reduced daily negative affect ratings. Most recently, Salin-Pascual and associates (1995, 1998) demonstrated that an acute and repeated administration of nicotine applied transdermally reduced symptoms of depression in clinically depressed individuals who were nonsmokers. These results suggest a relationship between nicotine-

induced biochemical alterations and subsequent negative affect reduction. Taken together, these experimental studies support the survey and clinical findings mentioned above and make a strong case for the notion that nicotine and tobacco smoking are associated with negative affect reduction.

## **B. Biological and Behavioral Links: Smoking, CNS Effects and Mood**

From the preceding sections it is evident that nicotine (smoking) withdrawal and administration produce changes in mood. The ability of smoking to modulate dysphoric moods may be related to its capacity to alter CNS function. In the central nervous system, nicotine has many actions. Following cigarette smoking, nicotine is rapidly extracted from smoke and enters pulmonary circulation (USDHHS, 1988). Within seven seconds it is contained within the blood stream and brain. Approximately one-quarter of the inhaled nicotine makes it across the blood brain barrier and once in the brain, it stimulates receptors, produces neurochemical changes and causes electrocortical effects (USDHHS, 1988).

One primary action of nicotine on the brain is its ability to produce electrophysiological effects that are associated with brain stimulation (Church, 1989). Electrocortical stimulation induced by nicotine involves an ascending midbrain cholinergic pathway and the release of acetylcholine (Armitage, Hall, & Sellers, 1969). Smoke inhalation and nicotine injected into the brain of animals or administered intravenously in human smokers results in electrocortical arousal as evidenced by the electroencephalogram (EEG) (Hudson, 1979; Lukas & Jasinski, 1983).

The EEG has long been used as an imaging tool to study the electrocortical effects of nicotine administration and deprivation in both animals and humans (Lambiase & Serra, 1957). The EEG is an amplified recording of brain electrical activity that consists of a signal that varies in frequency (Hz), amplitude ( $\mu\text{V}$ ), and phase. Quantification of electrocortical activity can be achieved by power spectral analysis which quantifies the EEG by the distribution and amplitude of brain waves at the following frequency bands: delta (under 4 Hz), theta (4-7 Hz), alpha (8-12 Hz), and beta (13 Hz and above).

#### 1. EEG Effects of Smoking

A number of reports have provided evidence in support of tobacco-induced increased cortical activation in humans. While these studies have varied greatly in recording methodology and study design, EEG investigations of smoking have more or less produced fairly consistent findings. Studies have demonstrated that cigarette smoking produces EEG changes characterized by increased EEG activation, consisting of desynchronized low-voltage, fast activity, in both non-deprived smokers and in deprived smokers (see review by Church, 1989; Pickworth et al., 1995).

In *non-deprived* smokers, an early study found that EEG desynchronization, with a decrease in voltage and an increase in alpha frequency, occurred in 80% of individuals that smoked one cigarette (Lambiase & Serra, 1957). Similarly, others found that smoking produced EEG desynchronization with an increase in peak alpha frequency (an



index of the alpha frequency containing the most power) of 1-2 Hz and increased beta power (Hauser, Schwartz, Roth, & Bickford, 1958).

Others have since elaborated on these influential observations. Knott (1989a, 1989b) examined EEG changes following each individual puff of a single cigarette in non-deprived smokers. Results showed that significant smoking-induced cortical excitation (e.g., increased alpha power and alpha frequency band and decreased delta/theta power) occurred soon after the fourth puff on a lit cigarette but not after puffing on an unlit cigarette. Thus, cortical activation produced by smoking was apparent as early as the first puff, slowly increased after the second and third puff, emerged fully by the fourth puff and was sustained until the end of cigarette smoking. Moreover, results indicated that the gradual EEG changes that occur during cigarette smoking were dependent upon the nicotine yield as the presence and onset of EEG changes were temporally delayed during the smoking of a cigarette with a low nicotine-yield relative to a medium-yield. That is, while significant delta and theta reductions, as well as alpha increments, were seen during the fourth puff of a medium-yield cigarette, delta reductions failed to occur during the puffing of a low-yield cigarette and a significant reduction of theta was seen during the fifth puff only.

The notion that the EEG activation produced by smoking is due to the absorption of nicotine from tobacco smoke is further bolstered by the findings of Robinson and colleagues (1992) who varied the nicotine yield of cigarettes while controlling for the tar and carbon monoxide (CO) yields. Results demonstrated that EEG activation was affected by only the cigarette containing a nicotine yield that is generally considered to be

found in a "light" cigarette" (0.6 mg) while the cigarette having a "lighter" nicotine yield (0.06 mg) produced no EEG effect.

The effects of smoking in *smoking-deprived* individuals has also been examined. Ulett and Itil (1969) measured EEG activity in males during a 24-hour period of abstinence from smoking and again following resumption of smoking. All changes observed during the abstinence period were reversed following consecutive smoking of three cigarettes. Knott and Venables (1977) similarly reported immediate reversal of EEG effects after the smoking of two cigarettes in smokers deprived of smoking for twelve hours.

Herning and colleagues (1983) examined heavy smokers who had been deprived of smoking for 10 to 19 hr. They reported that smoking one cigarette led to a decrease in theta and alpha power (with theta power being most influenced) and did not influence alpha frequency. The decrease in theta power was no longer evident 30 min after the last cigarette and was interpreted as a sign of withdrawal that may cue smokers to smoke another cigarette.

More recent studies have examined the EEG changes induced by smoking with topographical EEG maps. These studies have shifted away from using a limited number of scalp recording positions, as most of the above-mentioned studies recorded EEG profiles from only a few sites, to using an increased number of recording positions. The rationale for this shift is based on attempts to characterize more precisely the regional and hemispheric effects of nicotine. (Knott, 1989b; Domino, Riskalla, Zhang, & Kim, 1992; Pritchard, 1991).

Overall, these reports have shown that the spatial distribution of EEG smoking-induced changes vary in response to the nicotine yield of the cigarette smoked. For example, the findings of Knott (1989a) indicated that relative to the EEG changes induced by sham smoking, increasing the yield of a cigarette (low, medium, and high nicotine cigarettes) led to cortical spreading from posterior to anterior brain regions, with increases in alpha power and decreases in theta power.

Domino and colleagues (1992) similarly reported that smoking a cigarette with a high nicotine yield led to a decrease in power in the lower alpha band ( $\alpha_1$ : 8-10 Hz) but an increase in power in the higher alpha ( $\alpha_2$  10-12 Hz) and beta bands at midline electrodes in the frontal, parietal, and occipital regions. Increased activation in higher alpha frequency bands induced by nicotine has been reported by others (Knott, 1988). This finding is important because the lack of separation of alpha frequencies, which has been a common approach in most studies (i.e., grouping the frequencies into one bandwidth of 8-12 Hz), may explain the discrepant findings within the earlier literature of the alpha frequency band being reduced and not augmented.

Knott (1988, 1989a) has demonstrated that this characteristic response of smoking-induced alpha attenuation occurred in response to the administration of a novel or unfamiliar cigarette (the general approach within the EEG literature) and suggested that this response reflected a nonpharmacological effect where the smoker was emotionally or cognitively reacting to the unpleasant taste of a nonpreferred cigarette. Conversely, the smoking of a preferred cigarette was shown to induce an increase in alpha power and in peak alpha frequency, as well as power reductions in delta and theta

activity.

EEG effects of tobacco smoking have been linked to plasma blood levels of nicotine. For example, Benowitz and colleagues (1988) studied blood nicotine concentrations following the smoking of a single cigarette and reported that smoking produced an increase in blood nicotine levels of 10 to 15 ng/ml. Others have examined whether the smoking of a single cigarette in deprived smokers is correlated with different plasma nicotine levels (Domino, Matsuoka, & Kadoya, 1995; Kadoya, Domino, & Matsuoka, 1994). For example, one study found that changes in plasma levels were associated with decreases in delta, theta, and alpha<sub>1</sub> power and increases in alpha<sub>2</sub> and beta power (Domino et al., 1995). Moreover, a concentration of 10 ng/ml of plasma nicotine was required to significantly influence alpha<sub>2</sub> and beta activity whereas concentrations greater than 15 ng/ml produced an increase in beta effects.

Finally, Pritchard (1991) conducted a study in which deprived smokers performed a mental counting task and separated smokers into lighter and deeper-inhaling groups. Results indicated that in the light inhalers, smoking enhanced performance of tasks and produced EEG activation (reduction in delta, theta, and alpha frequency activity) while in deeper inhalers the only effect seen was an increase in beta<sub>2</sub> activity (18-28 Hz). These observed effects of smoking were thought to reflect the dual action of nicotine as evidenced in studies of smoking motives that have indicated that some smokers express that smoking helps them to think and concentrate while others report that it helps them to feel less anxious or stressed (Gilbert & Spielberger, 1987).

In this light, Pritchard (1991) hypothesized, according to the EEG/anxiolytic

literature, that the augmentation of beta2 in deep inhalers, which is similar to the increase in beta activity produced by anxiolytics, could be an index of smoking's negative-affect reducing properties. Further studies, however, have provided weak support for the association between increased beta2 activity and concomitant reductions in self-reported negative affect ratings (Pritchard & Robinson, 1994; Pritchard, Robinson, de Bethizy, Davis, & Stiles, 1995).

Finally, nicotine obtained from different administration mediums has been observed to produce a similar neuroelectric profile to that of tobacco smoking (Pickworth, Hering, & Henningfield, 1986). For example, ingestion of nicotine polacrilex gum (Pickworth et al., 1986) and transdermal nicotine (Knott, Bosman, Mahoney, Illivitsky, & Quirt, 1999; Palmer et al., 1992) have produced a similar EEG arousal profile as seen with the smoking of a cigarette in deprived smokers. Moreover, this activated response was blocked by the central nicotinic cholinergic antagonist mecamylamine (Pickworth, Hering, & Henningfield, 1988).

Taken together, an accumulation of evidence is consistent with the idea that acute cigarette smoking in deprived and non-deprived smokers typically produces a shift from predominantly lower to higher frequencies, with a reduction in power of the theta and alpha<sub>1</sub> bands, and an increase in the alpha<sub>2</sub> and beta bands.

## 2. EEG Effects of Smoking Abstinence

Many studies have investigated the effects of smoking abstinence on the EEG and have demonstrated that the brain waves of abstinent smokers are different than that of

non-abstinent smokers and nonsmokers. In one of the first investigations of the EEG effects of smoking deprivation, Brown (1968, 1973) reported that the spontaneous EEG significantly differed between smokers and nonsmokers only. The EEG of deprived smokers did not significantly differ. In marked contrast to this finding, however, were later reports indicating that the quantitative EEG of deprived smokers relative to nonsmokers and non-deprived smokers was significantly different with the deprived smokers showing reduced cortical arousal.

For example, Ulett and Itil (1969) reported increased activity in the slower EEG bands (delta and alpha) and a decrease in alpha frequency in 24 hr smoking abstinent male smokers. Additional changes that occurred during this period of withdrawal included subjective complaints of increased sleep, drowsiness, and increased levels of dysphoria. Knott and Venables (1977) similarly reported that the peak alpha frequency of 15 hr smoking-deprived smokers was significantly lower (9.3 Hz) than non-deprived smokers (10 Hz) and nonsmokers (10 Hz) in a passive eyes-closed condition. They hypothesized that smoking deprivation resulted in a cortical state that involved reduced arousal and that smokers smoked to reverse this arousal deficit.

Herning and colleagues (1983) examined a group of heavy smokers (who smoked over 1 1/2 packs of cigarettes per day) in an eyes-open condition. In abstinent smokers, EEG theta and alpha power were increased while peak alpha frequency was decreased. Moreover, these effects were most pronounced at the posterior recording site (Pz) and changes in theta power were significantly influenced by the deprivation time-period. For example, onset of increased theta power was evident 30 min after the last cigarette and

remained unchanged throughout the 19 hr of smoking deprivation. This immediate change in theta power induced by smoking deprivation was interpreted as an indicator of nicotine withdrawal.

In a study that examined the time course of EEG changes following smoking deprivation, results indicated that onset of EEG effects varied with time, condition, and recording site (Pickworth, Herning, & Henningfield, 1989). Deprivation-induced EEG changes over a 10 day period in deprived smokers relative to non-deprived smokers included: (a) a decrease in alpha frequency and an increase in theta power occurring 5 hr after the last cigarette and persisting for seven days; (b) decreased theta power which was most pronounced at the frontal (Fz) and posterior (Pz) recording sites; (c) a decrease in beta frequency with changes occurring more slowly than those of alpha frequency and sustained over seven days; (d) no significant changes between smoking deprived and non-deprived individuals were observed beyond 7 days, and (e) significant deprivation-induced alpha and beta frequency changes, as noted above, were evidenced during an eyes-closed but not during an eyes-open condition.

In summary, these studies show that smoking deprivation in heavy smokers results in EEG signs of electrocortical hypoarousal. While methodological differences exist among the studies cited, they generally report a slowing of the EEG frequencies accompanied by an increase in power of the lower frequencies (delta and theta bands) and a decrease in power of the higher frequency bands (alpha and beta). Moreover, the findings suggest that the EEG effects of smoking-deprivation are influenced by time, recording condition, and electrode position.

### 3. EEG, Smoking and Negative Affect

The preceding sections have provided considerable evidence indicating that individuals smoke to alter mood states and that nicotine administration (either via smoke inhalation, orally or transdermally) alters electrocortical processes. Smoking-induced cortical arousal is considered to be a mechanism that accounts for the strong association between daily smoking behavior and changes in subjective-mood states reported by habitual smokers. While each of these effects of smoking have been studied extensively, the intercorrelation between these two variables (e.g., smoking-induced changes in both EEG and mood) has not been examined or reported in the majority of studies reviewed above.

The concomitant measurement of EEG and mood-related effects of cigarette smoking have been reported more recently in a small number of studies (Gilbert, 1996; Knott, Harr, & Lusk-Mikkelsen, 1998). Knott and colleagues (1998) examined the morning effects of repeated cigarette smoking on quantitative EEG and mood in smokers (who were either deprived or non-deprived of smoking) and nonsmokers (Knott, Harr, & Lusk-Mikkelsen, 1998). Non-deprived smokers were instructed to smoke one cigarette at five consecutive 30 min intervals between 9:00 a.m. and 11:00 a.m. prior to each EEG and mood testing session. In non-deprived smokers, smoking during each of the five test sessions led to an increase in cortical arousal with a decrease in theta power and an increase in alpha power. These electrocortical effects were similar to those of nonsmokers but were significantly different from the EEG of deprived smokers who showed reduced cortical arousal (e.g., increased theta, less alpha, and a slower peak alpha frequency).



Accordingly, in non-deprived smokers nicotine-induced changes in mood ratings paralleled increased electrocortical changes such that the mood effects in non-deprived smokers were more similar to that of nonsmokers across all five test sessions. In contrast, the mood effects of deprived smokers relative to non-deprived smokers and nonsmokers was significantly different with deprived smokers showing lower scores on mood ratings of alertness, contentedness and calmness across the test sessions. Thus, these results are important because they not only indicate a lack of acute tolerance to the EEG and mood effects of repeated cigarette smoking but also show that increased cortical arousal in smokers and EEG slowing in abstinent smokers are associated with comparable changes in mood states.

Gilbert (1996) has similarly reported that EEG alterations can be associated with changes in mood. In his lateralized affective processor model, he suggests that nicotine may reduce negative affect by the differential activation of brain hemispheres, with nicotine-increased arousal of the left hemisphere relative to the right (Gilbert, 1996). Moreover, he posits that these lateralized effects influence neurotransmitters and other brain processes that underlie negative affect and depression.

Several studies have provided empirical evidence to support this model. For example, Gilbert (1987) found that smoking reduced right hemisphere more than left hemisphere (e.g., increased alpha power) arousal at parietal recording sites during stressful experimental conditions (while increased arousal of the right hemisphere dominated during non-stressful situations in non-depressed individuals). Studies that have examined the electrocortical effects of smoking in depressed or depression-prone (e.g.,

high neuroticism) individuals have observed that EEG asymmetries of depressed individuals, with lower left frontal activity and greater right frontal activity, were normalized by smoking one cigarette (Gilbert, Meliska, Wesler, & Estes, 1994).

Various scientific disciplines have also provided evidence of the different functions related to the hemispheres of the brain (Glass, 1987) and have lent some indirect support to this model. For example, asymmetric hemispheric brain effects have been reported for negative affect, with the right hemisphere being related to nonrational emotional states and the left hemisphere associated with rational and verbal-information processing (Davidson, 1993). Left-hemisphere dominant dopamine and acetylcholine systems have been found to covary with coping and approach behaviors while right-hemisphere dominant 5-HT and noradrenaline systems mediated withdrawal behaviors (Kinsbourne, 1989).

Studies that have examined cortical EEG arousal following the presentation of negative and positive affective stimuli to healthy individuals have provided results showing greater right/left EEG arousal with negative affective stimuli and increased left/right EEG arousal with positive affective stimuli (Davidson & Schwartz, 1979). These effects were most pronounced in frontal brain regions.

Clinical depression has similarly been characterized by a decrease in left and an increase in right frontal hemispheric activation (Henriques & Davidson, 1988) in some (but not all) studies. For example, Davidson and colleagues (1985) found that depression was mediated by decreased arousal in both the frontal region of the left hemisphere (e.g., increased alpha power) and in the posterior region of the right hemisphere. Others have

similarly reported reduced metabolism in the left prefrontal cortex, as evidenced by positron emission tomography (PET) scans, of depressed individuals (Martinot et al., 1990). Tucker and Williamson (1984) found that the effects of antidepressant medication were most pronounced in the right hemisphere compared to the left.

In summary, the lateralized affective-processor model offers a novel way of understanding nicotine's effects on negative affect and depression. Gilbert (1996) proposes that lateralized electrocortical effects are possibly mediated by nicotine's effects on various lateralized neurotransmitters. While this proposed lateralized neuromodulator mechanism is still highly speculative and in need of more study, there is some evidence suggesting that nicotine may reinforce smoking behavior by altering the release of several neuroregulators, which in turn may alleviate negative affect and thus improve mood. What is presently known about how nicotine in the brain might enhance affect will be examined next by briefly reviewing studies in laboratory animals.

#### 4. Neurochemical Effects of Nicotine and of Depressed Mood

A growing body of evidence indicates that nicotine affects the central nervous system functioning of cholinergic, peptidergic, monoaminergic and neurohormonal systems in animals (Balfour, 1982; Pomerleau & Pomerleau, 1984). More specifically, studies show that nicotine enhances the release of neuromodulators including acetylcholine, vasopressin, growth hormone, prolactin, endogenous opioids and glutamic acid (Wilkins, Carlson, van Vunakis, Hill, Gritz, & Jarvik, 1982; Pomerleau & Pomerleau, 1984), as well as the major biogenic amines in brain including dopamine,

norepinephrine, epinephrine, and 5-HT (Westfall, 1974; Balfour, 1982).

Studies that have examined the release of catecholamines in animals have shown nicotine-induced release and turnover of dopamine, norepinephrine and epinephrine for both whole brain and specific brain areas including the hypothalamus, striatum, cortex and cerebellum (Westfall, 1974). Catecholamine release has been observed after both acute and chronic nicotine injections in animals, at dose levels that are similar to those produced by smoking, and both in vitro and in vivo (Wonnacott, Drasdo, Sanderson, & Rowell, 1990).

Accordingly, in vitro studies using a slice or synaptosomal preparation show that nicotine is able to release both norepinephrine (Yoshida, Kato, & Imura, 1980) and dopamine via a calcium-dependent process (Westfall, Grant, & Perry, 1983). In vivo microdialysis studies have shown that acute systemic injections of a low dose of nicotine increase the extracellular concentration of the mesolimbic dopamine system of the brain (Benwell & Balfour, 1992) and of hippocampal norepinephrine (Brazell, Mitchell, & Gray, 1991). Local infusions of nicotine into the brain of rats stimulated the release of dopamine from the nucleus accumbens and striatum (Misfud, Hernandez, & Hoebel, 1989; Toth, Sershen, Hasim, Vizi, & Lajtha, 1992). Moreover, nicotinic receptors have been identified on the perikarya and terminals of the mesolimbic dopamine system (Clarke & Pert, 1985) and systemic injections of nicotine in rats increases the firing rate of dopaminergic activity when measured extracellularly (Yoon et al., 1986).

Nicotine-induced neurochemical release has prompted much speculation about the mechanisms by which nicotine might enhance affect. Pomerleau and Pomerleau (1984),

for example, have proposed that nicotine from tobacco smoke enhances the release of beta-endorphin, which in turn alleviates negative affect and ameliorates mood. These authors have provided some evidence to support this notion. In one study, they found that individuals treated with naloxone, an opiate antagonist, significantly smoked fewer cigarettes and had minimal urges to smoke. As well, they also showed blood concentrations of nicotine and beta-endorphin to be positively correlated. In contrast to these findings, however, others have not been able to reproduce the effects of naloxone on smoking reduction (Nemmeth-Coslett & Griffiths, 1986).

Another mechanism proposed to account for smoking's effects on mood involves nicotine's capacity to enhance dopaminergic neurotransmission. Activation of this system is believed to be important in the reinforcing actions of stimulant drugs (Fibiger & Philips, 1987). As noted above, there is some evidence to support enhanced dopamine release by nicotine.

Understanding the neurochemical basis of depression permits further theorizing about the neurobiological mechanisms by which nicotine might modulate affect. It has long been known that depressive illness is associated with abnormalities in brain monoamine neurotransmitter function. Early findings showed that the antidepressant drugs of the time, such as the monoamine oxidase inhibitors (which inhibit the breakdown of monoamines) and tricyclic antidepressants (which inhibit the re-uptake mechanism or inactivation of monoamines), exerted not only a beneficial effect on depressed individuals but also increased the amount of monoamine neurotransmitter

available at the synapse, thus enhancing neuronal activity of these systems (Sangdee & Franz, 1979).

As the reuptake of norepinephrine and 5-HT is highly influenced by tricyclic and reuptake inhibiting antidepressants, much research attention was initially, and continues to be, aimed at defining the role of central norepinephrine and 5-HT transmission in major depression. More recent findings implicate dysfunctional serotonergic and noradrenergic neuronal systems as significant (but not the only) causes of major depression (Heninger, Charney, & Price, 1988; Heninger, Delgado, & Charney, 1996).

As both nicotine and antidepressants alter the release of 5-HT, norepinephrine, and dopamine in the central nervous system (Balfour, 1989) and because there are distinct similarities between the clinical pictures of nicotine withdrawal and depression, some investigators have proposed that smoking (via nicotine) relieves dysphoria by increasing monoaminergic neurotransmitter release (Gilbert, 1996; Glassman, 1993; Pomerleau & Pomerleau, 1984). Similarly, the increase in depressed mood and negative affect which often accompany smoking abstinence could be mediated by decreased brain monoaminergic release (Balfour, 1989).

One molecular target that has been proposed to be altered by cigarette smoking is monoamine oxidase (MAO) (Fowler et al., 1996), a catabolic enzyme that oxidizes monoamines. Inhibitors of MAO are used in the treatment of depression and their nonselective antidepressant effects are associated with inhibition of the monoamines (Thase & Kupfer, 1996). Studies have now provided findings that smokers compared to non-smokers and/or former smokers have reduced brain levels of MAO-A and B as

evidenced with [<sup>11</sup>C]clorgyline and PET scans (Fowler et al., 1996). Other studies have shown reduced levels of both forms of MAO in peripheral blood measures of heavy smokers (Berlin, Said, Spreux-Varoquaux, Olivares, et al., 1995). However, in contrast to these observations are the findings of Carr and Basham (1991) indicating that cigarette smoke but not nicotine may be responsible for inhibition of MAO-A in the brain (Carr & Basham, 1991). While these findings are intriguing, and suggest that smoking inhibits MAO in a manner that is similar to the effects of the antidepressant monoamine oxidase inhibitors, they are still speculative and in need of more study.

More recently, attention has focused on the actions of nicotine on the brain serotonergic system. Examination of 5-HT as a mechanism by which nicotine might relieve negative affect is a reasonable choice of study as an abundance of data indicate that serotonergic neurotransmission is altered in depressed patients (Heninger et al., 1988; Heninger et al., 1996). In general, these studies have shown that the concentration of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin, is decreased in the hypothalamus, cortex, cerebrospinal fluid (CSF), and post-mortem brain in depressed patients relative to healthy individuals. As well the finding that efficacious antidepressant drugs, such as the selective serotonin reuptake inhibitors, that prolong the actions of serotonergic neurotransmission improve depressed mood states lends further support to the involvement of this neurochemical in depression.

That nicotine might affect brain 5-HT release is suggested by several studies. Acute systemic injections (Ribiero, Bettiker, Bogdanov, & Wurtman, 1993) and local administration of nicotine via a microdialysis probe (Toth et al., 1992) increase the

release of 5-HT in the frontal cortex of rats as measured by *in vivo* microdialysis.

Moreover, the effects of systemic nicotine on 5-HT release were blocked by pretreating animals with the nicotinic antagonist mecamylamine (Ribeiro et al., 1993).

Others have found that *in vitro* administration of dimethylphenyl piperazinium, an agent that stimulates nicotinic receptors, induced the release of 5-HT from brain slices of the striatum in rats (Westfall, Grant, Naes, & Meldrum, 1983). Nicotinic receptors on perikarya of the raphe nuclei have been observed in both humans (Benwell, Balfour, & Anderson, 1988) and rodents (Marks et al., 1992), and 5-HT axons in the rat hypothalamus and striatum have been shown to contain presynaptic nicotinic receptors (Schwartz, Lehmann, & Kelelar, 1984).

A decrease in the synthesis and concentration of 5-HT in rat hippocampus has also been reported following the chronic administration of nicotine (Benwell and Balfour, 1979). Recent studies examining the hippocampus of chronic smokers postmortem have similarly indicated a decrease in the synthesis and concentration of 5-HT as well as an increased number of 5-HT receptors (e.g., 5-HT<sub>1A</sub>) (Benwell, Balfour, & Anderson, 1990). Similarly, serotonergic neurons in the dorsal raphe nucleus showed an increased sensitivity to a 5-HT<sub>1A</sub> agonist drug in rats undergoing nicotine withdrawal (Rasmussen & Czachura, 1997). Thus, these results suggest that smoking withdrawal may be associated with changes in 5-HT function. Indeed, several studies have demonstrated that serotonergic agents are effective in reducing some of the symptoms of nicotine-withdrawal in both animals (Levin, Briggs, Christopher, & Rose, 1993) and humans (Pomerleau, Pomerleau, Morrell, & Lowenbergh, 1991; Spring et al., 1993; Spring,



Wurtman, Gleason, Wurtman, & Kessler, 1991).

In conclusion, the data suggest that the fundamental disturbances underlying smoking and depression may be mediated by similar neurochemical mechanisms. As reviewed above, antidepressants act on neurotransmitters in ways similar to that of nicotine. Further, the neurochemical changes underlying nicotine withdrawal may be similar to those underlying depression. Nicotine's complex effects on neurobiologic substrates suggest several potential mechanisms by which smoking may provide relief from negative affect and smoking abstinence. These findings, together with the observation that smoking abstinence is associated with negative affect, have recently yielded reports examining the use of various antidepressants as an aid in smoking cessation treatments. These studies are reviewed next.

#### **D. Pharmacologic Interventions for Smokers**

Medications that reduce symptoms of withdrawal and ease the withdrawal process may be beneficial in helping smokers quit smoking. In recent years there has been a growing interest in the use of non-nicotine treatments as potential aids for reducing withdrawal symptoms. These include nicotine antagonists (e.g., mecamylamine), aversive medications (e.g., silver acetate), and medications that help to ease the withdrawal process by reducing withdrawal symptoms (e.g., anxiolytics, clonidine, anorectics and antidepressants) (Cinciripini & McClure, 1998).

In the past ten years, use of anxiolytic and antidepressant medications in the treatment of smoking has received a great deal of attention due to the realization that

smokers who experience varying degrees of negative affect carry a higher risk for cessation failure and relapse (Cinciripini & McClure, 1998). The rationale for the therapeutic usage of antidepressant treatment is based on overlapping theoretical perspectives that include both psychological and physiological bases. From a psychological standpoint, treatment of depressed mood in those suffering from a current, historical, or subclinical depression may lead to increased self-esteem and confidence and improve overall compliance. Alternatively, from a physiologic standpoint antidepressant treatment may help to reduce cessation-related symptomatology, such as depressed mood and irritability, with secondary effects on neurobiologic substrates (Dalack et al., 1995; Glassman, 1993). Additionally, it may help smokers to quit and increase the chances of remaining abstinent by directly modulating neurochemical systems mediated by both nicotine and antidepressants (Dilsaver, 1986; Dilsaver, Hariharan, & Davidson, 1988).

Clonidine, an alpha-2 noradrenergic agonist often used in the treatment of anxiety, has been extensively researched as an aid to smoking cessation and has shown some promise. Several studies have noted clonidine to be most effective in women and less so in men, that are highly-dependent on nicotine (Covey & Glassman, 1990). In women, clonidine primarily reduced symptoms of craving and irritability with minimal effects on symptoms of hunger, depression, and difficulty concentrating.

In contrast to these studies, others have found that clonidine reduced anxiety, tension, irritability, craving, and hunger in heavy smokers that were either male or female (Gourlay & Benowitz, 1996). More recently, in a double-blind randomized dose-response study of transdermal clonidine, Niaura and colleagues (1996) observed that the use of

clonidine across a range of doses was more effective than placebo in helping smokers stop smoking on a short-term basis but not on a long-term basis as most smokers relapsed within 6-12 months. While this medication showed some promise, it requires further study.

Moclobemide, a monoamine oxidase (MAO-A) inhibitor, has recently been investigated as an aid to smoking cessation. The rationale for use of this drug is based on studies that have shown that smokers may have lower brain levels of MAO-A and therefore, may smoke to increase endogenous levels of monoamine transmitters and thus improve mood (Fowler et al., 1996). Based on a recent study involving 88 smokers, it was concluded that while moclobemide was not effective in reducing withdrawal symptoms, it did significantly enhance cessation rates at six-month post-treatment follow up but not at one year (Berlin, Said, Spreux-Varoquaux, Launay et al., 1995). As this is the only published study that has examined moclobemide, additional research is needed before any conclusions can be drawn about its potential effectiveness.

Bupropion hydrochloride (Zyban), an antidepressant with modest dopamine reuptake inhibiting properties, was recently approved (1998) by the Food and Drug Administration as an effective medication for smoking cessation. In a recent study that provided evidence to support the efficacy of bupropion, Hurt and colleagues (1997) examined 615 nondepressed smokers, with or without a history of depression, who were randomly assigned to receive either placebo, 150 mg or 300 mg of sustained-release bupropion therapy. Bupropion was initiated seven days prior to the participants target quitting date and lasted for an additional six weeks. Brief counseling was also offered and

was of low-intensity. At one year follow-up the cessation rates were 23.1% among participants that had received bupropion, irrespective of dosage, and 12.4% among those who had received placebo. Results indicated that bupropion, in combination with counseling, increased smoking cessation rates. While the effectiveness of this agent has thus far been demonstrated in only a few placebo-controlled clinical studies, further long-term treatment outcome studies are needed.

On a similar note, a few clinical trials have shown that the use of doxepin hydrochloride, a tricyclic antidepressant, was superior to placebo in controlling withdrawal symptoms (Edwards, Murphy, Downs, Ackerman, & Rosenthal, 1989). In one study, that compared individuals receiving doxepin to those receiving placebo, the doxepin-treated individuals reported less frequent and less intense symptoms during cessation; differences in actual smoking cessation, however, did not occur (Edwards et al., 1989). In another double-blind study with 19 adults receiving either 150 mg of doxepin or placebo, all individuals taking doxepin were significantly more likely to be abstinent one week after cessation and seven doxepin individuals remained abstinent nine weeks after cessation (Murphy, Edwards, Downs, Ackerman, & Rosenthal, 1990). Moreover, doxepin was most effective in relieving craving in comparison to other withdrawal symptoms. These results have provided evidence that doxepin aids smoking cessation and may do so by reducing the intensity of specific withdrawal symptoms.

Serotonergic drugs, such as the serotonin uptake inhibitors, have also been considered for application in treatment of smoking cessation. To date, only a few studies have endeavored to examine the efficacy of serotonergic uptake inhibitors in reducing

withdrawal symptoms and thus the data is considered preliminary. Clinical trials have suggested that fluoxetine hydrochloride (Prozac), a selective serotonin uptake inhibitor, is effective and may be beneficial primarily for smokers with a history of depression. For example, evidence from double-blind, randomized trials have produced results showing that fluoxetine can ameliorate affective symptoms in smokers prior to (Dalack et al., 1995) and following cessation and improve quit rates (Niaura et al., 1997).

In a multicenter, randomized, double-blind, dose response study Niaura and colleagues (1997) examined the efficacy of fluoxetine for smoking cessation in 989 healthy, nondepressed smokers and randomized them to receive placebo, 30 mg or 60 mg fluoxetine per day and nine sessions of cognitive-behavioral therapy. Participants were treated for 10 weeks and followed for 6 months following treatment. The primary outcome measure was survival time from quit day until self-reported smoking (e.g., drop-outs). Results indicated that the drop out rate (19.5%) by the end of 10 weeks did not differ by treatment condition. Survival analysis, however, indicated a significant treatment effect, with fluoxetine conditions outperforming placebo, but not one another. While the effects of fluoxetine on long-term abstinence rates were not reported in this preliminary trial, results suggested that fluoxetine may hold promise as an adjunctive aid to smoking cessation.

In a study that examined a three week treatment with fluoxetine or placebo (prior to quitting smoking) in a small group of heavy smokers with a history of major depression, results indicated a significant within-subject change for scores on the Beck Depression Inventory and the POMS subscales for the group treated with fluoxetine

(Dalack et al., 1995). Of the POMS subscales, the most significant reduction was observed with the anger subscale. While an ameliorating effect was found within the fluoxetine-treated group, the study failed to detect significant differences between the placebo and fluoxetine group. Methodologic shortcomings, including a small sample size and insufficient length of fluoxetine treatment, may have hampered a clearer understanding of these findings.

In another study that examined the effects of fluoxetine on the development of major depression after smoking cessation in nondepressed participants with or without a history of depression, Borelli and colleagues (1996) found that 7% of participants had developed major depression following three months of treatment. These participants all had a history of depression and while none of them had received the highest dose of fluoxetine (60 mg), 90% had received the lower dose of fluoxetine (30 mg) and 10% had received placebo. These results suggest that individuals with even minimal amounts of affective symptoms prior to treatment may potentially develop depression following smoking cessation. Moreover, a higher dose of fluoxetine may prevent the onset of depression following cessation.

Investigation of the effect of fluoxetine on smoking cessation has also involved alcoholic smokers with or without major depression (Naranjo, Kadlec, Sanhueza, Woodley-Remus, & Sellers, 1990; Cornelius et al., 1997). While the findings have been inconclusive it appears that selective 5-HT agonists, including fluoxetine, show promise in decreasing smoking among depressed alcoholic smokers with no efficacy in reducing smoking in nondepressed alcoholic smokers. For example, Naranjo and colleagues (1990)

examined the effect of fluoxetine on quitting smoking in nondepressed alcoholic smokers and found that fluoxetine did not significantly reduce smoking.

Further investigation of the use of fluoxetine has focused on its effectiveness in the reduction of nicotine withdrawal-induced weight gain. Increased caloric intake and weight gain frequently occur during smoking cessation. These symptoms have been shown to be related to 5-HT as increased neurotransmission of 5-HT is associated with satiety (Blundell, 1992) and reduced carbohydrate intake (Wurtman & Wurtman, 1979). Fluoxetine has been found to reduce nicotine withdrawal-induced weight gain in smokers subjected to reduced smoking (Pomerleau, Pomerleau, Morrell, et al., 1991). Accordingly, others have shown that increased caloric intake and weight gain during smoking abstinence was prevented with the use of d-fenfluramine, a serotonergic agonist (Spring et al., 1991).

In sum, pharmacologic treatment with antidepressants as an adjunct to smoking cessation offers intuitive appeal and has been shown to be effective for some smokers. Despite the fact that these pharmacologic aids have received limited examination, they may be beneficial for smokers with risk factors of past or current depression, use of tobacco smoking to manage dysphoric mood states, or previous unsuccessful smoking cessation attempts that were associated with dysphoric states or a clinical depression.

Finally, the above findings are intriguing because they suggest that pharmacologic treatments for smoking cessation may be effective and work via different neurobiologic substrates. For example, while the efficacy of clonidine appears to be related to its ability to reduce craving (Glassman et al., 1988), other aids such as fluoxetine, that have

minimal effects on craving, are potentially effective at reducing other cessation-related symptoms, such as depressed mood and weight gain (Dalack et al., 1995; Spring et al., 1991). These findings in combination with the fact that smokers are a heterogeneous group and smoking is a complex behavior suggest that cessation rates in smoking may be increased by tailoring treatment to individual smoker characteristics.

### **E. Mood Lowering Effect of Tryptophan Depletion**

Several neurochemical systems altered by antidepressant medications are being studied in an attempt to assess their role in smoking behavior. Serotonin, for example, has received a good deal of attention since alterations, and possibly dysfunctional neurotransmission, in this system has been associated with nicotine withdrawal and smoking behavior (Benwell et al., 1990; Ishikawa et al., 1999; Lerman et al., 2000). While interest in serotonin's role in smoking and smoking cessation is growing, laboratory studies examining the direct effects of brain serotonergic manipulation on smoking behavior in smokers is sparse.

One method for studying 5-HT function in humans is the acute tryptophan depletion strategy. Individuals ingest an amino acid mixture containing all of the essential amino acids except tryptophan or a similar mixture containing tryptophan. By temporarily lowering 5-HT levels through depletion of its precursor tryptophan, this research strategy theoretically provides information about the behavioral consequences of lowering brain 5-HT.



Due to its inability to cross the blood-brain barrier, 5-HT is produced within the brain. Its synthesis depends on intake of its precursor tryptophan, an essential amino acid acquired entirely from the diet. Studies have shown that increases and decreases of dietary tryptophan intake lead to corresponding changes in brain tryptophan and 5-HT levels in laboratory animals (Fernstrom & Hirsch, 1975). In lieu of dietary tryptophan depletion, administration of amino acid mixtures devoid of tryptophan to animals similarly leads to a temporary and marked reduction of plasma and brain tryptophan, brain 5-HT and 5-HIAA, and cerebrospinal fluid (CSF) levels of tryptophan and 5-HIAA (Biggio, Fadda, Fanni, Tagliamonte, & Gessa, 1974; Moja, Cipolla, Castoldi, & Tofanetti, 1989; Young, Ervin, Pihl, & Finn, 1989).

The onset of action of the tryptophan-depleting mixture in animals has been shown to be rapid, with maximal reduction in plasma tryptophan and brain 5-HT concentrations occurring within 2 hr of ingestion (Young et al., 1989; Moja et al., 1989). The mechanism of this effect is believed to be due to the depletion mixture inducing hepatic protein synthesis and causing rapid depletion of plasma tryptophan as the free tryptophan in plasma is incorporated into protein (Moja et al., 1991). This effect, in turn, is believed to cause a significant decrease in the rate of brain 5-HT synthesis.

In humans, it is also possible to induce a temporary and marked reduction of plasma tryptophan and brain 5-HT with the tryptophan depletion strategy. Ingestion of 50 to 100 g of a tryptophan-depleting amino acid mixture has been shown to produce a 70% to 90% reduction in free and total plasma tryptophan levels with maximal reductions occurring 5 hr after ingestion of the mixture (Young, Smith, Pihl, & Ervin, 1985).

Recently, Nishizawa and colleagues (1997) reported that acute tryptophan depletion lowered brain serotonin synthesis by approximately 90% (as evidenced by the use of PET imaging and (C11)-methyl tryptophan as a tracer) in 15 healthy young adults without personal or family psychiatric history. Similarly, Carpenter and coworkers (1998) reported that tryptophan depletion in healthy participants led to a significant reduction of CSF 5-HIAA during continuous sampling. Taken together, these data suggest that acute depletion of tryptophan in both animals and humans results in a marked reduction of brain 5-HT synthesis and turnover.

The short-term tryptophan depletion paradigm has been used to investigate many psychological and physiological functions such as cognition, sleep, memory and learning, anxiety, aggression, and the mechanism of action of psychotherapeutic agents (Benkelfat, Ellenbogen, Dean, Palmour, & Young, 1994; Cleare & Bond, 1995; Delgado et al., 1990; Moeller et al., 1996; Park et al., 1994; Smith, Pihl, Young, & Ervin, 1987). Other studies have investigated the effects of acute tryptophan depletion in: (a) healthy volunteers with (Benkelfat et al., 1994) and without a family history of affective disorders (Knott, Howson, Perugini, Ravindran, & Young, 1999; Smith et al., 1987; Young et al., 1985); (b) medication-free patients with major depression (Delgado et al., 1990); (c) autistic disorder (McDougle et al., 1996); (d) panic disorder (Goddard et al., 1994), and (e) obsessive-compulsive disorder (Smeraldi et al., 1996).

As the brain serotonergic system has been implicated in the regulation of mood, a number of studies have also examined the effects of acute tryptophan depletion on mood and have provided evidence to support its mood-lowering effects. For example, some (but

not all) studies have shown that in healthy young men acute tryptophan depletion induces transient, nonclinical, mild increases in depressive affect (Carpenter et al., 1998; Knott, Howson, et al., 1999; Young, Pihl, & Ervin, 1988; Young et al., 1985). In a study that examined gender differences during tryptophan depletion, results showed that healthy women had greater mood responses than men (Ellenbogen, Young, Dean, Palmour, & Benkelfat, 1999).

Others have reported that healthy volunteers with a positive history of major affective disorder showed significantly more depressed mood after tryptophan depletion than healthy individuals without such a family history (Benkelfat et al., 1994; Klaassen et al., 1999). Moreover, these mood changes were detected after 4 to 6 hr of tryptophan depletion and were no longer detected after 24 hr (Klaassen et al., 1999). Finally, others have indicated that depletion of tryptophan induced a depressive relapse in remitted patients with depression (Delgado et al., 1990).

In marked contrast to all of these studies, however, are those which have been unable to demonstrate a significant change in mood after acute tryptophan depletion. For example, Leyton and coworkers (2000) reported that tryptophan depletion did not have a significant mood-lowering effect in remitted depressed subjects who were also medication-free. Similarly, others have observed that tryptophan depletion did not induce significant behavioral or mood effects in healthy women (Moreno et al., 1999), obsessive compulsive disorder (Barr et al., 1994), medication-free patients with depression (Delgado et al., 1994), or panic disorder (Goddard et al., 1994).

Taken together, studies using the tryptophan depletion paradigm to investigate behavioral changes in humans have produced inconsistent findings. However, it is possible that the differential findings may have resulted from methodological differences among studies. Thus, the use of different mood measures and the timing of measures, the inclusion of individuals with varying or undetermined personal or family histories of psychiatric illness, examination of healthy individuals versus individuals with a psychiatric condition, and gender effects may have led to the mixed findings within the literature. Additionally, another possible explanation for these findings might be that it is more difficult to transiently disrupt brain neurochemical function in stable and healthy individuals than in individuals with a susceptibility to depression and/or perturbations of serotonergic brain functioning.

#### **F. Rationale And Objectives**

While there has been increased interest in the role of serotonergic activity in mediating the biobehavioral effects of smoking abstinence and acute smoking, as well as in the efficacy of serotonergic agents in treating smoking-withdrawal symptoms, to date there have been no laboratory studies examining the direct effects of serotonergic manipulations in smokers. Foremost among the present study's intentions was to better understand the extent to which 5-HT mediates the effects of acute smoking abstinence.

First, the present study sought to examine the modulating role of lowered levels of plasma and brain 5-HT (via a tryptophan-deficient amino acid mixture) on smoking abstinence effects. More specifically, negative mood states, the nicotine withdrawal

syndrome and EEG indices of cortical arousal were examined in response to the administration of the amino acid mixtures in smokers temporarily abstaining from cigarettes. As part of this objective, the study also examined whether resumption of cigarette smoking could significantly reduce and abate the mood, withdrawal and EEG deficits induced by both amino acid mixtures and return these levels to pre-mixture and pre-smoking abstinence baseline values.

Related to this objective, is the issue of the acute smoking response itself. Previous investigations have repeatedly shown that the smoking of a single cigarette results in EEG arousal and alterations in mood (Domino & Matsuoka, 1994; Domino et al., 1995; Knott 1990a, 1990b; Knott, Harr, & Lusk-Mikkelsen, 1998) as well as alteration of cognitive processes as evidenced by improved performance efficiency on a range of information processing tasks (Heischman et al., 1994; Pritchard & Robinson, 1998).

Pharmacological studies attempting to unravel the neurochemicals mediating these smoking-related changes have found pre-treatment with nicotinic cholinergic and dopaminergic antagonists to block some, but not all, aspects of the acute smoking response (Knott, Harr, Illivitsky, & Mahoney, 1998; Walker, Mahoney, Illivitsky, & Knott, 1999). This suggests, therefore, that these smoking-induced changes may be mediated by interactions with other neurotransmitters. As human studies to date have not examined the role of other neurotransmitter systems, including 5-HT, in mediating the acute smoking response, this study also examined the role of reduced 5-HT, via tryptophan depletion, in generating the acute smoking response.

Several critical variables were therefore manipulated. Plasma tryptophan levels were altered via ingestion of an amino acid mixture devoid of tryptophan. Participants also ingested a balanced control amino acid mixture containing tryptophan. Alterations in negative mood states, the nicotine withdrawal syndrome, and electrocortical activity were induced by temporary cigarette abstinence. Abstinence was induced by requiring participants to abstain from smoking for 5 hr beginning immediately after the collection of study measures at baseline. Hence, by manipulating abstinence *after* baseline study measures were collected the question of whether deficits induced by the tryptophan-deficient mixture in abstinent smokers returned to pre-smoking abstinence baseline levels following the resumption of smoking was assessed.

Several dependent measures were used to examine the effect of the amino acid mixtures on the biobehavioral changes of smoking abstinence and the acute smoking response. These included assessment of negative mood states, nicotine-withdrawal symptoms, electrocortical activity (as measured by quantitative EEG), total plasma tryptophan levels and expired-breath carbon monoxide (CO) concentrations.

With respect to the affect component of smoking abstinence, subjective mood and withdrawal effects following abstinence have been well documented. Increments in self-reported negative affect ratings (e.g., depressed mood, anger, irritability) and withdrawal symptoms (e.g., craving and drowsiness) have been observed immediately after the discontinuation of tobacco use (Hatsukami et al., 1984, 1987; Hughes & Hatsukami, 1986). Accordingly, smoking-induced mood alterations, including subjective alertness

(Knott, Harr, & Lusk-Mikkelsen, 1998) and calming effects (Russell et al., 1974) have been demonstrated with cigarette smoking.

EEG has also been employed in studies to assess the central impact of serotonergic compounds, including antidepressant drugs (Saletu, Grunberger, & Rajna, 1983; Saletu, 1987). Several studies have now provided evidence supporting the use of EEG as a sensitive indicator of both 5-HT bioavailability and of nicotine. For example, Knott and colleagues (1999) found that tryptophan depletion was associated with a slowing of the EEG, as evidenced by increases in slow (delta) wave amplitude, while fenfluramine, a serotonergic reuptake blocker and releaser, produced an increase in fast (beta) wave activity when administered after the tryptophan depletion mixture (Knott, Howson, et al., 1999). Moreover, an abundance of literature has shown that tobacco smoking and abstinence are associated with electrocortical arousal and hypoarousal, respectively (Knott, 1990a, 1990b; Knott, Harr, & Lusk-Mikkelsen, 1998; Pickworth et al., 1995; Pritchard, 1991).

### **G. Hypotheses**

Cigarette smokers were assessed during two separate test sessions with each participant serving as his own control. One test session followed ingestion of a balanced placebo amino acid mixture and another followed ingestion of the tryptophan-deficient amino acid mixture. The two test sessions were separated by a minimum of three days. In each of the test sessions ratings of mood and smoking withdrawal, and EEG measurement were taken in a standard sequence beginning at baseline, 5 hr after the ingestion of the

amino acid mixtures and during smoking abstinence, after sham smoking and again after the smoking of a single cigarette.

The study's primary hypothesis was that acute smoking-abstinence would induce increments in self-reported negative affect and withdrawal symptoms and alter EEG activity following ingestion of the tryptophan-depleted mixture relative to the balanced placebo mixture. Specifically, in abstinent smokers the tryptophan depletion mixture compared to the placebo mixture was expected to significantly increase withdrawal and dysphoria scores and produce a slowing of EEG activity (e.g., a shift in power from higher to lower frequencies with delta, theta, and alpha<sub>1</sub> increments and alpha<sub>2</sub> and beta decrements). That is, the smoking-induced withdrawal effects were expected to be relatively greater and more marked following the tryptophan depletion session. Thus, a finding that the acute lowering of plasma tryptophan alters the biobehavioral profile induced by smoking abstinence would point to 5-HT's potential involvement in nicotine's biobehavioral effects.

A second set of hypotheses revolved around the examination of the acute effects of cigarette smoking. It was anticipated that a cigarette smoking challenge would reduce any augmented withdrawal effects induced by the mixtures in abstinent smokers and in effect would reverse those changes to pre-treatment baseline values. Specifically, cigarette smoking was expected to attenuate smoking withdrawal effects and negative affect and to produce an EEG profile characterized by a shift in power from low to higher frequencies (alpha<sub>2</sub> and beta increments) in both the tryptophan depletion group and the balanced placebo group. Moreover, negative affect and withdrawal ratings and EEG



activity following smoking were expected to return to pre-treatment (e.g., before smoking abstinence and before ingestion of the amino acid mixtures) baseline values.

Although, to date, only a few EEG studies have examined lateralized cortical effects in smokers (as suggested by lateralized-affective processor models) and evidence of lateralized effects are tenuous, it was further hypothesized that following the tryptophan-depleted mixture relative to the balanced placebo mixture EEG alterations (theta and  $\alpha_1$  increments) would be more evident in frontal recording sites rather than posterior scalp sites and in the right cerebral hemisphere rather than the left. While the smoking abstinent state was expected to show relatively increased right-frontal and/or decreased left-frontal EEG activity, these effects were expected to be stronger in the tryptophan depletion condition relative to the placebo condition due to the induction of negative affect associated with reduced brain levels of 5-HT. In contrast, it was hypothesized that a cigarette smoking challenge would elevate left-frontal hemispheric arousal (e.g., smoking-related  $\alpha_2$  and beta increments) and decrease right-frontal activity in abstinent smokers and following ingestion of either amino acid mixture.

## **Method**

### **A. Participants**

Eighteen male smokers, with ages ranging between 18 and 33 years, were recruited through local newspaper advertisements. The study included male participants only, as studies have demonstrated that mood (Schechter, Bachman, Vaitukaitis, Phillips, & Saperstein, 1989), smoking behavior (Steinberg & Cherek, 1989), withdrawal

symptomatology (O'Hara, Portser, & Anderson, 1989) and EEG (Lamb, Ulett, & Masters, 1953) may be affected by menstrual cyclicity in women smokers (for review, see Pomerleau, Pomerleau, & Garcia, 1991). Accordingly, the rate of 5-HT synthesis induced by acute tryptophan depletion in human brain using PET scans has been shown to be significantly higher in normal males than females (Nishizawa et al., 1997). As a result of the potential variance attributable to gender, it was felt that the current study may have failed to find significant effects if genders were combined.

An initial telephone screen (Appendix B) was used to determine whether participants met initial criteria for entry into the study. For study inclusion all participants were required to be nicotine dependent, have smoked (with inhalation) a minimum of 15 cigarettes per day for the past three years, smoke within 15 min of awakening and continue smoking throughout the morning, have a history of nicotine withdrawal and experience withdrawal symptoms following smoking-abstinence throughout the morning, be within 15% of ideal body height in relation to weight, and have a minimum score of 7 on the Fagerstrom Tolerance Questionnaire (FTQ) (Fagerstrom, 1978). The FTQ (Appendix C) was used to determine the degree of nicotine dependence and the DSM-IV criteria for Nicotine Withdrawal (Appendix A) was used to determine a history of a nicotine withdrawal following smoking abstinence.

The FTQ is a widely used questionnaire that consists of eight items hypothesized to be related to physiological dependence to nicotine. The behaviors evaluated by the FTQ, such as, "smoking soon after awakening" and "rating the first cigarette of the day as the most difficult to do without" are proposed markers of nicotine dependence. The FTQ

was completed within five minutes and the responses to the questions were individually scored to provide an overall total score in the range of 0 -11 points. High dependence was defined as a score of seven and above and low dependence as a score of six or less. In a study that examined the validity of the FTQ, results showed that nearly all of the items discriminated smokers with high dependence from those with low dependence (Fagerstrom, 1978).

The DSM-IV criteria for nicotine withdrawal includes eight indicators of withdrawal of which four must be present. These indicators have been found to be related to greater tobacco dependence as several studies have reported a relationship between heavy smokers, as defined by the FTQ, and more withdrawal discomfort during abstinence (Fagerstrom, 1980; Hughes & Hatsukami, 1985, 1986).

After participants met these initial qualification criteria, written informed consent (see Appendix D) was obtained and they were scheduled for a more thorough medical screen with a psychiatrist who obtained their medical and psychiatric history. Those with significant past or present alcohol/drug abuse or dependence, psychiatric illness, or significant medical illness were excluded from the study. Further exclusion criteria were a history of allergies to the test substances, taking medications (prescription and non-prescription) that affected appetite or CNS functioning and past or current mood disorder in a first-degree relative.

Calculation of a suitable sample size (i.e., statistical power analysis) was according to information provided by Stevens (1992). Previous tryptophan depletion studies have obtained significant results with 12-15 participants per group (Knott,

Howson, et al., 1999; Leyton et al., 2000; Young et al., 1985). In the study by Knott and colleagues (1999) significant mood and EEG findings following tryptophan depletion occurred at the .05 level in a sample of 14 healthy males.

As tryptophan depletion effects have been generally found to be robust, particularly in vulnerable individuals with perturbed serotonergic functioning, it was believed that in the present study, tryptophan depletion in abstinent smokers would produce a greater effect size than that obtained with healthy nonsmokers. The means and standard deviations from previous studies which have utilized tryptophan depletion in nonclinical populations (Abbott et al., 1992; Knott, Howson, et al., 1999) were used to determine an effect size and sample size (Stevens, 1992). An effect size of .46 indicates that a sample size of 18 would provide .80 power with an alpha of .05 for a two-tailed test. All participants successfully completed the entire study protocol.

## **B. Study Design**

Participants attended the laboratory for one orientation session, to become familiar with the experimental protocol, and two additional test sessions which were separated by a minimum three day interval to reduce any potential interference from the amino acid mixture ingested in the first session. Each test session consisted of two test phases. In the first test phase EEG monitoring, mood and withdrawal ratings, CO levels and plasma total tryptophan blood levels were sampled before (at baseline) and 5 hr after ingestion of a tryptophan-deficient amino acid mixture drink or a nutritionally balanced placebo amino acid mixture containing tryptophan and during smoking. Each participant was

tested in both amino acid treatment conditions and served as his own control. The mixtures were administered double-blind and assigned in a counterbalanced order such that half of the participants were assigned randomly to receive the balanced treatment first and active tryptophan-deficient treatment second ( $n = 9$ ) and for the remaining participants the treatment was reversed ( $n = 9$ ). In the second test phase, EEG monitoring, mood and withdrawal ratings and CO levels were sampled after each individual smoked an unlighted cigarette (sham smoking) followed by the smoking of a single lighted cigarette.

### **C. Procedure**

Following the medical screen (on the same day) participants attended the laboratory orientation session with the purpose of familiarizing them with the study protocol. The orientation followed a fixed schedule as outlined in Table 1.

The orientation session included: (a) an explanation of the nature of the study including the possible risks and side effects of the tryptophan depletion mixture; (b) provision of a breath sample for the assessment of CO; (c) application of two scalp and two facial electrodes to allow participants to become familiar with the EEG recording procedure; (d) a practice EEG recording trial with eyes closed while resting and instructions on how to reduce non-cerebral artifacts during the recording; (e) a practice trial of smoking both an unlighted (sham smoking) and lighted cigarette, and (f) completion of smoking withdrawal and mood rating scales.

Table 1. Orientation Schedule

<b>Time</b>	<b>Procedure</b>
8:00 a.m.	Arrival at laboratory; briefing and consent
8:15 a.m.	Medical screen with physician
8:30 a.m.	Expired breath carbon monoxide sample
8:40 a.m.	EEG hook-up procedure
9:00 a.m.	Trial EEG recording
9:15 a.m.	Trial sham/cigarette smoking
9:30 a.m.	Trial (POMS & Smoking Withdrawal) Questionnaires
9:45 a.m.	Questions
10:05 a.m.	Appointments
10:10 a.m.	Final Instructions
10:20 a.m.	Departure

At the end of the orientation session, those interested in complying with the study protocol were scheduled for the experiment. Each participant was given an appointment card with the time and date of the two test sessions and was given pre-test session instructions to follow. Participants were instructed to abstain from eating, caffeine, alcohol, illicit drugs, prescriptive and non-prescriptive medications after midnight (beginning at 12:00 a.m.) on the evening prior to each test day. They were required to abstain from all food and beverage (except water) on each of the test mornings. This overnight fast helped to ensure that the effects of the amino acid mixtures would be more similar between individuals. Furthermore, participants were also instructed to continue smoking as usual (their own brand, ad libitum) on the morning of each test day and to smoke one cigarette immediately prior to their arrival at the laboratory. Each session followed an identical timetable.

Upon their arrival at the laboratory, each participant was seated in a reclining chair in isolation in a room and was prepared with electrodes for EEG monitoring. Approximately 15 min before the first (baseline) test battery assessment, participants were asked to smoke one of their own cigarettes in order to make certain that they were not deprived at baseline. Following electrode placement, participants were exposed to the procedural testing protocol as outlined in Table 2.

The pre-mixture/pre-smoking abstinence (baseline) test battery session (TB1) included: a 5 minute recording of EEG, completion of self-reports of mood state and withdrawal symptoms, an expired-breath CO sample, and blood sampling (5 ml) by a nurse for analysis of pretreatment baseline levels of total plasma tryptophan. Following

Table 2. Test Session Schedule

Time	Test Battery (TB) Assessments	Procedure
8:00 a.m.		Arrival
8:00 - 8:20 a.m.		EEG hookup and cigarette smoking <sup>a</sup>
8:30 - 8:50 a.m.	TB1 <sup>b</sup> (pre-mixture/pre-smoking abstinence baseline assessments)	
8:50 a.m.		Abstain from smoking
9:00 - 9:10 a.m.		Ingest amino acid mixture
9:10 a.m.- 2:10 p.m.		Post-mixture 5 hr waiting period
2:15 - 2:35 p.m.	TB2 <sup>b</sup> (post-mixture/post-smoking abstinence assessments)	
2:35 - 2:45 p.m.		Sham smoke <sup>d</sup>
2:45 - 3:05 p.m.	TB3 <sup>c</sup> (post-mixture/post-sham smoke assessments)	
3:05 - 3:15 p.m.		Cigarette smoke <sup>e</sup>
3:15 - 3:35 p.m.	TB4 <sup>c</sup> (post-mixture/post-cigarette smoke assessments)	
3:45 p.m.		Protein snack and Tryptan tablet <sup>f</sup>
4:00 p.m.		Departure

Note: <sup>a</sup>Participants were required to smoke one cigarette from their own brand. <sup>b</sup>TB1 and TB2 included CO breath and plasma tryptophan blood sampling, EEG and mood ratings. <sup>c</sup>TB3 & TB4 included CO breath sampling, EEG and mood ratings. <sup>d</sup>Sham smoking involved 10 puff inhalations (1 puff per 60 s) on a non-lighted cigarette. <sup>e</sup>Cigarette smoking involved 10 puff inhalations (1 puff per 60 s) on a lighted cigarette (from their own brand). <sup>f</sup>Participants were given a protein snack and a 1 g tablet of tryptophan (Tryptan).



completion of baseline assessments, the electrodes attached to the face and ears were removed as they could produce some discomfort when left on for an extended period of time. The baseline period lasted for approximately 20 min. Participants then ingested one of the two amino acid drink mixtures, either the balanced or tryptophan-depleting drink.

A second test battery session (TB2), identical to TB1 (see Table 2) was repeated 5 hr after participants ingested the experimental mixtures. This 5 hr interval was chosen on the basis of previous reports indicating that plasma tryptophan levels are reduced by as much as 70-90% and lowest within 5 hr of amino acid consumption (Benkelfat et al., 1994; Delgado et al., 1990; Young et al., 1985). During the 5 hr waiting period, participants were able to sit quietly in their testing room and were exposed to a standard selection of affectively neutral videos and magazines.

Immediately following the second test battery assessment (TB2), the second phase of testing began. Participants in both amino acid treatment conditions first inhaled on an unlighted cigarette (sham smoke) followed by the smoking of a lighted cigarette of their own brand. This was followed by the third (TB3) and final (TB4) test battery assessments. Test battery sessions TB3 and TB4 were similar to test battery sessions TB1 and TB2 except plasma tryptophan blood levels were not measured.

At the end of each test day, participants were given a 1 g tablet of L-tryptophan and a protein snack which contained foods expected to replete plasma tryptophan. This intervention has been shown to rapidly normalize depleted levels of tryptophan (Young et al., 1989). Participants were allowed to leave the laboratory if the scores on the POMS

depression scale did not show any evidence of depressed mood. Follow-up telephone calls in the evening and the following day were made. Participants were questioned about the presence or absence of adverse symptoms including their mood states.

#### **D. Drug Administration**

##### **1. Amino Acid Mixtures**

Two amino acid mixtures were used and both mixtures were given to each participant in a counterbalanced crossover design. The tryptophan depletion mixture contained 15 powdered amino acids not including tryptophan for a total quantity of 50 g of amino acids, while the balanced placebo mixture contained the same amino acids with 1.15 g of L-tryptophan for a total of 51.15 g, as described by Young and colleagues (1989). The formation of the mixtures is outlined in Appendix E. The balanced placebo mixture contained amino acids that were in proportion to the dose of amino acids contained in human milk. While human milk also includes the non-essential amino acids, aspartic acid and glutamic acid, they were omitted from the study mixtures as they can produce adverse effects at high doses (Young et al., 1989). Removing them was not expected to affect the ability of the tryptophan-depleting mixture to lower plasma tryptophan since tryptophan levels are significantly depleted when other nonessential amino acids are removed from the tryptophan depletion mixture (Moja et al., 1988). The 1.15 g of tryptophan that was used in the balanced placebo mixture was conservative relative to that used by others (Moja et al., 1988) and has been shown to not induce a

significant change in the levels of plasma tryptophan (Young, Tourjman, Teff, Pihl & Anderson, 1988).

The liquid mixtures were prepared a few minutes before consumption. The powdered amino acids were combined with 50 ml of chocolate syrup, 150 ml of water and a one-half packet of saccharin to form a chocolate-based drink. Since the mixtures were somewhat unpalatable, a nose plug was used to minimize olfactory cues and participants were asked to drink the mixtures quickly. Three of the less palatable amino acids, L-Methionine, L-Cysteine, and L-Arginine were encapsulated and taken with water immediately after drinking the mixture. This was followed by chewing on a half stick of cinnamon gum to cleanse the palate of any residual taste.

A 5 ml blood sample was taken before the ingestion of the mixtures for analysis of baseline tryptophan plasma levels and again at 5 hr after ingestion of the mixtures to quantify the degree of depletion of tryptophan manifested in the serum. The blood plasma was used to assess the extent of total tryptophan in plasma. Tryptophan in the ultrafiltrate and in plasma was analyzed by high performance liquid chromatography (Waters  $\mu$ Bondapak C18 reverse phase column) with fluorometric detection.

## 2. Cigarette Smoking

Each participant was tested under two smoking conditions: (a) Sham-smoke, required participants to inhale on an unlit cigarette puffing once every 60 s (as cued by a stopwatch) for a total of 10 puffs over a 10 min interval, and (b) Cigarette-smoke, required participants to inhale on a lit cigarette of their own preferred brand with an

inhalation rate of once every 60 s for 10 min. Sham-smoking served as a placebo condition to control for the behavioral and motoric aspects of smoking and has been employed in previous studies (Knott & Venables, 1977; Knott, 1988, 1989b). These conditions were not randomized and cigarette smoking followed sham smoking in all test sessions. An attempt was not made to utilize standard nicotine/tar yield cigarettes across all participants as their novelty and potentially unacceptable taste could have interfered with the EEG recordings (Knott 1989a).

In order to obtain a measure of degree of smoke inhalation/exposure across each test session, expired alveolar CO levels in parts per million (ppm) were assessed on tidal-breath samples at each test battery session and analyzed by an Ecolyser model 2000 meter.

### 3. L-Tryptophan

At the end of each test day, participants were given 1 g of L-tryptophan (Tryptan) and a protein snack to help normalize their biochemistry and to restore plasma tryptophan. Studies have indicated that the behavioral effects induced by the tryptophan-depleted mixture are reversed by a meal containing tryptophan or by tryptophan supplements (Young et al., 1989).

## **E. Measures**

### **1. Self-Report Measures**

#### **a. *Mood states***

The Profile of Mood States (POMS) self-report questionnaire (McNair et al., 1971) was used to monitor changes in mood throughout the experiment. The POMS is a widely used 65-item adjective checklist that is highly sensitive to mood changes in nonclinical populations. Participants were instructed to rate each adjective using a five-point scale with the labels “not at all,” “a little,” “moderately,” “quite a bit,” and “extremely” according to their feelings at that moment. Six clusters of adjectives (bipolar-mood scales) have been separated using factor-analysis. These include: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia and Confusion-Bewilderment.

The six separate scores were derived by scoring participant’s responses to the 65 adjectives. As well, a Total Mood Disturbance score was derived from the sum of the five POMS scores which reflected negative mood states (Tension-Anxiety, Depression-Dejection, Anger-Hostility, Fatigue-Inertia and Confusion-Bewilderment) minus the POMS score which reflected a positive aspect of mood (Vigor-Activity). Responses to items on the POMS were collected at each of the four test battery sessions (see Table 2).

The psychometric properties of the POMS have been shown to be adequate, with all POMS scales having internal consistent reliabilities in the .90 range. The predictive and construct validity of the POMS have been demonstrated in studies of controlled outpatient drug trials (McNair et al., 1971). As well, several studies have shown that the

POMS is sensitive to changes in mood following depletion of tryptophan in normal healthy males (Knott, Howson, et al., 1999; Smith et al., 1987; Young et al., 1985) and after tobacco abstinence in dependent smokers (Hatsukami et al., 1984, 1985).

*b. Smoking withdrawal*

A self-rated checklist, the Smoking Withdrawal Symptom Checklist (Hughes & Hatsukami, 1986), based on the DSM-IV criteria for Nicotine Withdrawal (APA, 1994) was used as a measure of the nicotine withdrawal syndrome. This scale has been widely used and consists of rating eight withdrawal symptoms on a four point scale (0 = not present, 1 = mild, 2 = moderate, and 3 = severe). Symptoms include: irritable, frustrated or angry, difficulty concentrating, restless, anxious, hunger, depressed mood, sad or feeling blue, and craving or urge to smoke. A total withdrawal score was calculated by the addition of all withdrawal ratings.

The validity of this rating scale has been demonstrated in studies showing that abstinent smokers (e.g., 80%) reported a minimum of four or more DSM-IV nicotine withdrawal indicators, thus implying that a tobacco withdrawal syndrome would have been diagnosed (Cottler et al., 1995). This measure has shown significantly elevated nicotine-withdrawal scores among smokers attempting to quit smoking in either their natural environment or within controlled clinical settings (Hughes, 1991; Hughes & Hatsukami, 1986). Studies have confirmed an increase in these self-reported withdrawal symptoms following acute tobacco abstinence (Hughes et al., 1984, 1987; Hughes & Hatsukami, 1986; Shiffman & Jarvik, 1976) such that withdrawal symptoms were rated

mild during abstinence and increased by 0.5-1.0 point on the three point scale (Hughes et al, 1984; Hughes & Hatsukami, 1986). These self-reported ratings by abstaining smokers have been confirmed by observer ratings (Hatsukami et al., 1987; Hughes & Hatsukami, 1986) and have been shown to remain consistent across repeated trials (Hughes et al., 1984).

## 2. Quantitative EEG

### a. *EEG recording*

During each test session, electroencephalographic activity was recorded at four separate intervals: before (baseline) and 5 hr after the ingestion of the mixtures and smoking abstinence and immediately after sham and real cigarette smoking. At each test battery session, five minutes of EEG activity was recorded with eyes closed while participants were seated in a comfortable chair. Prior to each recording, participants were instructed “to close their eyes, to relax, and remain still”. They were also reminded of the importance of remaining awake and of the possible contaminating effects of eye movements, muscular contractions and body movements on EEG activity.

Electroencephalographic activity was recorded from 6 scalp sites using a monopolar fronto-occipital derivation based on the 10-20 international system (Jasper, 1958). Electrodes were attached to scalp sites, C3, C4, F3, F4, O1, and O2 and electrode impedances below 5 kohms were obtained. These sites were selected in view of previous studies which have shown that frontal recording sites are sensitive to negative-affective states (Gilbert, 1988) and frontal, central and occipital recording sites are sensitive to

acute smoking and smoking abstinence manipulations (Knott, 1989a). The electrode sites were positioned on homologous left (F3, C3, O1) and right (F4, C4, O2) frontal, central and occipital hemispheric regions. Two linked ear electrodes were used as references for the scalp electrodes and additional electrodes, positioned near the orbital ridges and external canthi of the right eye, were used to monitor vertical and horizontal electro-oculographic (EOG) eye movement activity. Electrical activity was collected at a sampling rate of 256 Hz, a high-pass filter setting of 0.3 Hz and a low-pass filter setting of 30 Hz, and a discrete 60 Hz notch filter to reduce background electrical noise.

*b. EEG data reduction*

Electrical activity was digitized at 256 Hz, stored to disk, and analyzed off-line after completion of the study. Digitized EEG segments were visually analyzed and all segments associated with muscle activity, body movement or vertical/horizontal ocular contaminations were omitted from analysis. For each test battery session, a minimum of thirty, artifact-free, two second epochs of EEG activity were processed via Fast Fourier Transform (FFT) analysis for determination of band amplitude values for the following frequency bands: delta (0.75-3.75 Hz), theta (3.75-7.75 Hz), alpha<sub>1</sub> (7.75-10.75 Hz), alpha<sub>2</sub> (10.75 -13.75 Hz) and beta (13.75- 20 Hz). The EEG bands for alpha were split into two subfrequency ranges in view of previous reports which showed that acute smoking may differentially affect these two subfrequency ranges (Domino & Matsuoka, 1991; Domino et al., 1992; Knott, Hooper, Lusk-Mikkelsen, & Kerr, 1995; Pritchard, 1991). Prior to statistical analyses, EEG absolute values were log-transformed to



approximate normal distributions on the basis of the following log transformation:  $(\log(x))$  where  $x$  = absolute amplitude in band) (Prichep & Easton, 1987).

## **F. Statistical Analyses**

For each dependent measure, the effect of the amino acid mixtures was examined via separate univariate analysis of variance (ANOVA) procedures for repeated measures. Dependent variables included: (a) total plasma tryptophan concentrations; (b) expired-breath CO (ppm) levels; (c) ratings on the POMS and Smoking Withdrawal Symptom Checklist, and (d) EEG frequency band amplitudes.

Factors used in ANOVA procedures were: (a) treatment condition (two levels: balanced mixture, tryptophan depletion mixture), and (b) time (four test battery sessions: pre-mixture/pre-smoking abstinence (TB1), post-mixture/post-smoking abstinence (TB2 hr), post-mixture/post- sham smoke (TB3), and post-mixture/post-cigarette smoke (TB4) (except for plasma tryptophan levels where only two time points were used: TB1 and TB2).

Analyses of mood ratings were carried out by separate 2 (treatment condition) x 4 (test battery sessions) repeated measures ANOVAs for each of the six subscales of the POMS and for total mood disturbance. Similarly, separate 2 (treatment condition) x 4 (test battery session) repeated measures ANOVAs were used to analyze each item and the total withdrawal discomfort score from the Smoking Withdrawal Symptom Checklist.

For the EEG analyses additional factors were employed including hemisphere (two levels: right [F4, C4, O2] vs left [F3, C3, O1]) and topographic region (three levels:

frontal [F3, F4] vs. central [C3, C4] vs. occipital [O1, O2]). Amplitude indices for each EEG band were subjected to separate 2 (treatment condition) x 4 (test battery session) x 2 (hemisphere) x 3 (region) repeated measures ANOVA.

Validation of the biochemical effect of the pharmacological manipulations carried out in the study were assessed by analyzing changes in: (a) blood samples for total plasma tryptophan concentrations both before and 5 hr after administration of amino acid mixtures, and (b) expired breath CO (ppm) samples both before and after ingestion of amino acid mixtures and after sham and cigarette smoking. To determine if plasma tryptophan levels would be lowered following the ingestion of the tryptophan-deficient mixture but not the balanced control mixture, a 2 (treatment condition) x 2 (test battery session) repeated measures ANOVA was performed. To assess whether changes occurred in expired-breath CO levels over the course of the experiment, a 2 (treatment condition) x 4 (test battery sessions) repeated measures ANOVA was computed.

Due to the large number of statistical tests, significance levels for EEG analyses were adjusted, where appropriate, with the more conservative Greenhouse-Geisser corrected probability levels to protect against alpha inflation and violation of Type I errors. Also, in an effort to reduce the number of statistical tests significant hemisphere and region effects were followed up only if they interacted with the amino acid conditions and/or time effects.

When ANOVAs revealed any significant ( $p < .05$ ) main and/or interaction effects, these were further assessed via t-tests. To further protect against Type I errors, Bonferroni-adjusted significance levels, in which the alpha level of each pairwise

comparison was divided by the total number of comparisons, were utilized in all post-hoc tests. Although the primary interest of these follow-up analyses was focused on measures exhibiting significant interactions involving treatment condition, significant effects of time were also followed up if they were significant on their own, or in the case of EEG measures, if they interacted with condition, region, and/or hemisphere.

Follow-up of main effects of time were specifically carried out to determine: (a) whether acute smoking abstinence resulted in significant withdrawal effects [for acute abstinence, comparisons were limited to t-tests which compared pre-mixture/pre-smoking abstinence values with post-mixture/post-smoking abstinence values (see Table 3)]; (b) whether the abstinence-induced effects were altered and reduced with the smoking of a single cigarette [for the acute effects of smoking, comparisons were limited to t-tests which compared post-mixture/post-smoking abstinence with post-mixture/post-sham smoke and post-cigarette smoke (see Table 3)], and (c) whether the abstinence-induced effects were reversed by cigarette smoking as indicated by the return of post-cigarette smoking measurements to pre-abstinence baseline measurements [for the reversal effect, comparisons were limited to analyses which compared pre-mixture/abstinence baseline values to post-mixture cigarette smoke values (see Table 3)]. When significant time effects not involving treatment conditions were analyzed post-hoc these follow-up tests were carried out with data from the two amino acid conditions being averaged at each separate test battery session.

**Table 3. Schemata of Post-hoc t-test Comparisons Following Significant Main Effects of Time used to Determine the Effects of Smoking Abstinence and the Acute Effects of Cigarette Smoking over the Course of the Study**

<b>Hypothesized Effect</b>	<b>Post-hoc Comparison of Test Battery (TB) Assessments to Determine Hypothesized Effect</b>
Smoking Abstinence Effects	TB1 vs. TB2
Reduction of Abstinence Effects by Cigarette Smoking	TB2 vs. TB4 TB2 vs. TB3 TB3 vs. TB4
Reversal of Abstinence Effects by Cigarette Smoking to Pre-mixture/Pre-Smoking Abstinence Baseline Values	TB1 vs. TB4 TB1 vs. TB3

**Note:** TB1 = pre-mixture/pre-smoking abstinence (baseline); TB2 = 5 hr post-mixture/post-smoking abstinence; TB3 = post-mixture/post-sham smoke; TB4 = post-mixture/post-cigarette smoke.

Exploratory analysis utilizing the Pearson product moment correlation statistic was also performed to examine the relationship between mood and EEG changes and the degree of change in plasma tryptophan levels. Analyses were performed on change score data for the tryptophan depletion condition in order to examine the net effects of the mixtures and smoking abstinence. Change scores were computed by subtracting values measured at pre-mixture/pre-smoking abstinence baseline (TB1) from values measured at post-mixture/post-smoking abstinence (TB2).

## **Results**

### **A. Sample Characteristics**

Presented in Table 4 are participants' demographic and smoking characteristics. The sample was comprised of males between the ages of 18 and 33 years (mean = 24), with the majority having some university education (94%) and approximately half of the sample being Caucasian (45%). All participants were paid for their participation in the study and came from different universities and colleges within the community.

Participants smoked an average of 18 cigarettes per day with their preferred brands having a mean nicotine yield of 1.26 mg per cigarette, and had been smoking with some degree of regularity for over six years. Smokers in this study were not smoking novices and were considered to be smokers who were highly dependent on nicotine (FTQ mean score = 7.8). As expected, participants expired breath CO concentrations collected during the orientation phase of the study were consistent with that of a cigarette smoker (mean CO level = 15.38 ppm).

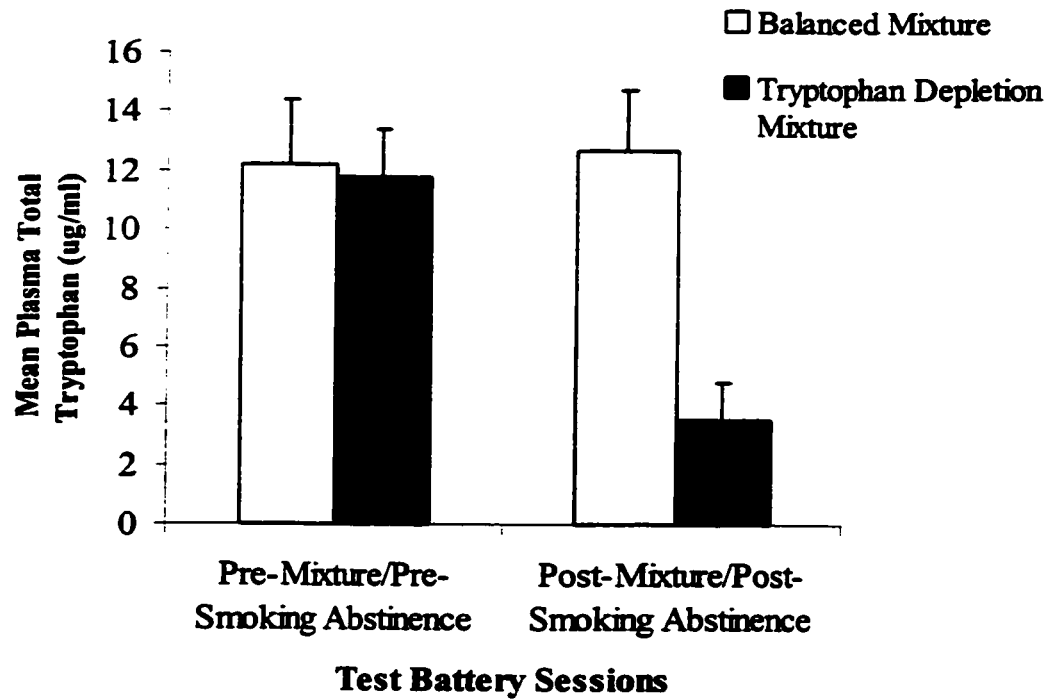
### **B. Plasma Tryptophan**

Figure 1 illustrates the mean changes in total plasma tryptophan before and 5 hr after the ingestion of the balanced and tryptophan-deficient mixtures. Results indicated that while both conditions exhibited similar pretreatment baseline plasma tryptophan levels, highly significant differences were observed 5 hr after ingestion of the amino acid mixtures. The analysis yielded a highly significant main effect for condition,  $F(1,17) = 152.09, p < .00001$ , with the tryptophan-deficient mixture relative to the balanced placebo

Table 4. Demographic and Smoking Characteristics

Variable	Smokers N = 18
Age (yrs)	
Mean	24
SD	(5.32)
Sex (%)	
Male	100
Race (%)	
Caucasian	45
Non-Caucasian	55
At least some university education (%)	94
CO values (ppm)	
Mean	15.38
SD	4.64
<sup>a</sup> Cigarettes per day (#)	
Mean	18
SD	4.0
<sup>b</sup> Nicotine yield per cigarette (mg)	
Mean	1.26
SD	0.31
Years smoking current amount (#)	
Mean	4.21
SD	3.16
Years smoking regularly (#)	
Mean	6.20
SD	3.52
<sup>c</sup> FTQ Score (total)	
Mean	7.8
SD	1.1
<sup>d</sup> History of DSM-IV smoking withdrawal symptoms (total #)	
Mean	5.79
SD	0.31

**Note.** <sup>a</sup>Average reported number of cigarettes smoked per day. <sup>b</sup> Average nicotine yield of regular preferred brand of cigarettes. <sup>c</sup> Average Fagerstrom Tolerance Questionnaire total score. <sup>d</sup> Average reported number of DSM-IV smoking withdrawal symptoms following smoking abstinence in the morning and during previous attempts to quit smoking. Nicotine withdrawal is indicated when a minimum of four of eight withdrawal symptoms are present.



**Figure 1.** Mean ( $\pm$ SE) plasma total tryptophan levels at baseline (pre-mixture/pre-smoking abstinence) and 5 hr after (post-mixture/post-smoking abstinence) the balanced placebo and tryptophan depletion mixtures. Plasma total tryptophan levels following the tryptophan depletion mixture at the post-mixture/post-smoking abstinence test battery session is significantly different from levels derived at all other sessions.

mixture causing a sharp decline in total plasma tryptophan. In addition, the ANOVA revealed a highly significant time effect,  $F(1,17) = 132.79, p < .00001$ , confirming that there was a change in plasma tryptophan from baseline pretreatment to the 5 hr post-treatment period, respectively. Additionally, there was a significant condition by time interaction,  $F(1,17) = 160.58, p < .00001$ , indicating that the change in tryptophan levels occurred differentially depending upon the amino acid mixture ingested.

Follow-up analyses using t-tests, at the .05 alpha level, revealed that the tryptophan-deficient mixture resulted in a marked 71% decrease in plasma total tryptophan levels over time,  $t(17) = 15.93, p < .00001$ , whereas with placebo testing a lack of an effect was observed with ingestion of the balanced mixture producing no change in plasma total tryptophan levels over time,  $t(17) = .92, p < .37$  (see Figure 1). Thus, it is clear from these analyses that 5 hr after ingestion of the tryptophan-deficient mixture, but not the balanced placebo mixture, a significant decrease in plasma total tryptophan occurred. Such a conclusion is consistent with previous research.

### **C. Expired Breath Carbon Monoxide (CO) Levels**

Presented in Table 5 are subjects' expired-breath CO (ppm) levels prior to and following the ingestion of the balanced and tryptophan depletion mixtures and the post-sham and post-cigarette smoking test battery sessions. A statistically significant main effect for time was observed,  $F(3,51) = 136.92, p < .0001$ , but there was neither a significant main effect for condition,  $F(1,17) = 1.74, p < .20$ , nor a significant interaction,



$F(3,51) = .52, p < .60$ . Thus, the lack of a significant condition effect indicated that the amino acid mixtures had no effect on CO levels.

**Table 5. Expired-Breath Carbon Monoxide (CO) Levels Taken Before and After Smoking-Abstinence for the Two Amino Acid Conditions.**

	Balanced Mixture	Tryptophan-Depleted Mixture
Pre-mixture/pre-smoking abstinence	16.33 (6.02)	15.61 (5.12)
Post-mixture/post-smoking abstinence	8.21 (3.75)	7.04 (3.72)
Post-mixture/post-sham smoke	8.16 (3.38)	7.38 (3.43)
Post-mixture/post-cigarette smoke	14.85 (4.85)	13.91 (4.25)

**Note.** CO values are given as mean (ppm) and standard error in parentheses.

Post-hoc comparisons using t-tests with Bonferroni correction (collapsed across the two amino acid conditions) at the .008 alpha level revealed that CO (ppm) levels at baseline and following cigarette smoking significantly differed from CO (ppm) levels following the 5 hr post-mixture/post-smoking abstinence,  $t(17) = 3.05, p < .007$ , and post-sham smoke test battery sessions,  $t(17) = 3.02, p < .008$  (See Table 5). Thus, CO values following test sessions involving cigarette smoking (e.g., TB1 and TB4) differed significantly from testing sessions involving smoking abstinence (e.g., TB2 and TB3). Moreover, the resumption of smoking during the post-cigarette smoking test battery session markedly increased (compared to post-sham smoking) expired-air CO (ppm)

concentrations and returned CO (ppm) values to baseline. Thus, CO concentrations following cigarette smoking did not differ from concentrations observed at the pre-mixture/pre-smoking abstinence (baseline) session,  $t(17) = 1.49, p < .14$ .

#### **D. Self-Report Measures**

##### **1. Profile of Mood States (POMS)**

Mean and standard error of scores on the subscales of the POMS before and after the administration of the balanced and tryptophan-deficient drinks to abstinent smokers are shown in Table 6 while the ANOVA findings are summarized in Table 7. Analyses revealed that the tryptophan-deficient amino acid mixture had no significant effect on mood ratings. As shown in Table 7, no significant main effects for condition were obtained for POMS tension-anxiety ( $p < .20$ ), anger-hostility ( $p < .46$ ), vigor-activity ( $p < .25$ ), fatigue-inertia ( $p < .20$ ), confusion-bewilderment ( $p < .43$ ), and Total Mood Disturbance ( $p < .87$ ). A main effect for condition for the depression-dejection subscale ( $p < .07$ ) approached significance with scores after the tryptophan-depletion mixture showing a greater increase (mean score = 5.17) relative to scores seen with the balanced mixture where depression scores tended to be lower (mean score = 4.5). Accordingly, there was no significant condition by time interaction for any of the subscales. As shown in Table 6, the mean POMS scores 5 hr after the tryptophan-deficient drink were similar to baseline mean scores, and to participants' scores after the balanced amino acid drink.

Table 6. Mean and Standard Error of Scores on the Profile of Mood States Following the Ingestion of a Balanced and Tryptophan-Depleted Amino Acid Drink in Smoking Abstinent Participants

Variable	Test Battery (TB) Session	Balanced Amino Acid Mixture	Tryptophan Depleted Mixture
		Mean Score	Mean Score
Tension - Anxiety	TB1	6.33 (0.93)	6.94 (1.19)
	TB2	9.83 (1.30)	10.39 (1.43)
	TB3	8.39 (1.30)	10.79 (1.29)
	TB4	7.28 (0.92)	7.17 (1.17)
Depression - Dejection	TB1	3.44 (0.90)	4.11 (0.96)
	TB2	4.50 (0.94)	5.17 (1.18)
	TB3	4.72 (1.04)	5.39 (1.29)
	TB4	3.83 (1.02)	4.67 (1.21)
Anger - Hostility	TB1	2.56 (0.64)	3.17 (1.15)
	TB2	4.83 (1.43)	4.22 (1.16)
	TB3	3.89 (1.03)	4.95 (1.79)
	TB4	3.00 (0.93)	3.55 (1.15)
Vigor - Activity	TB1	13.57 (1.48)	14.28 (1.78)
	TB2	10.11 (1.11)	11.17 (1.36)
	TB3	9.83 (1.32)	12.06 (1.49)
	TB4	12.39 (1.71)	12.17 (1.63)
Fatigue - Inertia	TB1	4.39 (0.95)	4.22 (0.70)
	TB2	6.00 (0.81)	5.28 (0.94)
	TB3	5.33 (0.69)	4.33 (0.81)
	TB4	4.28 (0.84)	4.61 (0.82)
Confusion - Bewilderment	TB1	5.39 (0.64)	5.00 (0.65)
	TB2	6.56 (0.78)	6.56 (0.72)
	TB3	6.83 (0.99)	6.39 (0.56)
	TB4	5.94 (0.89)	5.33 (0.73)
TMD	TB1	8.55 (3.76)	9.17 (3.73)
	TB2	21.61 (4.44)	20.44 (4.03)
	TB3	19.33 (3.86)	19.77 (4.26)
	TB4	11.94 (4.35)	13.17 (4.34)

Note. TMD = Total Mood Disturbance. Test battery (TB) sessions are labeled as: TB1 = pre-mixture/pre-smoking abstinence, TB2 = post-mixture/post-smoking abstinence, TB3 = post-mixture/post-sham smoke, TB4 = post-mixture/post-cigarette smoke. Except for the vigor-activity mood dimension, increasing numerical values reflect an increase in negative affect. Standard error in parentheses.

Table 7. ANOVA Results for the Profile of Mood States

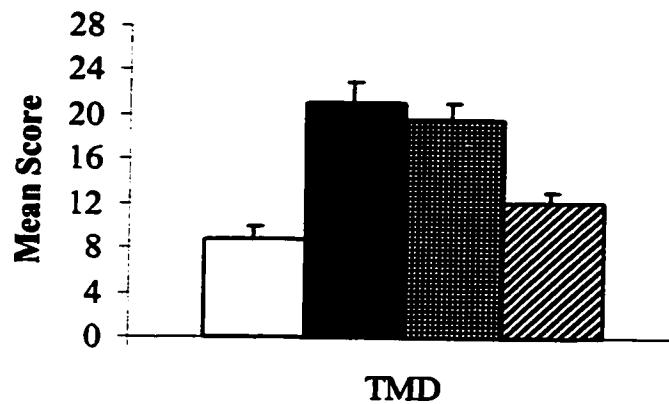
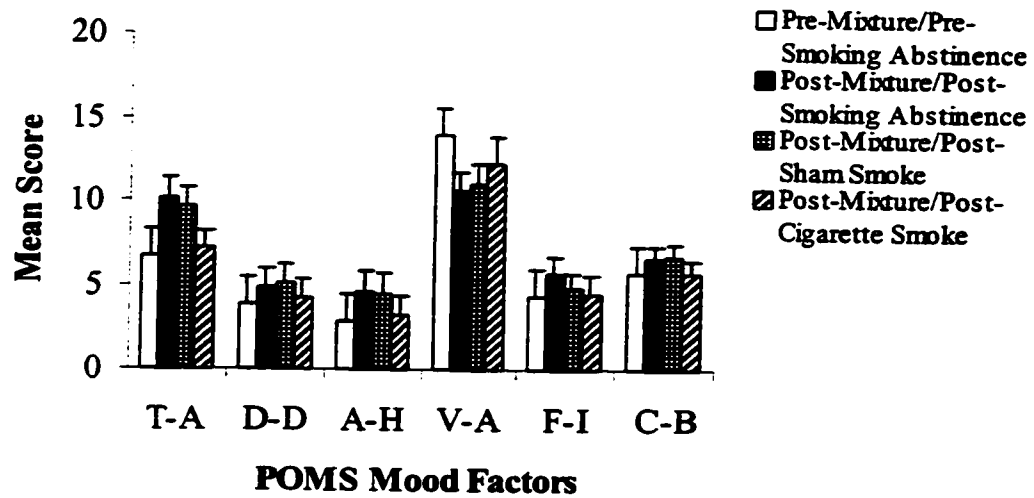
Variable	Effect	<i>F</i>	<i>p</i>
Tension - Anxiety	Condition	1.70	.20
	Time	6.09	.007 *
	C x T	1.22	.31
Depression - Dejection	Condition	3.43	.08
	Time	6.88	.002 *
	C x T	0.13	.88
Anger - Hostility	Condition	0.56	.46
	Time	4.69	.01 *
	C x T	0.85	.39
Vigor - Activity	Condition	1.39	.25
	Time	5.84	.003 *
	C x T	1.46	.24
Fatigue - Inertia	Condition	0.40	.53
	Time	1.86	.16
	C x T	1.09	.35
Confusion - Bewilderment	Condition	0.64	.43
	Time	3.86	.02 *
	C x T	0.24	.84
TMD	Condition	0.03	.87
	Time	12.93	.00001 *
	C x T	0.21	.88

**Note.** TMD = Total Mood Disturbance. Condition = Balanced, tryptophan-depletion mixtures. Time = (TB1) pre-mixture/pre-smoking abstinence, (TB2) post-mixture/post-smoking abstinence, (TB3) post-mixture/post-sham smoke and (TB4) post-mixture/post-cigarette smoke. C x T = condition by time interaction. Degrees of freedom are 1, 17 for between-condition analyses; degrees of freedom for between-session and interaction analyses are 3, 51. Asterisks indicate a statistically significant difference at  $p < .05$ .

Although no significant condition or condition by time interaction effects were observed for any of the subscales, there was a significant main effect for time reflecting alterations in mood states over the course of the experiment (Table 7, Figure 2). Statistically significant time effects were observed for 5 of the 6 individual mood states: tension-anxiety ( $p < .007$ ), depression-dejection ( $p < .002$ ), anger-hostility ( $p < .01$ ), vigor-activity ( $p < .003$ ), and confusion-bewilderment ( $p < .02$ ). The Total Mood Disturbance score also showed a significant main effect for time ( $p < .00001$ ). The only item which did not change over time was the fatigue-inertia subscale ( $p < .15$ ).

Post-hoc comparisons using the Bonferroni procedure at the .008 alpha level were conducted for each mood item as shown in Table 8. The balanced and tryptophan-deficient amino acid conditions were collapsed to produce an average of these two conditions at each separate test battery session. This was done to determine the occurrence of specific withdrawal effects following smoking abstinence and to examine whether nicotine relieved and reversed the tobacco withdrawal syndrome (see Table 3).

After 5 hr of smoking abstinence (i.e., post-mixture/post-smoking abstinence), tension-anxiety,  $t(17) = -3.06, p < .007$ , and depression-dejection,  $t(17) = -3.24, p < .005$ , ratings increased significantly and were accompanied by a significant decrease in feelings of vigor,  $t(17) = 3.36, p < .004$ , (see Table 8, Figure 2). The Total Mood Disturbance score was also significantly higher after the post-mixture/post-smoking abstinence session relative to the pre-mixture/pre-smoking abstinence session. A similar trend ( $p > .03$ ) was seen for the anger-hostility subscale where anger-hostility ratings were also higher after



**Figure 2.** Mean ( $\pm$  SE) scores (averaged across the balanced placebo and tryptophan depletion mixtures) for the six mood factors (T-A = Tension-Anxiety; D-D = Depression-Dejection; A-H = Anger-Hostility; V-A = Vigor-Activity; F-I = Fatigue-Inertia; C-B = Confusion Bewilderment) and Total Mood Disturbance (TMD) extracted from the Profile of Mood States (POMS) at each of the four test battery sessions.

Table 8. Post-hoc t-tests for the Profile of Mood States Ratings Over the Test Battery Sessions

<u>Mood State</u>	<u>Test Battery (TB) Sessions</u>					
	TB1 vs 2	TB1 vs 3	TB1 vs 4	TB2 vs 3	TB2 vs 4	TB3 vs 4
Tension - Anxiety	*	*				
Depression - Dejection	*	*				*
Anger - Hostility					*	
Vigor - Activity	*	*				
Fatigue - Inertia						
Confusion - Bewilderment						
TMD	*	*			*	*

**Note.** Mood state ratings were averaged across amino acid mixtures at each test battery (TB) session. TB sessions include: TB1 = pre-mixture/pre-smoking abstinence, TB2 = post-mixture/post-smoking abstinence, TB3 = post-mixture/post-sham smoke and TB4 = post-mixture/post-cigarette smoke. Asterisks indicate a statistically significant difference ( $p \leq .008$ ) according to Bonferroni-adjusted  $p$ -values.

post-mixture/post-smoking abstinence (mean score = 4.50) than pre-mixture/pre-smoking abstinence (mean score = 2.85).

As shown in Table 8, significant alterations in mood were also evident following cigarette smoking. Post-hoc analysis revealed that increased feelings of anger-hostility,  $t(17) = 3.04, p < .007$ , and Total Mood Disturbance,  $t(17) = 3.01, p < .008$ , induced by smoking abstinence were removed by acute cigarette smoking (TB2 vs TB4). This analysis also revealed that mean comparisons of post-mixture/post-smoking abstinence to post-sham (TB2 vs TB3) and post-cigarette-smoking (TB2 vs TB4) was significant only following cigarette smoking with no significant differences for mood ratings after sham smoking ( $ps > .05$ ). Analyses also revealed that for the three other mood states which changed for the worse during the post-mixture/post-smoking abstinence session (e.g., tension-anxiety, depression-dejection, and vigor-activity), and were not significantly affected by cigarette smoking, there was a trend for reduced ratings of tension-anxiety and depression-dejection after cigarette smoking.

To ascertain whether participants returned to pre-abstinent levels of mood states following cigarette smoking, post-hoc analyses were conducted on the following test sessions: baseline pre-mixture/pre-smoking abstinence versus post-mixture/post-cigarette smoking (TB1 vs TB4) and pre-mixture/pre-smoking abstinence versus post-mixture/post-sham smoking (TB1 vs TB3). As shown in Table 8, the analyses revealed that POMS ratings after cigarette smoking did not significantly differ from pre-abstinence baseline ratings indicating that abstinence symptoms after cigarette smoking returned to baseline pre-deprivation levels. As frequently observed, increased feelings of tension-



anxiety,  $t(17) = -3.04, p < .007$ , depression-dejection,  $t(17) = -3.35, p < .004$ , and total mood disturbance,  $t(17) = 3.01, p < .008$ , and decreased feelings of vigor-activity,  $t(17) = 3.45, p < .003$ , remained following sham smoking and were not returned to pre-mixture/pre-smoking abstinence baseline levels.

## 2. Smoking Withdrawal Symptom Checklist

Mean and standard error of scores of individual withdrawal items and total withdrawal score before and after the administration of the balanced and tryptophan-deficient drinks are shown in Table 9 while the ANOVA findings are summarized in Table 10. Analyses revealed that the tryptophan-deficient mixture had no significant effect on withdrawal ratings. There were no significant main effects for condition, nor any condition x time interactions for any of the withdrawal items (see Table 10). As was found with the depression-dejection subscale of the POMS, the item designated as depressed/sad/feeling blue was the only item from the checklist that approached a statistically significant main effect for condition,  $F(1, 17) = 3.51, p < .07$ , with scores after ingestion of the tryptophan-deficient mixture being marginally higher (mean = 0.72) than scores for the balanced placebo mixture (mean = 0.56). As illustrated in Table 9, the mean smoking withdrawal symptom ratings 5 hr after the tryptophan-deficient load were similar to baseline scores, and to participants' ratings after ingestion of the balanced amino acid mixture.

Although no significant condition or condition by time interaction effects were observed, ratings did increase significantly over time and there were significant time main

**Table 9. Mean and Standard Error of Ratings on the Smoking Withdrawal Symptom Checklist Following the Ingestion of a Balanced and Tryptophan-Depleted Amino Acid Drink in Smoking Abstinent Participants**

Variable	Test Battery (TB) Session	Balanced Amino Acid Mixture	Tryptophan Depleted Mixture
		Mean Rating	Mean Rating
Irritable/ Frustrated/ Angry	TB1	0.22 (0.10)	0.16 (0.09)
	TB2	0.67 (0.16)	0.61 (0.17)
	TB3	0.72 (0.18)	0.72 (0.17)
	TB4	0.50 (0.14)	0.50 (0.15)
Difficulty Concentrating	TB1	0.55 (0.12)	0.56 (0.18)
	TB2	0.94 (0.20)	1.00 (0.16)
	TB3	0.83 (0.18)	1.00 (0.16)
	TB4	0.66 (0.84)	0.89 (0.21)
Restless	TB1	0.66 (0.16)	0.88 (0.17)
	TB2	1.33 (0.18)	1.33 (0.21)
	TB3	1.33 (0.18)	1.33 (0.22)
	TB4	1.11 (0.18)	1.11 (0.17)
Anxious	TB1	0.44 (0.14)	0.33 (0.14)
	TB2	0.94 (0.18)	1.11 (0.25)
	TB3	1.00 (0.19)	1.00 (0.21)
	TB4	0.88 (0.17)	0.78 (0.15)
Hunger	TB1	1.38 (0.84)	1.11 (0.17)
	TB2	2.05 (0.98)	1.95 (0.15)
	TB3	2.17 (0.12)	1.94 (0.15)
	TB4	2.05 (0.19)	1.94 (0.17)
Depressed/ Sad/Feeling Blue	TB1	0.22 (0.42)	0.33 (0.14)
	TB2	0.55 (0.14)	0.72 (0.19)
	TB3	0.44 (0.12)	0.72 (0.19)
	TB4	0.27 (0.10)	0.55 (0.17)
Craving/Urge to Smoke	TB1	1.22 (0.15)	0.88 (0.22)
	TB2	1.94 (0.15)	2.17 (0.15)
	TB3	2.06 (0.17)	2.11 (0.19)
	TB4	1.17 (0.18)	1.33 (0.84)
Total Withdrawal Discomfort	TB1	4.72 (0.53)	4.27 (0.68)
	TB2	8.44 (0.63)	8.89 (0.57)
	TB3	8.56 (0.66)	8.83 (0.63)
	TB4	6.67 (0.74)	7.11 (0.81)

**Note.** Test battery (TB) sessions are labeled as: TB1 = pre-mixture/pre-smoking abstinence, TB2 = post-mixture/post-smoking abstinence, TB3 = post-mixture/post-sham smoke and TB4 = post-mixture/post-cigarette smoke. Increasing numerical values reflect an increase in self-reported abstinence symptoms. Standard error in parentheses.

Table 10. ANOVA Results for the Smoking Withdrawal Symptom Checklist

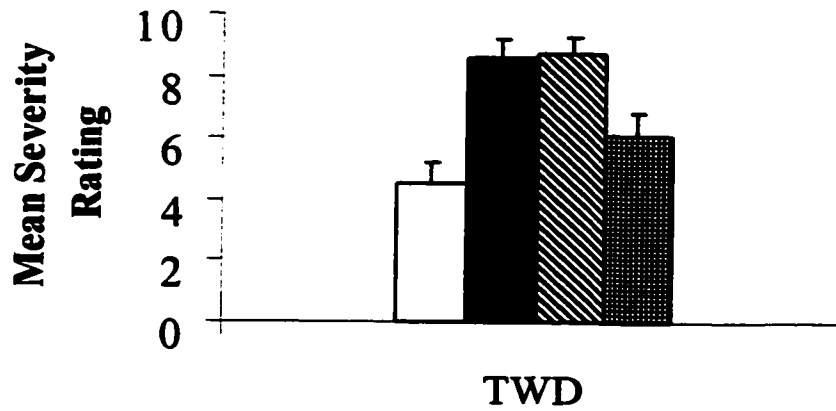
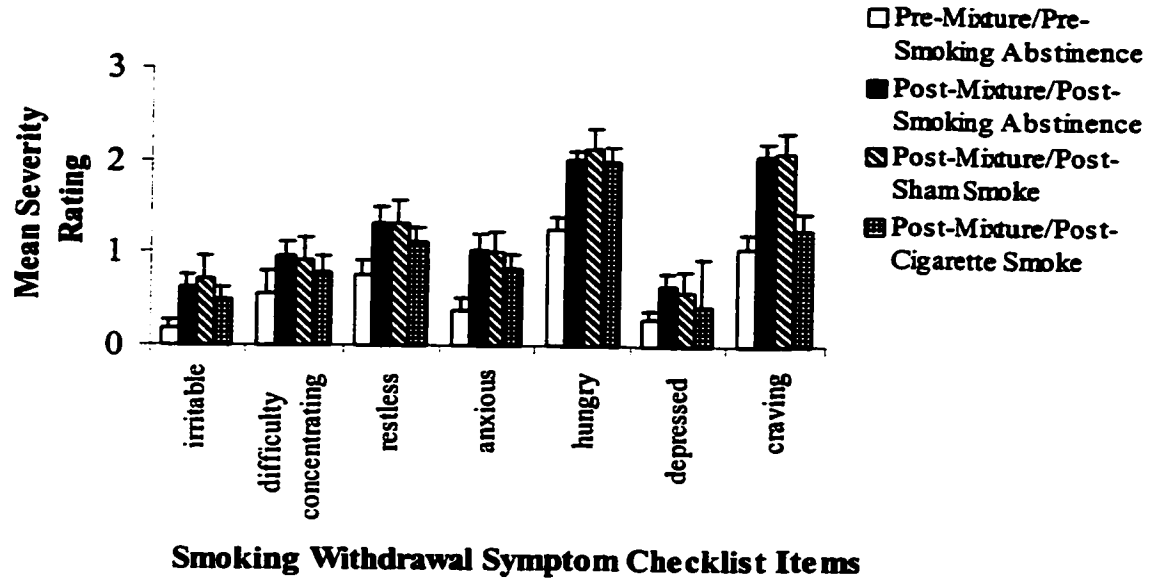
Variable	Effect	<i>F</i>	<i>p</i>
Irritable/ Frustrated/ Angry	Condition	0.09	.76
	Time	7.62	.001*
	C x T	0.10	.92
Difficulty Concentrating	Condition	0.70	.42
	Time	2.75	.06
	C x T	0.50	.65
Restless	Condition	0.31	.58
	Time	6.39	.003*
	C x T	0.60	.54
Anxious	Condition	0.02	.89
	Time	7.91	.001*
	C x T	1.46	.24
Hunger	Condition	1.57	.22
	Time	19.31	.0001*
	C x T	0.28	.75
Depressed/Sad/ Feeling Blue	Condition	3.51	.08
	Time	6.63	.001*
	C x T	0.44	.67
Craving/Urge to Smoke	Condition	0.06	.81
	Time	20.79	.0001*
	C x T	2.86	.06
Total Withdrawal Discomfort	Condition	0.34	.56
	Time	32.03	.00001*
	C x T	1.31	.27

**Note.** Condition = Balanced, tryptophan-depletion mixtures. Time = (TB1) pre-mixture/pre-smoking abstinence, (TB2) post-mixture/post-smoking abstinence, (TB3) post-mixture/post-sham smoke and (TB4) post-mixture/post-cigarette smoke. C x T = condition by time interaction. Degrees of freedom are 1, 17 for between condition analyses; degrees of freedom for between session and interaction analyses are 3, 51. Asterisks indicate a statistically significant difference at  $p < .05$ .

effects (Table 10, Figure 3). As seen in Table 10, significant time effects were obtained for six of the seven individual items: irritable/frustrated/angry ( $p < .001$ ), restlessness ( $p < .003$ ), anxious ( $p < .001$ ), hunger ( $p < .0001$ ), depressed/sad/feeling blue ( $p < .001$ ), and craving/urge to smoke ( $p < .0001$ ). The total withdrawal discomfort rating also showed a significant main effect for time ( $p < .0001$ ). The only item which did not change over time was difficulty concentrating: this item achieved borderline statistical significance ( $p > .05$ ).

Post-hoc multiple comparisons using t-tests with Bonferroni correction at the .008 alpha level are shown in Table 11. Post-hoc analysis revealed numerous differences between the four test battery sessions. As seen in Table 11, the baseline pre-mixture/pre-smoking abstinence ratings were significantly different from the 5 hr post-mixture/post-smoking abstinence ratings (TB1 vs TB2) for the following items: irritable/frustrated/angry,  $t(17) = -4.19$ ,  $p < .0006$ , restless,  $t(17) = -3.08$ ,  $p < .007$ , anxious,  $t(17) = -3.64$ ,  $p < .002$ , hunger,  $t(17) = -5.53$ ,  $p < .00001$ , depressed/sad/feeling blue,  $t(17) = -3.71$ ,  $p < .002$ , craving/urge to smoke,  $t(17) = -5.27$ ,  $p < .0001$ , and total withdrawal discomfort,  $t(17) = -8.00$ ,  $p < .00001$ . These abstinence symptoms were evident after 5 hr of smoking abstinence and continued into the post-sham smoke session. Abstinence symptoms were generally rated mild at baseline and increased by 0.5 - 1.0 point on the four-point scale following smoking abstinence.

Significant alterations in withdrawal ratings were also observed following resumption of cigarette smoking (Table 11, Figure 3). Analysis of craving/urge to smoke yielded significant differences between post-smoking abstinence and cigarette smoking



**Figure 3.** Mean ( $\pm$  SE) severity ratings (averaged across the balanced placebo and tryptophan depletion mixtures) for the seven individual items and Total Withdrawal Discomfort (TWD) extracted from the Smoking Withdrawal Symptom Checklist at each of the four test battery sessions.

Table 11. Post-hoc t-tests for the Smoking Withdrawal Symptom Checklist Ratings Over the Test Battery Sessions

<u>State</u>	<u>Test Battery (TB) Sessions</u>					
	TB1 vs 2	TB1 vs 3	TB1 vs 4	TB2 vs 3	TB2 vs 4	TB3 vs 4
Irritable/ Frustrated Angry	*	*	*			
Difficulty Concentrating						
Restless	*	*				
Anxious	*	*	*			
Hunger	*	*	*			
Depressed/ Sad/ Feeling Blue	*	*				
Craving/Urge to Smoke	*	*			*	*
Total Withdrawal Discomfort	*	*	*		*	*

Note. Smoking abstinence ratings were averaged across the balanced and tryptophan-deficient amino acid mixtures at each test battery (TB) session. TB sessions include: TB1 = pre-mixture/pre-smoking abstinence, TB2 = post-mixture/post-smoking abstinence, TB3 = post-mixture/post-sham smoke and TB4 = post-mixture/post-cigarette smoke. Asterisks indicate a statistically significant difference ( $p \leq .008$ ) according to Bonferroni  $p$ -values.

test sessions (TB2 vs TB4), and between cigarette and sham smoking test sessions (TB3 vs TB4), such that participants reported high levels of craving following 5 hr of smoking abstinence,  $t(17) = -5.27, p < .0001$ , and reported less craving following cigarette smoking,  $t(17) = 4.33, p < .0005$ , as compared to sham smoking,  $t(17) = -0.25, p > .80$ . A similar pattern was seen with the total withdrawal discomfort ratings which illustrated that cigarette,  $t(17) = 3.29, p < .004$ , but not sham smoking,  $t(17) = -0.10, p > .05$ , produced an immediate reduction in total withdrawal discomfort. Feelings of irritability, difficulty concentrating, restlessness, anxiety, and depressed mood showed similar trends, but none were statistically significant (all  $p$ 's  $> .008$ ). Participants did not report changes in hunger following sham or cigarette smoking.

Post-hoc analyses also revealed that several withdrawal ratings were returned to the pre-mixture/pre-smoking abstinence baseline levels following cigarette but not sham smoking. Nonsignificant differences between baseline and cigarette smoking (TB1 vs TB4) were obtained for ratings of restlessness, depressed mood and craving, (all  $p$ 's  $> .008$ ), indicating that these feelings did return to pre-smoking abstinence baseline levels after cigarette smoking. However, baseline and cigarette smoking test sessions were observed to differ significantly with respect to ratings of irritability,  $t(17) = -3.05, p < .007$ , anxiety,  $t(17) = -3.33, p < .003$ , hunger,  $t(17) = -4.47, p < .0003$ , and total withdrawal discomfort,  $t(17) = -4.46, p < .0003$ , indicating that acute smoking did not return these ratings to non-abstinent baseline values. As expected, analysis of individual withdrawal ratings (with the exception of difficulty concentrating) yielded a significant difference between the pre-smoking abstinence baseline and post-sham smoking test

session (TB1 vs TB3), such that withdrawal symptoms did not return to baseline levels following sham smoking (see Table 11).

### **E. Quantitative EEG**

Mean and standard error EEG amplitude values for each amino acid condition at each test battery session are shown in Table 12 while the ANOVA findings are summarized in Table 13. Analysis of amplitude values revealed a significant main effect for amino acid condition for only one of the five EEG bands. A significant effect was observed for condition at  $\alpha_2$ ,  $F(1, 16) = 4.70, p < .04$ , such that the tryptophan-deficient mixture (mean score= 95.91) relative to the balanced placebo mixture (mean score = 105.25) induced a significant decrease in  $\alpha_2$  amplitude (see Figure 4). Additionally, a significant condition by region interaction was also observed for  $\alpha_2$  amplitude,  $F(2, 32) = 3.48, p < .04$  (see Figure 5).

Follow up analyses of the significant condition by region interaction observed at  $\alpha_2$ , with Bonferroni-adjusted significance levels at .008, revealed that  $\alpha_2$  varied with region within each amino acid condition. As illustrated in Figure 5,  $\alpha_2$  amplitude was reduced in frontal relative to central,  $t(17) = -5.72, p < .00001$ , and occipital,  $t(17) = -8.04, p < .00001$ , regions following the balanced placebo mixture and a similar pattern was observed following the tryptophan depletion mixture (frontal relative to central,  $t(17) = -4.76, p < .0002$ , and occipital,  $t(17) = -7.79, p < .00001$ , regions). Analyses also revealed a trend between the balanced placebo and tryptophan-depleted EEG amplitude



**Table 12. Mean and Standard Error EEG Frequency (Hz) Amplitude Following the Ingestion of a Balanced and Tryptophan-Depleted Amino Acid Drink in Smoking Abstinent Participants**

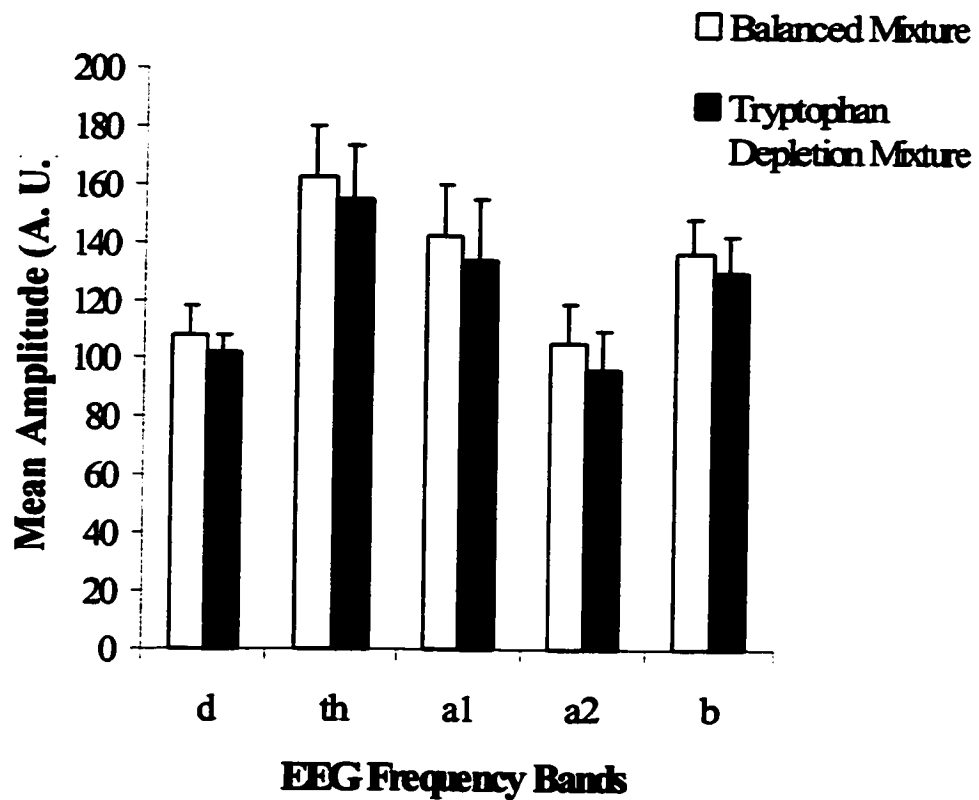
Frequency	Test Battery (TB) Session	Balanced Amino Acid Mixture	Tryptophan Depleted Mixture
		Mean Value	Mean Value
Delta	TB1	107.68 (7.58)	99.70 (4.97)
	TB2	118.53 (17.41)	107.25 (6.92)
	TB3	113.03 (11.76)	105.49 (6.25)
	TB4	91.83 (3.82)	95.11 (5.32)
Theta	TB1	149.91 (14.05)	144.43 (15.35)
	TB2	172.12 (22.05)	157.84 (18.67)
	TB3	172.59 (19.12)	162.42 (19.80)
	TB4	154.95 (14.92)	154.49 (18.92)
Alpha <sub>1</sub>	TB1	130.81 (14.85)	127.55 (18.57)
	TB2	147.49 (21.11)	133.94 (20.34)
	TB3	151.90 (20.48)	142.34 (23.58)
	TB4	138.35 (14.91)	132.04 (20.56)
Alpha <sub>2</sub>	TB1	101.85 (12.42)	95.72 (13.49)
	TB2	102.48 (13.79)	90.48 (13.39)
	TB3	104.47 (13.49)	93.17 (13.04)
	TB4	112.21 (13.58)	104.27 (13.49)
Beta	TB1	129.93 (10.33)	123.96 (11.43)
	TB2	136.26 (12.24)	125.84 (11.91)
	TB3	136.59 (10.56)	129.86 (12.98)
	TB4	143.17 (12.87)	139.56 (14.28)

**Note.** Test battery (TB) sessions are labeled as: TB1 = pre-mixture/pre-smoking abstinence, TB2 = post-mixture/post-smoking abstinence, TB3 = post-mixture/post-sham smoke and TB4 = post-mixture/post-cigarette smoke. Increasing numerical values reflect an increase in EEG amplitude values. Standard error in parentheses.

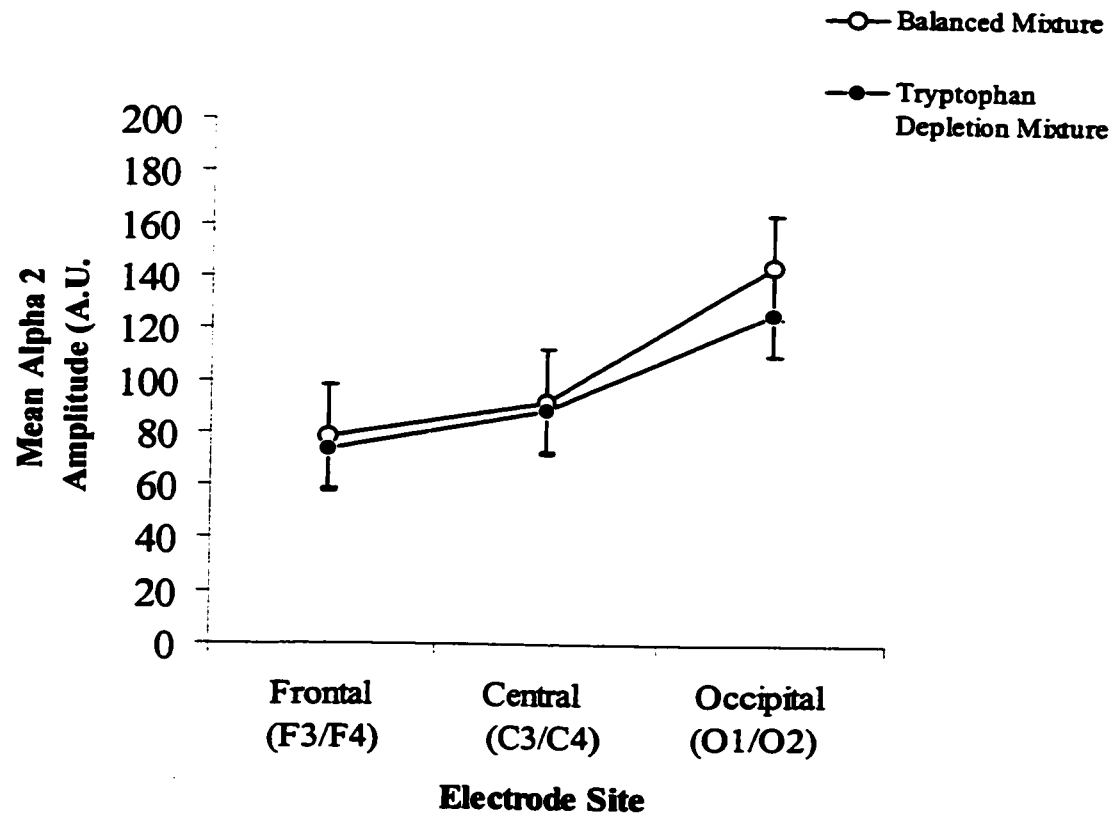
Table 13. ANOVA Results for EEG Amplitude

ANOVA Factor	Frequency Band														
	Delta			Theta			Alpha <sub>1</sub>			Alpha <sub>2</sub>			Beta		
	df	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Condition	1, 16	0.14	.71	1.30	.27	4.17	.06	4.70	.04*	2.93	.10				
Time	3, 48	10.33	.00001*	4.39	.02*	1.20	.31	3.67	.01*	3.76	.03*				
Region	2, 32	12.06	.0001*	23.16	.00001*	12.44	.001*	42.96	.00001*	6.37	.01*				
Hemisphere	1, 16	0.44	.51	0.03	.87	0.12	.73	1.53	.23	2.83	.11				
C x T	3, 48	0.99	.37	0.29	.72	0.23	.79	0.14	.85	.44	.66				
C x R	2, 32	1.68	.20	1.40	.25	3.55	.06	3.48	.04*	1.15	.32				
C x H	1, 16	0.90	.35	1.90	.18	2.83	.08	0.78	.39	.02	.89				
T x R	6, 96	0.64	.59	3.55	.01*	3.79	.01*	0.76	.54	.53	.63				
T x H	3, 48	2.71	.07	2.07	.14	4.89	.007*	2.78	.07	2.21	.14				
R x H	2, 32	29.08	.00001*	25.01	.00001*	7.73	.005*	3.45	.05*	21.16	.0001*				
C x T x R	6, 96	0.45	.75	0.52	.67	1.65	.17	0.54	.67	2.83	.12				
C x T x H	3, 48	2.85	.09	2.95	.09	2.62	.10	2.69	.07	2.54	.10				
C x R x H	2, 32	2.32	.10	2.53	.07	1.49	.24	0.68	.50	1.01	.37				
T x R x H	6, 96	1.21	.31	2.51	.09	1.29	.28	1.16	.19	1.19	.18				
C x T x R x H	6, 96	1.02	.39	1.89	.15	1.05	.38	0.30	.81	1.62	.19				

Note. Condition (C) = Balanced mixture, tryptophan-depletion mixture. Time (T) = (TB1) pre-mixture/pre-smoking abstinence, (TB2) 5 hr post-mixture/post-smoking abstinence, (TB3) post-mixture/post-sham smoke and (TB4) post-mixture/post-cigarette smoke. Region (R) = frontal (F3, F4), central (C3, C4) and occipital (O1, O2). Hemisphere (H) = left (F3, C3, O1) and right (F4, C4, O2). Asterisks indicate a statistically significant difference ( $p \leq .05$ ) according to Greenhouse-Geisser  $p$ -values.



**Figure 4.** Mean ( $\pm$  SE) EEG amplitude scores following the balanced placebo and tryptophan depletion mixtures for delta (d), theta (t), alpha<sub>1</sub> (a1), alpha<sub>2</sub> (a2) and beta (b) EEG frequency bands (averaged across F3, F4, C3, C4, O1, and O2 scalp sites and across the four test battery sessions). A significant between condition difference ( $p < .05$ ) was observed for alpha<sub>2</sub> (a2) amplitude. A.U. = arbitrary units.



**Figure 5.** Mean ( $\pm$  SE) EEG amplitude indices for Condition  $\times$  Region interaction for  $\alpha_2$  following the balanced placebo and tryptophan depletion mixtures (averaged across the four test battery sessions). Frontal means reflect the average of amplitudes at F3 and F4 sites, central means reflect the average of amplitudes at C3 and C4 sites and occipital means reflect the average of O1 and O2 sites. A.U. = arbitrary units.

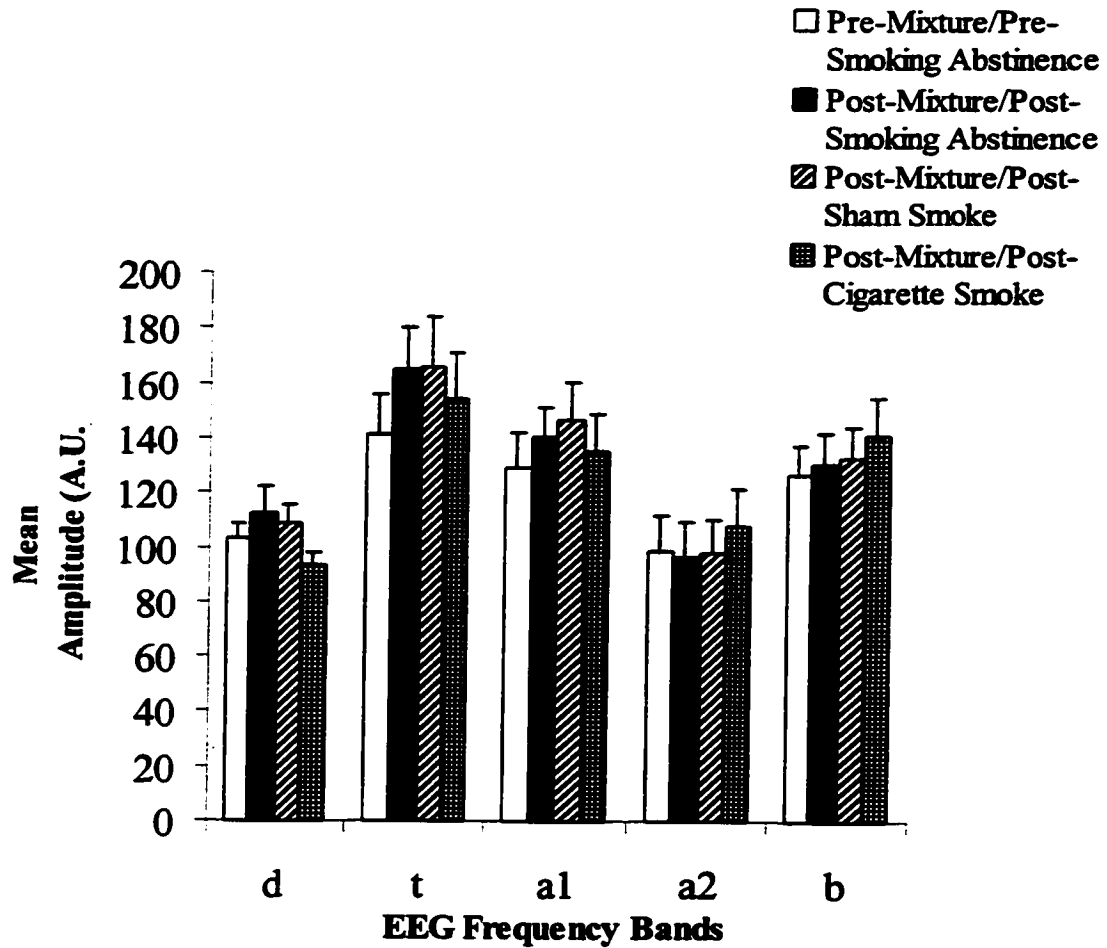
the balanced placebo condition at occipital regions,  $t(17) = 2.43$ ,  $p < .03$  (see Figure 5). This effect, however, did not attain the .008 (Bonferroni) level of statistical significance.

A similar trend was observed for  $\alpha_1$  (condition,  $F(1, 16) = 4.17$ ,  $p < .06$ , condition by region,  $F(2, 32) = 3.55$ ,  $p < .06$ , and condition by hemisphere,  $F(3, 48) = 3.02$ ,  $p < .08$ , interaction effects) such that the tryptophan-deficient mixture appeared to reduce  $\alpha_1$  amplitude (mean score = 133.96) relative to the balanced placebo mixture (mean score = 142.14). No condition main effects or interaction effects involving condition were observed for the other three frequency bands.

As shown in Table 13, significant main effects for time were observed for the following frequency band indices: delta ( $p < .00001$ ), theta ( $p < .02$ ),  $\alpha_2$  ( $p < .01$ ), and beta ( $p < .03$ ). The only frequency band which did not change over time was  $\alpha_1$  ( $p < .31$ ). Alterations in mean EEG amplitude scores across the four test battery sessions are illustrated in Figure 6.

Follow up analyses using t-tests with Bonferroni-corrected significance values at the .008 alpha level revealed several differences between the four test battery sessions. As shown in Table 14 and Figure 6, mean theta amplitude at baseline (TB1) was significantly different from 5 hr of smoking abstinence (TB2) such that increments in theta were evident following smoking abstinence,  $t(17) = -3.44$ ,  $p < .005$ . Theta amplitude increments continued at the later sham smoking session, with the greatest increment after 5.5 hr of smoking abstinence (TB3),  $t(17) = -3.57$ ,  $p < .002$ .

Mean EEG comparisons of post-mixture/post-smoking abstinence versus post-cigarette (TB2 vs TB4) and post-sham smoking (TB2 vs TB3) revealed that smoke



**Figure 6.** Mean ( $\pm$  SE) EEG amplitude scores for delta (d), theta (t),  $\alpha_1$  ( $a_1$ ),  $\alpha_2$  ( $a_2$ ), and beta (b) frequency bands (averaged across F3, F4, C3, C4, O1, and O2 scalp sites and across the balanced placebo and tryptophan depletion mixtures) at each of the four test battery sessions. A.U. = arbitrary units.

Table 14. Post-hoc t-tests of Significant Time Effects for EEG Frequency

<u>Frequency</u>	<u>Test Battery (TB) Sessions</u>					
	<u>TB1 vs 2</u>	<u>TB1 vs 3</u>	<u>TB1 vs 4</u>	<u>TB2 vs 3</u>	<u>TB2 vs 4</u>	<u>TB3 vs 4</u>
Delta			*		*	*
Theta	*	*				
Alpha <sub>1</sub>						
Alpha <sub>2</sub>			*		*	*
Beta			*			

**Note.** EEG frequencies were each averaged across the balanced and tryptophan-deficient amino acid mixtures at each test battery (TB) session. TB sessions include: TB1 = pre-mixture/pre-smoking abstinence, TB2 = post-mixture/post-smoking abstinence, TB3 = post-mixture/post-sham smoke and TB4 = post-mixture/post-cigarette smoke. Asterisks indicate a statistically significant difference ( $p \leq .008$ ) according to Bonferroni  $p$ -values.

inhalation relative to sham smoking significantly reduced delta,  $t(17) = 3.30, p < .004$ , and increased alpha<sub>2</sub>,  $t(17) = 3.22, p < .002$ , amplitude values. Cigarette smoking was also found to return theta to pre-abstinent baseline scores (TB1 vs TB4). However, comparisons indicated that for delta, alpha<sub>2</sub> and beta, smoke inhalation failed to return amplitude scores to levels corresponding to pre-smoking abstinent baseline. Thus, although following acute smoking a smoking-induced EEG arousal profile in abstinent smokers was observed, smoke inhalation did not fully return participants to their baseline EEG values.

Several interactions were also observed to be significant between time and electrode site at theta,  $F(3, 32) = 3.55, p < .02$ , and between time and hemisphere, at alpha<sub>1</sub>,  $F(3, 38) = 4.89, p < .007$  ( see Table 13). Post-hoc comparisons using t-tests with Bonferroni-adjusted significance values at the .008 alpha level revealed that, compared to pre-mixture/pre-smoking abstinence, sham-smoking was associated with significant theta amplitude increments in frontal,  $t(17) = -4.15, p < .0007$ , and central,  $t(17) = -3.93, p < .001$ , regions. These results indicate that the greatest amplitude increments in theta occurred after 5.5 hr of smoking abstinence, although increments were evident at the earlier 5 hr post-mixture/post smoking abstinence session. Follow-up tests of the time by hemisphere interaction for alpha<sub>1</sub> did not reach the .008 level of significance and failed to reveal any statistical differences in alpha<sub>1</sub> hemisphere effects following 5 hr of smoking abstinence versus sham and cigarette smoking.



## **F. Relationship Between Plasma Tryptophan Depletion, Self-Report Measures and EEG**

A number of Pearson correlations (see Tables 15 and 16) were computed to determine the relationship between the degree of change in plasma tryptophan level and change in smoking abstinence-induced effects. The dependent variables were as follows: (a) the POMS; (b) the Smoking Withdrawal Symptom Checklist; (c) level of plasma total tryptophan, and (d) amplitude in the five EEG frequency bands. The analysis was carried out on change score data in order to ascertain the net effects of the tryptophan depletion mixture. Change scores were computed by subtracting values measured at baseline pre-mixture/pre-smoking abstinence from values measured 5 hr after ingestion of the tryptophan depletion mixture and smoking abstinence, for each of the above-noted variables.

Of the correlations carried out between total plasma tryptophan concentrations and abstinence symptoms, negative mood states and EEG, none showed significant relationships. The degree of total plasma tryptophan depletion failed to correlate significantly with changes in POMS mood states scores and withdrawal symptom ratings after tryptophan depletion. Similarly, correlations with EEG indices did not reach the .05 level of significance.

Table 15. Correlation of Changes in Total Plasma Tryptophan Levels with Profile of Mood States and Smoking Withdrawal Symptom Checklist Ratings for the Tryptophan Depletion Condition

Measure	State	r	p
POMS	Tension-Anxiety	.26	ns
	Depression-Dejection	-.26	ns
	Anger-Hostility	.20	ns
	Confusion-Bewilderment	.13	ns
	Fatigue-Inertia	.06	ns
	Vigor-Activity	.30	ns
	Total Mood Disturbance	.21	ns
Smoking Withdrawal Symptom Checklist	Irritable/Angry	.16	ns
	Difficulty Concentrating	.28	ns
	Restless	-.22	ns
	Anxious/Tense	.12	ns
	Hunger	-.11	ns
	Depression/ Sad/ Feeling Blue	.10	ns
	Craving/Desire to Smoke	.30	ns
	Total Withdrawal Discomfort	.25	ns

Note: Correlations above .35 are statistically significant,  $p < .05$ .

Table 16. Correlation of Changes in Total Plasma Tryptophan Levels with EEG Amplitude for the Tryptophan-Depletion Condition

Frequency Band	r	p
delta	-.29	ns
theta	-.24	ns
alpha <sub>1</sub>	.06	ns
alpha <sub>2</sub>	-.18	ns
beta	-.14	ns

Note: Correlations above .35 are statistically significant,  $p < .05$ .

## Discussion

The findings within this study, one of the first to examine the separate and combined effects of 5-HT depletion and cigarette smoking on smoking withdrawal-induced effects, failed to support the hypotheses postulated a priori. Before proceeding into a detailed discussion of the results, an overview of the main outcomes related to the three hypotheses will be provided.

First, the data suggest that the tryptophan depletion mixture relative to the balanced control mixture did not exacerbate mood states and abstinence symptoms in abstaining smokers (hypothesis one). Thus, these results revealed that lowered plasma tryptophan and brain 5-HT levels were not associated with an exacerbation of the nicotine withdrawal syndrome. Although the tryptophan depletion condition was not associated with significant mood-lowering and nicotine withdrawal symptoms, it was associated with a statistically significant alteration in EEG. Specifically, significant amplitude decreases were evident in  $\alpha_2$ , and although  $\alpha_1$  and beta reductions were also evident, they were somewhat more variable and nonsignificant. Thus, while the depletion mixture failed to augment subjective mood-related abstinence symptoms or alter cortical hypoarousal induced by smoking abstinence, it did have an effect on brain arousal per se.

Hypothesis two stated that resumption of cigarette smoking would relieve abstinence symptoms, negative affect and EEG indices of hypoarousal in the presence of depleted tryptophan as well as reverse to baseline any worsened abstinence symptoms induced by tryptophan depletion. Cigarette smoking was observed to reduce and reverse some of the mood and abstinence-related symptoms, as well as EEG hypoarousal.

However, these effects were unrelated to the tryptophan depletion mixture and were evident in both tryptophan depleted and non-depleted states.

A final hypothesis was that following the tryptophan depletion mixture, relative to the balanced placebo mixture, EEG alterations (delta, theta and  $\alpha_1$  increments) would be more evident in frontal recording sites rather than posterior scalp sites and in the right cerebral hemisphere rather than the left. While both the balanced placebo and the tryptophan depletion conditions were expected to show relatively increased right-frontal and/or decreased left-frontal EEG arousal as a result of smoking abstinence, these effects were expected to be stronger in the tryptophan depletion condition relative to the placebo condition due to the induction of negative affect associated with reduced brain levels of 5-HT. In contrast, it was hypothesized that a cigarette smoking challenge would elevate left-frontal hemispheric arousal (e.g., smoking-related  $\alpha_2$  and beta increments) and decrease right-frontal arousal in smokers. Again, the results indicated that reduced brain levels of 5-HT were not associated with exacerbation of negative mood and withdrawal effects as well as hemispheric asymmetry in abstinent smokers. Although, taken together, the findings suggest that acute reduction of plasma tryptophan and brain 5-HT levels do not appear to alter the signs and symptoms associated with acute smoking abstinence effects, they do have potential implications and significance for smoking cessation and smoking behavior.

### **A. Effect of Tryptophan Depletion on the Tobacco Withdrawal Syndrome, Mood and EEG Following Smoking Abstinence**

Any conclusions regarding the relationship between 5-HT function and the signs and symptoms of smoking abstinence are contingent upon whether the experimental manipulations were successful in inducing a tobacco withdrawal syndrome and in altering brain 5-HT. A number of investigations have found that for many smokers abrupt cessation of tobacco smoking produces a reliable constellation of symptoms including irritability, anxiety, difficulty concentrating, restlessness, increased appetite/hunger, increased urges to smoke, depressed mood, and so on (Hughes & Hatsukami, 1986; Hughes et al., 1990, 1991; West & Russell, 1988). The present study was highly successful in demonstrating that cigarette deprivation led to clear time-dependent increases in subjective mood states and withdrawal symptoms and it replicated the results of previous studies of cigarette abstinence.

After 5 hr of smoking abstinence (e.g., post-mixture/post-smoking abstinence session), various mood states, as measured by the Smoking Withdrawal Symptom Checklist and the POMS, were altered with significant increases in feelings of irritability, restlessness, tension/anxiety, hunger, depressed mood, urge to smoke, reduced vigor-activity, total withdrawal discomfort, and POMS total mood disturbance (see Figures 2 and 3). Nonsignificant increases were seen in feelings of the POMS anger-hostility and the item difficulty concentrating on the Smoking Withdrawal Symptom Checklist. Craving was generally reported as the most troublesome withdrawal symptom followed by increased hunger, anxiety/nervousness, irritability, restlessness, depressed mood and

difficulty concentrating. These results are consistent with previous investigations of individual withdrawal symptoms as well as with several studies indicating that craving for tobacco is one of the more prevalent withdrawal symptoms reported by acutely deprived smokers (Hughes & Hatsukami, 1986; Hughes et al., 1991).

The absence of a statistically significant withdrawal-induced effect on the remaining variables may have been due to one or a combination of the following: (a) a longer abstinence period was required; (b) there was a large between-subject variability in tobacco withdrawal effects (Hughes et al., 1991); (c) the induction of abstinence in a controlled environment may have minimized the intensity of tobacco withdrawal symptoms (Falk, 1970), and/or (d) they are not true withdrawal phenomena.

Although the absolute magnitude of the significant changes in withdrawal ratings and POMS scores were small, they are in keeping with those reported in previous studies of smoking abstinence (Hatsukami et al., 1984; Hughes & Hatsukami, 1986; Hughes et al., 1991; Parrott et al., 1996). The results are also consistent not only with previous research on smoking abstinence in cigarette smokers, but also with reports indicating that abstinence from other nicotine-containing products such as smokeless tobacco (Hatsukami et al., 1987) and nicotine itself (e.g., as in nicotine chewing gum) induce similar withdrawal profiles (Hughes, Hatsukami, Mitchell, et al., 1986; West & Russell, 1985). Thus it is likely that a tobacco withdrawal syndrome was induced in study participants.

The present study was also successful in producing the EEG effects of tobacco abstinence in the direction of decreased cortical arousal. Following the post-treatment

(TB2 and TB3) test sessions, smoking abstinence significantly increased theta amplitude; and although delta and  $\alpha_1$  increments were also evident, they were variable and nonsignificant. No significant changes in  $\alpha_2$  and beta amplitude were observed. For the most part, the present EEG findings generally replicated the results of earlier studies that characterized the slowing of the EEG frequencies and increases in power of the slower (delta and theta) frequency bands in abstinent smokers. Results are consistent with reports showing that EEG changes induced by tobacco abstinence have an onset as soon as 30 min after the last cigarette (Herning et al., 1983) and are evident after 5 hr of smoking abstinence (Pickworth et al., 1989).

Moreover, the present study replicated the results of several other studies that demonstrated an association between EEG signs of cortical hypoarousal and subjective mood complaints that are typically associated with smoking abstinence (Hughes & Hatsukami, 1986; Knott, Harr, & Lusk-Mikkelsen, 1998). Thus, it is concluded that the types of changes seen in the present study -- worsening of mood states, withdrawal symptoms and lowered cortical arousal -- are consistent with the induction of smoking withdrawal effects after 5 hr of temporary smoking abstinence.

The present study set out to investigate whether the lowering of brain 5-HT through alterations in tryptophan availability influenced the signs and symptoms of smoking abstinence. The results indicated that compared to the balanced placebo mixture, the depletion mixture did not exacerbate tobacco withdrawal symptoms, subjective mood states, or electrocortical activity. A number of possible explanations exist for these findings. One possible interpretation for the negative results in this study is that brain 5-

HT synthesis was not altered. Although in the present study indirect measures (plasma tryptophan and EEG) of 5-HT metabolism were used to indicate what changes might be occurring in the brain, there is an abundance of circumstantial evidence suggesting that the nutritional manipulations did alter brain 5-HT in the expected way.

First, the tryptophan depletion mixture reduced total plasma tryptophan levels by more than 70% (Figure 1). Studies in experimental animals have shown that a reduction of total plasma tryptophan of 70-90%, similar to the magnitude of effect observed in participants in the present study, caused a reduction of brain 5-HT to approximately 50% of control values (Biggio et al., 1974). In humans, acute tryptophan depletion has been shown to lower brain 5-HT synthesis, as evidenced by PET imaging, by approximately 90% in healthy volunteers (Nishizawa et al., 1997).

In agreement with other reports, the tryptophan depletion mixture was associated with a significant reduction of total plasma tryptophan 5 hr after ingestion of the tryptophan depletion mixture whereas the balanced control mixture, which contained tryptophan, produced a slight nonsignificant increase of this amino acid. Moreover, the depletion mixture induced significant changes in electrocortical activity which were characterized by a reduction in  $\alpha_2$  amplitude with a regional decrease in  $\alpha_2$  at prefrontal sites. The modification of electrocortical activity associated with the tryptophan depletion condition confirms a central action of the mixture in study participants. Thus, there is good evidence, from the results of the present study, as well as from circumstantial evidence reviewed above, that the nutritional manipulations should have had the expected effect on brain 5-HT metabolism.



If, as suggested, central levels of 5-HT were reduced in the expected way, it is pertinent to ask whether 5-HT function was altered. Evidence suggests that a change in brain 5-HT metabolism is not necessarily accompanied by a corresponding change in 5-HT function (Young, 1986). In order for a functional change to occur, there must be a change in 5-HT release and neurotransmission. As brain 5-HT release in humans cannot be examined directly, alterations in 5-HT are based upon indirect measures which are thought to be influenced by 5-HT. For example, in a previous study, investigators concluded that since mood states and EEG activity were altered by the depletion of tryptophan, then brain 5-HT levels must have been affected (Knott, Howson, et al., 1999). Others have found the depletion mixture to influence other measures, including sleep regulation, aggression, obsessive-compulsive symptoms, and autism, all of which are thought to be influenced by 5-HT (Cleare & Bond, 1995; Huwig-Poppe et al., 1999; Smeraldi et al., 1996; McDougle et al., 1996).

Although the reduction in plasma tryptophan did not result in behavioral alterations, as evidenced by the absence of change in mood and withdrawal ratings 5 hr after ingestion of the tryptophan-depleted mixture, it was associated with altered brain state arousal as indicated by the reduction in alpha<sub>2</sub> amplitude. Pharmacologically-induced reductions in arousal/alertness/vigilance are characterized by a series of EEG stages, with mild decrements in arousal being associated with a stage of diffuse, widespread increases in alpha followed by a stage of localized (posterior) diminished alpha. More progressive behavioral arousal decrements are associated with lower

frequency band changes, with activity in theta and then delta being increased diffusely across the scalp (Herrmann & Schaerer, 1986).

However, as alpha reduction/alpha desynchronization has also been associated with increased behavioral/emotional arousal, as would be reflected in states of task-induced activation and/or experimentally-induced anxiety, the alpha<sub>2</sub> reduction seen with the tryptophan depletion mixture may reflect either sedating or stimulating effects. Regardless, the alpha<sub>2</sub> alterations induced in male smokers in the present study are in contrast to the slow wave delta increments observed with the depletion of tryptophan in male nonsmokers (Knott, Howson, et al., 1999). The somewhat milder sedating actions of tryptophan depletion observed in the present study in male smokers may reflect altered 5-HT neurotransmission/receptor sensitivity and/or receptor density resulting from chronic smoking. Accordingly, studies have shown that chronic nicotine administration to rats causes a regionally selective decrease in the concentration and biosynthesis of 5-HT in the hippocampus (Benwell & Balfour, 1982). Similarly, results of a postmortem study showed a significant decrease in the serotonergic activity in the hippocampus as well as an increase in hippocampal 5-HT 1A binding sites in heavy smokers (Benwell et al., 1990).

Given the reduction in plasma tryptophan and the alpha<sub>2</sub> decrements associated with the tryptophan depletion condition, it is likely that 5-HT function was altered in the present study. However, it is possible that lowering tryptophan levels may have affected the levels of other potentially psychoactive tryptophan metabolites, such as tryptamines (Young & Gauthier, 1981), melatonin (Zimmermann et al., 1993), as well as brain protein

synthesis which may have altered EEG activity. Moreover, it may well be that tryptophan depletion and the consequent reduction in brain 5-HT may have altered some critical balance between 5-HT and other neurotransmitter systems (e.g., dopamine).

The present results, which suggest that abstinence-induced subjective mood states and EEG are not influenced by lowering 5-HT, is in contrast to various studies within the animal literature showing that: (a) nicotine withdrawal and administration lead to alterations in serotonergic neurotransmission (Rasmussen & Czachura, 1997; Ribeiro et al., 1993), and (b) behavioral consequences of nicotine withdrawal and certain effects of nicotine are attenuated by serotonergic drugs (Carboni, Acquas, Leone, & Di Chiara, 1989; Costall et al., 1990; Levin et al., 1993). Furthermore, the current results do not support indications from human studies that deficient 5-HT functioning may underlie some of the signs and symptoms of smoking withdrawal, particularly negative affect and depressed mood, and that serotonergic agonists may be useful as a pharmacotherapy for smoking cessation.

Explanations for the disparity between previous animal studies of nicotine withdrawal-induced effects and the present study may be attributed to the differing experimental populations and operationalizations of abstinence. Most of the studies on 5-HT and nicotine withdrawal or nicotine administration have used rats as the experimental population and have largely focused on behavioral assessments, and to a lesser extent, neurobiologic effects as operational definitions of the nicotine withdrawal reaction. These have included changes in conditioned responses (Corrigal, Herling, & Coen, 1989), changes in body weight or food and water consumption (Levin et al., 1993), changes in

abstinence signs of locomotion, gasps, shakes, teeth chatters, yawns, and ptosis (Hildebrand, Nomikos, Bondjers, Nisell, & Svenson, 1997), and changes in withdrawal-induced serotonergic neuronal firing (Rasmussen & Czachura, 1997).

Conceptually, it is a big leap from animal models of withdrawal to a model involving human smoking abstinence. First, the chronic treatment period in animals is fairly short when compared with the length of tobacco smoking in humans. Thus the behavioral reactions that follow upon cessation or antagonism of nicotine treatment in the rat is not necessarily related to abstinence in habitual smokers. Moreover, the rat has a limited behavioral repertoire with which it can respond to withdrawal of nicotine. Whereas, humans experience a much wider range of subjective, behavioral, and physiological effects, including subjective mood states, which can not be directly assessed in animal research.

At a more basic level, smoking abstinence in humans involves smoking history, inhalation of nicotine, smoking topography, smoking motives, personality factors and so on that are not likely to be factors in the animal models. It may be that the results derived from animal studies are specific in that the serotonergic system may play a role in modulating only some symptoms of nicotine withdrawal, such as in weight gain and increased food consumption (Levin et al., 1993). However, in the present study no alteration by tryptophan depletion was found in ratings of hunger.

The Profile of Mood States and Smoking Withdrawal Symptom Checklist are well-established measures of smoking withdrawal and mood states and it seems unlikely that the lack of behavioral change following tryptophan depletion is due to the use of

these instruments (Hughes & Hatsukami, 1986; McNair et al., 1971). A possible explanation may be that the induction of negative affect following smoking deprivation may have obscured an effect of tryptophan depletion on mood states. Along these lines, the test conditions and session length may have also been sufficiently aversive to obscure any effect of the mixture on mood ratings. The study involved day-long participation, overnight and day-long fasting, smoking deprivation, repeated blood sampling, frequent testing, and a 5 hr waiting period spent mostly alone with minimal stimulation. Therefore, it is conceivable that the induction of tobacco withdrawal symptoms and/or the participants reactions to aversive testing conditions obscured an effect of the depletion mixture on negative mood states.

The purpose of tryptophan depletion in this investigation was based on its specificity in altering central 5-HT function. It is possible that the implied relationship between 5-HT, mood and smoking abstinence noted within the literature is due more to its functional interaction with multiple neurochemical systems and due less to a direct effect of 5-HT. For example, the nigrostriatal and mesolimbic dopamine systems receive substantial input from 5-HT-containing projections and these serotonergic afferents may exert inhibitory effects on the regulation of dopamine systems (Jacobs & Azmitia, 1992). Of relevance is evidence indicating that the reinforcing effects of nicotine are associated with the release of dopamine in the mesolimbic dopamine pathway (Fibiger & Philipps, 1987). Moreover, behavioral studies have provided strong evidence indicating that decreasing the activity of serotonergic neurotransmission can reliably potentiate dopamine-dependent behaviors (Jacobs & Azmitia, 1992). The fact that tryptophan

depletion decreases 5-HT levels and does not directly affect the other neurochemical systems (such as dopamine) for which 5-HT functions primarily as a modulator, may account for the subtle and nonsignificant effects of tryptophan depletion on mood, abstinence and EEG in abstinent smokers.

The few clinical trials investigating 5-HT involvement as a pharmacologic intervention in smoking cessation in humans are far from conclusive on the matter. There have been several reports that serotonergic medications attenuate acute withdrawal symptoms in humans. For example, fluoxetine, a 5-HT reuptake inhibitor, has been found to: (a) reduce smoking withdrawal-induced weight gain in abstainers (Pomerleau, Pomerleau, Morrell, et al., 1991; Spring et al., 1995); (b) reduce withdrawal symptoms and improve smoking quit rates for nondepressed chronic smokers (Niaura et al., 1996), and (c) reduce dysphoric mood and prevent symptoms of withdrawal in the first week after quitting smoking in chronic smokers (Spring et al., 1993). Additional clinical trials have indicated that fluoxetine is beneficial primarily for smokers attempting to quit with a history of or current depression (Niaura et al., 1996). Others, however, found that fluoxetine did not decrease smoking in nondepressed alcoholics (Naranjo et al., 1990) and in patients with a past (but not current) episode of depression (Dalack et al., 1995).

These preliminary data suggest that serotonergic agents may deserve a place in the treatment of smoking cessation, but additional controlled trials are needed before any firm conclusions about 5-HT's role in smoking abstinence can be drawn. Moreover, the bulk of these studies are correlational in nature, have investigated different outcome measures, and used differing experimental populations (e.g., chronic smokers with or without past

or current depression, and alcoholics). Again, a direct comparison of these approaches with the present study may be questionable. It may well be that the results derived from the experimental design utilized in the present study may give a more adequate representation of serotonergic involvement in smoking abstinence in a nonclinical population.

The anticipated overall mood-lowering effect of tryptophan depletion in abstinent smokers was not observed in this study. While several studies have found a mood-lowering effect of tryptophan depletion in healthy individuals (Cleare and Bond, 1995; Knott, Howson, et al., 1999; Smith et al., 1987; Young et al., 1985), as evidenced by changes in the POMS depression scale, others have found that in some individuals tryptophan depletion did not result in any lowering of mood (Abott et al., 1992; Benkelfat et al., 1994; Delgado et al., 1990). Benkelfat and co-workers (1994) found that tryptophan depletion induced mild depressive symptoms only in male participants with personal or family histories of mood disorder but did not affect healthy males without personal or family histories of mood disorder. The authors suggest that the mood response to tryptophan depletion is primarily seen in those individuals at risk for depression.

Accordingly, a number of recent studies have also found that the mood-lowering response to tryptophan depletion is significantly greater in: (a) those individuals with, than without, a personal or family history of depression (Ellenbogen et al., 1999; Leyton et al., 2000); (b) individuals with paternal alcoholism (LeMarquand, Benkelfat, Pihl, Palmour, & Young, 1999); (c) individuals whose baseline depression scale scores are in the high average range (Delgado et al., 1990) as opposed to the below average range (e.g.,

euthymic individuals) (Abbott et al., 1992), and (d) vulnerable individuals with conditions thought to be related to low 5-HT neurotransmission as in the case of remitted depressed patients (Delgado et al., 1990), obsessive-compulsive disorder (Barr et al., 1994), and autistic disorder (McDougle et al., 1996).

These findings suggest that the mood-lowering response to acute tryptophan depletion may occur preferentially in subgroups of vulnerable individuals with a susceptibility to lowered mood or depression and at high genetic risk for disorders associated with affective lability. Consistent with this notion, is a study (conducted after the current investigation) by Spring and colleagues in 1998 that showed that tryptophan depletion lowered mood only in smokers with a personal or family history of depression. A comparison of these findings with those of the present study indicate that methodological differences led to a subject sample that was less vulnerable to the depressogenic effects of tryptophan depletion (i.e., the exclusion of individuals with a personal or family history of affective illness).

Another reason why tryptophan depletion may not have had the expected effect in the present study was that 5-HT may have been sufficiently lowered following smoking abstinence so that any further lowering was not possible or obscured an effect of tryptophan depletion on mood states. Accordingly, the different findings between the current study and that of Spring and colleagues (1998) suggest that abstinent smokers without a family or personal history of depression show a different response to tryptophan depletion than smokers who have such a history, due to either differences in tryptophan and/or 5-HT metabolism or a different emotional responses to similar metabolic changes.



Alternatively, it may well be that a worsening of symptoms following depletion could have been elicited had the depletion lasted longer. Hence, tryptophan depletion for five hours may be too short to exacerbate smoking withdrawal symptoms. In support of this possibility are the findings of Moreno and colleagues (1999) who reported that in participants with a history of depression, tryptophan depletion caused some individuals to experience increases in mood ratings at 5 hr, others at 7 hr after testing, and others not until the following day. In a study investigating the effects of depletion on 5-HIAA concentration in the cerebrospinal fluid (CSF) in humans, it was also shown that CSF 5-HIAA concentrations were lowest 8 to 12 hr after ingestion of the mixture (Carpenter et al., 1998). Thus, it appears that the brain biochemical effects of tryptophan depletion may be achieved later than the occurrence of peripheral (plasma) tryptophan changes and therefore, it is possible that tryptophan depletion would have an effect on the study measures but was not detected due to the study design.

Taken together, data from studies using the tryptophan depletion paradigm suggest that individuals with a vulnerability to depressive illness have a heightened sensitivity to changes in 5-HT concentration and appear to be more sensitive than other individuals to the transient depressogenic effects of tryptophan depletion. Therefore, it is possible that participants in the current study might have experienced greater change in abstinence-related symptoms if the study had included healthy non-depressed smokers with an enhanced susceptibility for affective disorders or if the depletion session had lasted longer. As it offers great potential in revealing the mechanism subserving

smoking's effects on mood in specific populations of smokers, the tryptophan depletion paradigm, with proper modifications, can, and should, be utilized in subsequent inquiries.

### **B. Effect of Tryptophan Depletion on the Tobacco Withdrawal Syndrome, Mood and EEG Following Cigarette Smoking**

The hypothesis that a cigarette smoking challenge would reduce and reverse any augmented abstinence, mood and EEG effects induced by the tryptophan depletion mixture in abstinent smokers was not supported. While a cigarette challenge decreased and reversed some of the symptoms and EEG effects associated with smoking abstinence, these effects were evident in both tryptophan-depleted and non-depleted states and were independent of the depletion mixture. As these mood-related and EEG changes following cigarette smoking were evident and similar regardless of the amino acid mixture ingested, they indicate a lack of an effect of tryptophan depletion on the acute smoking response. Therefore, these findings may suggest that in chronic smokers different neurochemical pathways other than 5-HT may modulate subjective states and the EEG response to acute smoking.

Cigarette smoking was observed to reduce some of the symptoms associated with the tobacco withdrawal syndrome. Ratings on items assessing craving/urges to smoke and total withdrawal discomfort of the Smoking Withdrawal Symptom Checklist were found to decrease following cigarette smoking, as compared to sham smoking. These ratings were low at baseline (pre-mixture/pre-smoking abstinence), showed a reliable time-dependent increase after 5 hr of cigarette deprivation, remained elevated following sham

inhalation, and decreased immediately following the smoking of a single cigarette. Moreover, following cigarette smoking the item craving/urge to smoke, but not total withdrawal discomfort, returned to pre-abstinence baseline values suggesting that craving was relieved by the smoking of a single cigarette.

Several POMS mood factors were also significantly affected by resumption of smoking. Anger-hostility and total mood disturbance ratings decreased during cigarette smoking relative to 5 hr of smoking deprivation and sham smoking. However, no significant smoking-induced effects were observed for the remaining factors. Taken together, the results of the subjective smoking withdrawal and mood ratings suggest that in abstinent individuals smoking affects dimensions related to both physiological and subjective mood states.

With respect to EEG changes, the resumption of cigarette smoking, as compared to sham-smoking, in both tryptophan-depleted and non-depleted participants induced changes in electrocortical activity similar to the results obtained in earlier smoking studies (Domino et al., 1992; Domino & Matsuoka, 1994; Knott et al., 1995). More specifically, the effects of smoking a single cigarette produced a psychostimulant-like EEG profile characterized by amplitude reductions in slow frequencies (delta) and amplitude increases in fast frequencies ( $\alpha_2$ ), a trend which is consistent with acute smoking deprivation studies. In addition, smoking not only reversed a state of cortical hypoarousal induced by tobacco abstinence but it more or less returned EEG activity to pre-smoking abstinence levels.

The efficacy of smoking in alleviating the tobacco withdrawal syndrome has been assessed in a multitude of studies using the Smoking Withdrawal Symptom Checklist (Hughes & Hatsukami, 1986; Hughes et al., 1984, 1987). Studies have confirmed an increase in self-reported withdrawal symptoms following acute tobacco abstinence and the resumption of smoking has been shown to reduce the increase in these symptoms and to return them to baseline values (Hughes et al., 1984, 1991). This reduction in withdrawal symptomatology has also been confirmed by participants' scores on the POMS (Hughes et al., 1984).

It is notable that of the tobacco withdrawal and mood measures found to be significantly reduced following cigarette smoking in previous studies of acute smoking abstinence, only a few were found to change significantly following acute smoking in this study. Abstinence-induced feelings of irritability, restlessness, anxiety, hunger, and depressed mood of the Withdrawal Symptom Checklist, as well as POMS tension-anxiety, depression-dejection, vigor-activity, fatigue-inertia and confusion-bewilderment, all showed a nonsignificant reduction following cigarette smoking.

There was also a trend for reduced ratings of POMS tension-anxiety and depression-dejection and depressed/sad/feeling blue of the Withdrawal Symptom Checklist after cigarette smoking. These effects, although they would have been significant at the .05 level, were not significant at the .008 (Bonferroni) level of statistical significance. Overall, acute cigarette smoking tended to nonsignificantly reduce the scores of most mood and smoking withdrawal ratings. While acute smoking did reduce and return most of the POMS mood ratings and several ratings of the Withdrawal

Symptom Checklist to pre-smoking abstinence baseline levels, other withdrawal-effects were not returned to baseline values.

There may be several reasons for the inability of an acute cigarette challenge to completely relieve and reverse the abstinence-induced effects. One reason may be that the smoking of a single cigarette following 6-7 hr of smoking abstinence may not be sufficient to completely relieve withdrawal effects. As nicotine blood levels were not assessed in the present study, the relative amount of nicotine obtained by cigarette smoking could not be determined. Daytime plasma nicotine levels in habitual smokers have been shown to average around 35 ng/ml (Benowitz, 1988). In a study that examined the relationship between plasma levels of nicotine and EEG effects results showed that smoking-induced EEG profiles were evident only when plasma nicotine concentrations reached a threshold level that was greater than 10 ng/ml (Domino et al., 1995). In light of this, it may be that higher blood nicotine levels were needed in order to suppress additional symptoms of withdrawal discomfort in the present study.

Alternatively, nicotine intake following smoking may have varied across participants due to differences in inhalation and smoking topography. Inspection of the CO values for individual participants following cigarette smoking showed that the mean CO values varied between 13 ppm to 21 ppm. While CO levels were on average 8.5 ppm following 5 hr of smoking abstinence, they increased significantly (>10 ppm) following acute cigarette smoking relative to sham-smoking. Moreover, smoking returned CO values to near-baseline values. As the present design did not assess the possibility that participants controlled the subjective (e.g., sedating versus stimulant) effects of nicotine

by taking stronger or weaker inhalations, it is difficult to tell whether the depth of inhalation and thus behavioral arousal varied substantially among participants.

The present study set out to investigate whether a cigarette smoking challenge would relieve and reverse any augmented smoking withdrawal, negative affect and cortical hypoarousal changes in the presence of depleted tryptophan. Related to this objective was the issue of the acute smoking response itself and whether serotonergic depletion would modulate the EEG response to cigarette smoking. Previous investigations have repeatedly shown that smoke-inhaled nicotine via cigarettes results in EEG and mood alterations (Knott 1990a, 1990b; Knott, Harr, & Lusk-Mikkelsen, 1998) as well as activation of cognitive processes as evidenced by improved performance efficiency on a range of information processing tasks (Heischman et al., 1994; Pritchard & Robinson, 1998).

Pharmacological studies attempting to unravel the neurochemical systems mediating these smoking-related changes have found that pre-treatment with nicotinic cholinergic and dopaminergic antagonists block some, but not all, aspects of the acute smoking-induced EEG response profile. For example, nicotinic blockade inhibited the smoking/nicotine-induced reductions in the slow-frequency response (Knott, Harr, Illivitsky, et al., 1998) whereas dopaminergic blockade inhibited the smoking-induced increase of beta amplitude (Walker et al., 1999). These findings suggest that smoking-induced changes in slow and fast frequency bands are differentially affected by the actions of other neurochemical systems and are not mediated solely by nicotinic systems.

The alpha band activities have been less influenced by the blockade of cholinergic and dopaminergic systems suggesting that one or more noncholinergic systems acting separately or in concert with nicotinic and/or dopaminergic systems may mediate the emergence of the arousal profile. In this respect, it is of interest to note that in the present study the tryptophan depletion condition relative to the balanced control mixture was associated with a significant reduction in alpha<sub>2</sub> activity. Although this effect failed to interact with the smoking abstinence or acute smoking conditions, the importance of clarifying the role of 5-HT in modulating the smoking-induced EEG profile cannot be underestimated. It therefore falls to future research to determine whether this working hypothesis is supported. Future studies employing central serotonergic blockers alone or in combination with nicotinic and dopaminergic antagonists may prove useful in this regard.

### **C. Effect of Tryptophan Depletion on Lateralized EEG Following Smoking Abstinence and Cigarette Smoking**

Among the present study's intentions was to examine the extent to which reduced brain levels of 5-HT mediated lateralization of EEG effects following smoking abstinence and cigarette smoking. It was hypothesized that following 5 hr of smoking abstinence both tryptophan depleted and non-depleted states would be associated with relatively more right-than-left frontal EEG activity due to the induction of smoking abstinent states. These effects were expected to be stronger in the tryptophan depletion condition relative to the placebo condition due to the induction of negative affect associated with reduced

brain levels of 5-HT. In contrast, it was expected that a cigarette smoking challenge would eliminate negative mood states and normalize EEG asymmetry by inducing increases in right-relative-to-left frontal EEG activity.

The current study yielded data inconsistent with the notion of right-frontal-hemisphere effects in smoking deprived individuals. Specifically, data showed that smoking abstinence was associated with tobacco withdrawal effects and reduced cortical arousal that was nonlateralized and nonlocalized. Accordingly, cigarette smoking was shown to reduce and reverse some of the abstinence-related effects and to induce EEG arousal effects in a nonlateralized and nonlocalized fashion. Moreover, further analyses demonstrated that the abstinence and smoking-induced effects were not affected by the tryptophan depletion and balanced placebo treatments. Several explanations can be offered for these negative results.

First, there is a small body of literature indicating that lateralized EEG and subjective effects of smoking and smoking abstinence vary as a function of individual differences (Gilbert, 1988; Gilbert et al., 1994; Gilbert et al., 1995). For example, lateralized EEG effects have been associated with smokers who were high in neurotic traits (e.g., depression, anxiety, anger) and in social alienation (e.g., psychoticism, impulsivity, low conscientiousness and agreeableness) (Gilbert, 1988).

In a previous study on male and female smokers, the initial baseline depression and neuroticism scores were significantly higher among smokers compared to nonsmokers and depression scale scores were significantly higher among female smokers than male smokers, and male and female nonsmokers (Gilbert et al., 1994). Moreover,



abstinent smokers scoring high in neuroticism and depression exhibited smaller electrocortical and hormonal responses to nicotine and showed more activated right-than-left hemisphere effects. Thus, the initial state of the individual and the role of individual differences seems to be important in determining the response to smoking abstinence and cigarette smoking.

In the present study, the extensive screening done to exclude participants with a past or present personal or family history of psychiatric disorders resulted in a population that showed low baseline POMS and Smoking Withdrawal Symptom Checklist ratings for all aspects of negative affect. Given that lateralized EEG effects and the ability of tryptophan depletion to lower mood seems to be strongly associated with a vulnerability to mood-lowering, the absence of lateralized and mood-lowering effects in the present study's sample of euthymic smokers is not surprising.

Moreover, in accordance with the present findings others have also failed to show nicotine/smoking-induced lateralized EEG effects in abstinent and non-abstinent smokers (Knott, Bosman, et al., 1999; Pritchard, 1991). Thus, it is difficult to compare the results presented here with those of previous studies as smokers were not grouped according to personality traits and the methods of analysis for asymmetrical EEG effects differed from those of Gilbert and co-workers (1994). Moreover, the studies differed in terms of nicotine yield, delivery and total number of cigarettes smoked. Therefore, all of these factors could potentially have contributed to the differences in findings between studies.

Second, the current study differs from others in that it included male smokers only. As indicated above, studies examining lateralized EEG effects in female smokers

have reported that electrocortical and subjective responses to smoking and smoking abstinence varied as a function of gender (Gilbert et al., 1994). These studies have reported associations between depression, anger, and smoking, especially among female smokers, and suggest that these personality traits may influence one's decision to smoke.

These observed patterns of results appear to be compatible with the findings of other authors who report women are more likely to smoke to manage negative affect and those with a history of depression are less likely to quit than men (Borrelli et al., 1996). Additionally, others have indicated that healthy women are more sensitive to the effects of tryptophan depletion than men (Ellenbogen, Young, Dean, Palmour, & Benkelfat, 1996) as well as show greater tryptophan-induced brain biochemical effects during PET imaging studies (Nishiwaza et al., 1997). Taken together, these studies generally suggest that females may be more vulnerable to alterations in 5-HT neurotransmission and smoking abstinence effects. Thus, it is possible that inclusion of a comparison group of female smokers in the present study could have produced more significant findings. Future work in this area should consider examining acute tryptophan depletion effects in female smokers at high risk for the development of mood disorders.

#### **D. Limitations**

Before drawing conclusions from the present study's findings, the results should be interpreted while considering the following limitations. First, the sample size was relatively small in this study and consisted only of young adult male smokers. The sample did not include female smokers or smoker subtypes (e.g., negative affect reduction versus

positive affect smokers). Second, it is quite possible that the smoking abstinence-induced effects observed within the present study were confounded by abstinence from caffeine, food or alcohol. Third, the absence of controls (e.g., a group of non-abstinent smokers) makes it difficult to assess whether the apparent induction of an abstinence syndrome reflected a specific nicotine effect or placebo factors. Involvement in this study included day-long participation, time periods spent alone, and frequent testing. Therefore, it is quite possible that smoking abstinence symptoms were influenced by these nonpharmacological factors. Fourth, the sham and cigarette smoking conditions were not randomized or counterbalanced. Furthermore, while the sham smoking condition served as a control for the motoric/behavioral effects of smoking, it lacked control of the inhalation and sensory features associated with smoke influencing olfactory and gustatory receptors. Fifth, as plasma nicotine levels were not assessed to monitor central bioavailability, it is difficult to know whether the smoking-induced effects observed within the present study were entirely nicotine-related.

Finally, the study required participants to smoke their preferred cigarette brand and in using this approach it relinquished control over several potentially important variables. The present design did not allow for assessment of the possibility that smokers controlled the subjective (stimulant vs. sedating) effects of nicotine by taking in more or less of it. Therefore, nicotine intake following smoking may have varied across participants due to differences in inhalation rate and smoking topography. Accordingly, others have found that cigarettes tend to be smoked more intensively in a laboratory setting than in a natural setting (Russell, 1987). Furthermore, because smokers were

aware that they would eventually resume smoking, expectancy effects may have affected their mood states. Future work in this area should employ a placebo-control group to assess some of these issues as well as to verify the extent to which nicotine itself, as opposed to other nonnicotine components and nonpharmacological factors, is responsible for any EEG and mood state changes.

### **E. Conclusions**

The working hypothesis of this study was that 5-HT plays a role in some of the signs and symptoms associated with smoking abstinence and acute smoking behavior. Based on studies showing that central 5-HT function may be reduced in experimental animals following nicotine withdrawal and serotonergic agents help attenuate some of the symptoms of smoking abstinence, it was posited that: (a) acute depletion of tryptophan and presumably brain 5-HT, would result in an exacerbation of tobacco withdrawal symptoms and negative affect, and reduce EEG arousal in abstinent smokers; (b) a cigarette smoking challenge would reduce and reverse behavioral and EEG effects in the presence of depletion of tryptophan, and (c) induction of smoking withdrawal effects following the ingestion of both amino acid mixtures, and exacerbation of these effects by tryptophan depletion, would result in lateralized and localized EEG effects and the resumption of smoking would normalize these effects.

The study demonstrated that acute smoking deprivation led to clear time-dependent increases in subjective measures of negative affective states and tobacco withdrawal symptoms and reduced cortical arousal. However, the depletion of tryptophan

did not exacerbate this withdrawal in chronic smokers. While a cigarette smoking challenge reduced and reversed some of the withdrawal-induced subjective effects and increased EEG arousal, again these effects were not influenced by the tryptophan depletion mixture. Finally, lateralized and localized EEG effects were not observed following smoking abstinence and were unaffected by the depletion of tryptophan. Although many questions are raised, the conclusions of this study are that acute reduction of plasma tryptophan and brain 5-HT levels do not appear to alter the signs and symptoms associated with acute smoking abstinence or acute smoking behavior.

These conclusions, however, cannot be considered as a definitive proof against the 5-HT hypothesis of smoking abstinence/smoking behavior. As only relatively young male smokers were examined the sample is not representative of the general population of smokers. Rather, the possibility of individual differences in 5-HT dysfunction among smokers is supported by the findings of Spring and co-workers (1998) who reported that tryptophan depletion induced depressive symptoms in smokers at risk for depression but did not affect smokers who were not at risk. Additionally, recent molecular studies have indicated that individuals with poorly transcribed genes for the brain 5-HT transporter and a high level of neuroticism have an increased risk of smoking and a decreased ability to quit (Hu et al., 2000; Lerman et al., 2000). Thus, these findings suggest that in specific populations of smokers, serotonergic dysfunction may be a contributing factor to smoking behavior.

Further exploration of variables that may affect the individual smoker and varying responses to 5-HT function are required. Future studies may consider using the

tryptophan depletion paradigm to study other populations of smokers including heavy smokers, males and females at high risk for affective disorders, individuals who smoke to manage their negative affect, and those with high levels of neuroticism. Further elucidation of the influence of genes involved in synthesis, release, and receptor function for 5-HT and other neurotransmitters in smokers may enhance our understanding of the genetic contributions to individual differences in smoking behavior. Additionally, as there is increasing evidence that 5-HT interacts with mesolimbic dopamine pathways that are critical for the rewarding and reinforcing properties of nicotine (Epping-Jordan, Watkins, Koob, & Markou, 1998; Parsons & Justice, 1993; Pontieri, Tanda, Orzi, & Di Chiara, 1996) investigation of serotonergic blockers alone or in combination with dopamine and cholinergic antagonists may prove useful in examining the role of this system in modulating behavioral and electrophysiologic processes underlying smoking withdrawal and smoking behavior. Thus, continued investigation into the role of 5-HT and other neurotransmitter systems in the neurobiology of smoking abstinence is warranted.

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**Appendix A**  
**Diagnostic Criteria for Nicotine Withdrawal (DSM-IV)**

- A. Daily use of nicotine for at least several weeks.
- B. Abrupt cessation of nicotine use, or reduction in the amount of nicotine used, followed within 24 hours by four (or more) of the following signs:
- (1) dysphoric or depressed mood
  - (2) insomnia
  - (3) irritability, frustration, or anger
  - (4) anxiety
  - (5) difficulty concentrating
  - (6) restlessness
  - (7) decreased heart rate
  - (8) increased appetite or weight gain
- C. The symptoms in criterion B cause clinically significant distress or impairment in social, occupational or other important areas of functioning.
- D. The symptoms are not due to a general medical condition and are not better accounted for by another mental disorder.

**Appendix B**  
**Telephone Screening Questionnaire**

Participant Ref #: \_\_\_\_\_  
Date: \_\_\_\_\_

The following questions will help us to determine whether you qualify for the study. All information given will be kept strictly confidential.

Are you between the ages of 18-40?  
Are you within the normal range of body weight for your height?  
Are you right or left-handed? R L.

**Drug/Alcohol Use**

Do you smoke? How long have you been smoking?  
Do you experience any discomfort if you abstain from smoking?  
Are you currently taking any prescription or nonprescription medication?  
Do you drink coffee? If yes, how much.  
Do you use illicit drugs? If yes, how much.  
Do you drink alcohol? How much?

**General Health History**

Are you currently consulting with a physician? Y N.  
Are there any medical conditions (for e.g. epilepsy) that may prohibit your taking part in this study?  
Often get headaches? Y N.  
History of migraines? Y N.  
Trouble sleeping? Y N.  
Problem with hearing? Y N.  
Have you ever had a serious head-injury?

**Mental Health**

Are you consulting with a psychologist or psychiatrist, or have you in the past?  
Have you experienced periods in your life where you felt depressed or unhappy?  
Does anyone in your family (a parent or a sibling) suffer from depression or other mental health illness?

**Appendix C**  
**Fagerstrom Tolerance Questionnaire**

Questions	Answers	Points
1. How many cigarettes a day do you smoke?	15 or less	0
	16-25	1
	26 or more	2
2. What is the nicotine level of your usual brand of cigarette?	0.9 mg or less	0
	1.0-1.2 mg	1
	1.3 mg or more	2
3. Do you inhale?	Never	0
	Sometimes	1
	Always	2
4. Do you smoke more during the morning than during the rest of the day?	Yes	1
	No	0
5. How soon after you wake up do you smoke your first cigarette?	Within 30 min	1
	After 30 min	0
6. Which cigarette would you hate most to give up?	The first one in the morning	1
	Any other	0
7. Do you find it difficult to refrain from smoking in places where it is forbidden (e.g., in church, at the library, in cinema, etc.)?	Yes	1
	No	0
8. Do you smoke if you are so ill that you are in bed most of the day?	Yes	1
	No	0

**FTQ Assumptions:**

The FTQ is a scale for measuring nicotine dependence. It was hypothesized that nicotine dependence should be related to the following:

1. How often the drug was used (number of cigarettes/day).
2. Smoking cigarette brands with high doses of nicotine.
3. Effective utilization of nicotine (via inhalation).
4. Smoking more in the morning (the rate of nicotine use within the first hour or two after awakening). Plasma nicotine levels should be low in the morning thus affecting nicotine-seeking.

5. Smoking almost directly after getting up in the morning.
6. Rating the first cigarette in the morning as the “best” or most valuable. The morning cigarette should be reported as important due to its power to alleviate nicotine-specific withdrawal symptoms.
7. More internal stimulus control relative to external control (manifested as frequent urges to smoke in forbidden places, e.g., church).
8. Smoking while ill.

#### Scoring the Tolerance Questionnaire

Higher points are always given for answers indicating greater nicotine dependency. In the first question smokers are divided as light <15, moderate 16-25, and, heavy >25 smokers. In the second question, the brands are classified into three categories with low, medium, and high nicotine levels. In the third question the frequency of inhalations are divided into three categories. The rest of the questions are scored zero or one, with one point for yes answer given on question 4. In the fifth question one point is given to smoking within 30 minutes. In the sixth question one point is assigned for answering “the first cigarette in the morning”. The seventh and eighth questions are scored with one point for a yes answer.

The questionnaire has a range of 0-11 points, with 0 indicating minimum nicotine dependence and 11 points maximum nicotine dependence (Fagerstrom, 1978).



## Appendix D

### INFORMED CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY

**Title of Study:** Acute Plasma Tryptophan Depletion and Smoking  
Abstinence: Withdrawal, Mood and Quantitative EEG  
Correlates and the Acute Smoking Response

**Principal Investigator:** Mary A. Perugini (Ph.D student)  
School of Psychology, University of Ottawa

**Associate Investigators:** V. Knott, Ph.D.

**Description of Study:** Smokers deprived of cigarette smoking often experience a number of adverse effects including frustration, irritability, anger, fatigue, and craving. These smoking withdrawal effects are believed to prevent many smokers from quitting smoking and to contribute to the high relapse rate among unsuccessful quitters. Various treatments and medications exist to help smokers quit smoking. Some of these medications are non-nicotine medications. They work by attempting to normalize brain chemicals that are influenced by the nicotine in tobacco smoke and produce similar effects on mood and behavior as does smoking. One common chemical found in both the body and brain that may be influenced by smoking is serotonin. Serotonin is involved in controlling many aspects of our behavior, mood and physiology. Although much is known about serotonin, there is little information about how changes in the level of this chemical affect smoking withdrawal symptoms. Your participation in this study will help to reveal how changes in brain serotonin levels affect mood, smoking withdrawal symptoms and brain activity as measured by computerized EEG (electroencephalogram) techniques. You are participating in this study as an individual who smokes cigarettes on a daily basis and experiences clear discomfort when refraining from smoking for a few hours.

The main objective of this study is to examine the effects of a temporary reduction in serotonin in smokers deprived of cigarette smoking. A reduction in serotonin will be accomplished by reducing the levels of tryptophan in both the blood and brain. Tryptophan is one of several amino acids found in a number of foods such as turkey and milk. Amino acids are molecules which the body uses to build proteins and brain chemicals. In the brain, tryptophan produces serotonin. By lowering the amount of tryptophan in the blood the levels of serotonin in the brain can also be lowered.

A secondary objective of this study is to examine whether cigarette smoking can relieve the effects of decreased brain serotonin. A number of studies have suggested that smokers may smoke to reverse some of the effects of decreased serotonin (e.g., increased frustration and irritability). In other words, smokers smoke to feel better and experience a positive mood. This study will examine whether the resumption of cigarette smoking can reverse the effects induced by reduced serotonin in abstaining smokers.

**Procedures Involved:** Three sessions are involved. During the first "orientation session" you will be introduced to the procedures that will be carried out in the subsequent two test sessions. The two test sessions will take place from 8:00 a.m. - 3:00 p.m. You will have to abstain from food, caffeine, alcohol, street drugs and all medications (e.g. aspirin) including herbal medications beginning at midnight on the night prior to each test session. You will also have to abstain from cigarette smoking (from approximately 8:30 a.m. to 2:30 p.m.) during each of the two testing days. You will not be allowed to consume any food or beverage except water throughout the test sessions. During each test session brain waves will be assessed 4 separate times by computerised EEG while you rest with your

eyes closed. Sensors will be placed on your scalp and around your eyes. These sensors record the electrical activity that is generated from your brain. You will also be asked 4 separate times to complete questionnaires which measure your mood state. In addition to these assessment measures, blood samples will be collected twice, each collection separated by 5 hours, in order to measure the levels of tryptophan in your blood. Each sampling will involve the collection of a small quantity of blood (5 millilitres) which will be drawn by a nurse experienced in this procedure. As well, expired breath samples will be taken for the assessment of carbon monoxide levels following both smoking abstinence and cigarette smoking.

During the test session you will be given a beverage to drink together with several capsules to swallow. The beverage will contain a mixture of amino acids that may or may not contain tryptophan. The mixtures are identical except one of them will contain tryptophan. The order of administration of the beverages will be random and neither you nor the researcher will know what beverage you are receiving until after the entire study is completed. The capsules will also contain amino acids but they are in capsule form because of their unpleasant taste. Approximately 5.5 hours after ingestion of the beverage and capsules you will be asked to inhale on an unlit cigarette puffing once every 30 seconds for a total of 10 minutes. You will then be asked to inhale on a lit cigarette of your own preferred brand inhaling once every 30 seconds for 10 minutes.

After the test assessments are completed the sensors will be taken off your scalp and face. You will be given a light snack and a tablet containing 1 gram of L-tryptophan (trade name TRYPTAN) which will help restore your tryptophan to normal levels.

**Possible Risks Involved:** The risks are minimal. A small number of participants experience slight nausea, drowsiness, headaches, or diarrhea after the amino acid mixtures. These side effects are of short duration, and they typically disappear in several minutes to several hours. Some participants also experience a mood-lowering effect from the mixture which is also of short duration. In a previous study that we conducted in our laboratory with 15 male volunteers that received a tryptophan depletion mixture that decreased serotonin no side effects and no adverse long-term effects were reported. The amino acid mixtures were well tolerated. They are a safe and nontoxic method of lowering tryptophan and serotonin levels.

With respect to L-tryptophan (TRYPTAN), daily dose levels 12 times the amount you will be receiving produce side effects such as nausea, vomiting, gastric pain/heartburn, anorexia, dizziness, headache, drowsiness/slowness, tremors, dry mouth, and constipation.

It should be realized that while this list of side effects experienced by some participants is quite extensive, they are temporary (usually not lasting longer than 24 hours maximum) and it is unlikely participants in this study will experience all of them and it is quite possible none of them will be experienced.

The drawing of blood may be uncomfortable for some, however the procedure is quick and generally painless. Only a small amount of blood (5 millilitres) is taken each time blood is drawn (twice during each test session).

The EEG monitoring is very similar to what is carried out in general hospitals except that it is analyzed by computer. The sensors placed on your scalp and skin may cause a mild and temporary irritation and redness of the skin which will disappear after a few hours.

Any possibility of the need for immediate medical attention during the testing will be covered by the study doctor (Dr. V. Ilivitsky).

### OTHER INFORMATION

**Participation:** Your participation is strictly voluntary and you will be free to withdraw from the study at any moment or refuse to participate without any penalty. The research personnel involved in this study reserve the right to terminate your participation in this study at any time for scientific reasons. In the case that either of these should cause your withdrawal from the study you will be paid for the time you have put in up until that point. You will receive \$7.00 per hour payment for your time. Your payment will be \$100.00 for fully completing the study requirements.

**Confidentiality:** Information will be coded and your privacy will be protected. Any scientific publications from this work will be designed so that your anonymity will be preserved.

**Problems or Questions:** The principal investigator is Mary Perugini (doctoral student). You may contact her (722-6521, ext: 6411 or 6757) or her supervisor, Dr. Knott (722-6521, ext: 6843), if you have any concerns or questions about this study. The address is the Royal Ottawa Hospital, Neurophysiology Laboratory, 1145 Carling Avenue, Ottawa, Ontario, K1Z 7K4.

### CONSENT FORM

**Study Name:** Acute Plasma Tryptophan Depletion and Smoking Abstinence:  
Withdrawal, Mood and Quantitative EEG Correlates and the Acute  
Smoking Response

I have read and understood the Informed Consent Statement for this study and all my questions have been answered. I voluntarily consent to participate in this study and I understand that I can withdraw consent at any time.

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PARTICIPANT	WITNESS	INVESTIGATOR
_____ Print Name	_____ Print Name	_____ Print Name
_____ Signature	_____ Signature	_____ Signature
_____ Date	_____ Date	_____ Date

I wish to receive a summary of the results of this study which will be available in \_\_\_\_\_  
at the following address: \_\_\_\_\_

THANK-YOU FOR YOUR PARTICIPATION.

**Appendix E**  
**Formation of Amino Acid Mixtures**

Amino acids included in the balanced placebo (B) and tryptophan depletion (T-) mixtures.

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	<u>B(gram)</u>	<u>T- (gram)</u>
L- Alanine	2.75	2.75
L-Arginine*	2.45	2.45
L-Cysteine*	1.35	1.35
Glycine	1.6	1.6
L-Histidine	1.6	1.6
L-Isoleucine	4.0	4.0
L-Leucine	6.75	6.75
L-Lysine monohydrochloride	5.5	5.5
L-Methionine*	1.5	1.5
L-Phenylalanine	2.85	2.85
L-Proline	6.1	6.1
L-Serine	3.45	3.45
L-Threonine	3.25	3.25
L-Tryptophan	1.15	0
L-Tyrosine	3.45	3.45
L-Valine	4.45	4.45
<b>TOTAL:</b>	<b>51.15</b>	<b>50</b>

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\*not included in the mixture but were administered in capsules due to bitter taste.

Each amino acid mixture was blended with:

- 150 ml water
- 50 ml of non-dairy chocolate syrup
- one-half packet of saccharin