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Effects of tryptophan depletion on acute smoking abstinence symptoms and the acute smoking response

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Abstract

Given the putative role of serotonin in the modulation of smoking withdrawal and the central actions of nicotine, this study examined the affective and neuroelectric correlates of smoking abstinence and cigarette smoking following depletion of the serotonin precursor, tryptophan. In a randomized, double-blind two session (tryptophan depletion [TD] vs. nondepletion), placebo-controlled design, spectrally analyzed electroencephalogram (EEG), self-ratings of withdrawal symptoms and mood states were assessed in 18 male cigarette smokers before smoking abstinence, 5 h postsmoking abstinence and again following sham smoking and the smoking of one cigarette. Compared to a nutritionally balanced amino acid (AA) mixture containing tryptophan (i.e., placebo mixture), oral ingestion of a similar mixture devoid of tryptophan resulted in a 70% reduction of plasma tryptophan but failed to alter the appearance or reversal (by acute cigarette smoking) of withdrawal symptoms, negative mood states and increased slow wave EEG in male smokers deprived of cigarettes. These results, although not supporting a role for the serotonergic system in acute smoking and early smoking abstinence symptoms, are discussed in relation to the neuropharmacology of smoking behavior and suggestions for future work.

Keywords: Smoking; Nicotine; Smoking withdrawal; Serotonin; Tryptophan; Mood; Electroencephalography (EEG)

1. Introduction

Biobehavioral models of smoking behavior have ascribed nicotine's central actions, particularly those which result in increases in central (electroencephalographic: EEG) arousal, mood (i.e., subjective alertness) control, and enhancement of attention and information processing efficiency, a critical motivational role in the initiation and maintenance of cigarette dependence (Gilbert, 1995). Nicotine has been shown to exert its effects primarily via presynaptic nicotinic acetylcholine receptors (nAchRs) situated on axon terminals (Le Houezec, 1998), which not only increase acetylcholine transmission but also release various neurotransmitters, each of which appear to be associated with different functions (Watkins et al., 2000). nAchRs have also been found on serotonergic (5-hydroxytryptamine: 5-HT) axons of the raphe nuclei (Benwell et al., 1988; Marks et al., 1992), hypothalamus and striatum (Schwartz et al., 1984). While acute systemic nicotine injections (Ribiero et al., 1993), local application of nicotine via microdialysis probe (Toth et al., 1992) and administration of the nAchR agonist dimethylphenyl piperazinium (Westfall et al., 1983), have resulted in the increased release of 5-HT from frontal cortex and striatum brain slices, nicotine-induced 5-HT release has been blocked by pretreatment with the nicotinic antagonist mecamylamine (Ribiero et al., 1993). In addition to its putative role in the mood modulating actions of acute smoke-inhaled nicotine (Benowitz, 1999), 5-HT activity has also been implicated in the appearance and intensity of withdrawal symptoms and negative affect elicited during smoking cessation. Thus, increased 5-HT_{1A} receptor sensitivity (presumably secondary to reduced synaptic availability of 5-HT) has been

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shown in rats undergoing nicotine withdrawal (Rasmussen and Czachura, 1997) and serotonergic agents have evidenced efficacy in reducing some nicotine withdrawal symptoms in both animals (Levin et al., 1993) and humans (Niavra et al., 1997; Pomerleau et al., 1991; Spring et al., 1991, 1993).

One possible strategy for elucidating the role of 5-HT mechanisms in nicotine's positive and negative reinforcing actions in habitual cigarette smokers is to investigate the functional effects of the serotonin precursor, tryptophan. Tryptophan depletion (TD) is an experimental procedure used to temporarily lower tryptophan availability and 5-HT function in the brain. The dietary TD methodology (Reilly et al., 1997) involves administration of a single tryptophan-free amino acid (AA) mixture that induces protein synthesis (which requires tryptophan and other AAs) and results in lowered plasma tryptophan (Harper et al., 1970). Depending on the dosage and time of administration, the resulting plasma tryptophan reduction (9–55% of baseline values) reaches its lowest level 5–7 h after AA ingestion (Van der Does, 2001) and is paralleled by lowered cerebral spinal fluid (CSF) indices of central tryptophan and 5-hydroxyindoleacetic acid (5-H1AA) (Carpenter et al., 1998; Williams et al., 1999) and by changes in brain 5-HT function, including altered postsynaptic receptor sensitivity (Bell et al., 2001).

In addition to increased nicotine craving, the smoking withdrawal profile accompanying smoking abstinence has been associated with a cluster of physiological, mood and cognitive-behavioral symptoms which appear to develop within a few hours of smoking cessation, reach maximal peak intensity within 1 to 3 days and, for the most part, are immediately alleviated with the administration of nicotine or the resumption of cigarette smoking (Hughes et al., 1991, 1990; Hughes and Hatsukami, 1981). As both smoking withdrawal and its abatement have been profiled in the laboratory during acute brief (morning- and day-long) smoking deprivation periods (Knott, 1995; Knott and Harr, 1998), the purpose of this study was to examine the effects of TD on smoking withdrawal symptoms and mood states following 5 h of smoking abstinence and following a subsequent acute smoking period.

Quantitative EEG monitoring was examined as a neurobiologic marker of TD, smoking withdrawal and smoking, and for the purpose of providing some insight into the neurophysiologic mechanisms by which 5-HT putatively regulates the positive and negative (i.e., withdrawal) reinforcing actions associated with smoking behavior (Watkins et al., 2000). EEG was specifically selected as it is an established methodology for pursuing pharmacodynamic profiles of psychoactive agents (Knott, 2000) and, in our laboratory, it has been consistently shown to be sensitive to the central actions of nicotine, smoking withdrawal (Knott, 2001) and serotonergic manipulations, including TD (Knott et al., 1999a,b). Smoking withdrawal (Knott, 2001) and TD (Knott et al., 1999a,b) have both resulted in EEG deactivation (i.e., shifting of power from fast to slow frequencies), while smoking/nicotine (Knott, 2001) and 5-HT agonists (Knott et al., 1999a,b; Saletu, 1993) have produced signs of EEG activation (i.e., shifting of power from slow to fast frequencies). Of particular interest in this study was the intention to examine the notion that regional balances in right (R) and left (L) frontal EEG activation reflect affective states such as depressed mood, which is frequently associated with lowered 5-HT functioning (Deakin, 1998), is often observed during smoking cessation (Gilbert, 1996) and has been linked with frontal activation asymmetry (R>L) in nonclinical and clinical populations (Davidson, 1995). Smoking abstinence has been reported to induce a similar asymmetric pattern in the frontal cortex of smokers scoring high in psychometrically assessed trait depression (Gilbert et al., 1999), while nicotine has elevated mood and normalized frontal EEG asymmetries (Gilbert et al., 1994), but it is unclear as to whether these actions involve serotonergic systems.

2. Method

2.1. Experimental subjects

Eighteen right-handed healthy male (45% Caucasian) cigarette smokers voluntarily participated in this study following written consent to an investigation, which was approved by the hospital research ethics committee. The study only included male participants as previous investigations have shown mood (Schechter et al., 1989), smoking behavior (Steinberg and Cherek, 1989), withdrawal symptoms (O'Hara et al., 1989) and EEG (Lamb et al., 1953) may be affected by menstrual cyclicity. The means and standard deviations of plasma tryptophan levels from previous TD studies in nonclinical populations (Abott et al., 1992; Knott et al., 1999a,b) were used to determine effect and sample size (Stevens, 1992). An effect size of 0.46 indicates that a sample size of 18 would provide 0.80 power with an α of .05 for a two-tailed test. For study inclusion subjects had to be smoking a minimum of 15 cigarettes per day for the past 3 years, smoke their first cigarette within 15 min of awakening, meet DSM-IV criteria for nicotine withdrawal (American Psychiatric Association, 1994) and exhibit a minimum score of 7 (out of 11-point scale) on the Fagerstrom Tolerance Questionnaire (FTQ), a putative psychometric index of the degree of nicotine dependence (Fagerström, 1978). All were medically and psychiatrically screened by a psychiatrist (VI), were required to be within 15% of ideal body weight in relation to height, and those with significant past or present alcohol/drug abuse or dependence (other than nicotine dependence), psychiatric illness or significant medical illness were excluded from the study. Subjects were also excluded if they had been taking medications (prescription and nonprescription) that affected appetite or CNS functioning or reported a past or current

Table 1
Male smoker characteristics

	Mean	S.D.
Age	24.3	5.3
Years smoking regularly	6.2	3.5
Years smoking current amount	4.2	3.2
Cigarettes smoked per day	18.8	4.0
Nicotine yield (mg) per cigarette	1.3	0.3
FTQ score[a]	7.8	1.1
DSM-IV smoking withdrawal symptoms[b]	5.8	0.3

[a] Fagerstrom Tolerance Questionnaire total score.
[b] Average reported number of DSM-IV smoking withdrawal symptoms during previous morning smoking abstinence periods and previous attempts to quit smoking.

mood disorder in a first-degree relative. Subject characteristics are shown in Table 1.

2.2. Study design

Subjects attended the laboratory for one "orientation" session for familiarization with study procedures, and for two additional "test" sessions in which they received, under randomized double-blind conditions, either a nutritionally balanced (B) placebo AA mixture containing tryptophan or a tryptophan-deficient (T-) AA mixture. The test sessions were separated by a minimum 3-day interval and the mixtures were administered in a counterbalanced (incomplete) order such that half ($n=9$) of the participants (randomly selected) were assigned to receive the B treatment first and the T- treatment second, while the remaining participants received the treatments in the reverse order. Study test battery measures were collected prior to AA treatment (TB1) and on three subsequent occasions (TB2, TB3, TB4) following AA treatment.

2.3. Study procedures

Test sessions were carried out in the morning following overnight (beginning at 12:00 a.m.) abstinence from food, beverages (except water), caffeine, alcohol, drugs and medications. So as not to induce smoking withdrawal prior to the study period, all subjects were 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 test session followed an identical timetable as outlined in Table 2.

Upon arrival at the laboratory, subjects were taken to a sound-attenuated electrically shielded recording chamber situated immediately adjacent to the control room housing computers, monitors, amplifiers and recording equipment. They were prepared for EEG hook-up and, at the same time, so as not to induce withdrawal, were required to smoke (in their usual manner) one cigarette from their own brand. Following electrode placement, subjects were assessed for the first time with a test battery (TB1), lasting approximately 20 min, which involved (in the following fixed order): a 5-min EEG recording, completion of self-reports of mood state and withdrawal symptoms, collection of an expired-breath carbon monoxide (CO) sample and a sampling of blood (5 ml) for total plasma tryptophan measurements. After a brief 10-min break, subjects were administered one of the two AA drink mixtures and waited 5 h for absorption. The absorption interval was chosen on the basis of previous reports indicating that plasma tryptophan levels were reduced by as much as 70–90% within 5 h of AA consumption (Benkelfat et al., 1994; Delgado et al., 1990; Young et al., 1985) During the 5-h waiting period subjects were allowed to sit quietly in the chamber and were given a standard selection of emotionally neutral videos and magazines. Immediately after the 5-h absorption period, subjects were administered the identical test battery for the second time (TB2). A 10-min sham smoking period was then initiated and this was followed by a third test battery assessment (TB3) which did not include blood sampling. A subsequent 10-min cigarette-smoking period then preceded the final assessment (TB4), which again did not include blood sampling. At the end of each test session, subjects were given a 1-g tablet of Tryptan and a protein snack which contained foods expected to replete plasma tryptophan (Young et al., 1989). Subjects were then allowed to leave the laboratory if they were experiencing no unusual physical symptoms and if they were not evidencing any depressed mood. Follow-up telephone calls in the evening and following day were made to check subject status.

2.4. Tryptophan depletion

The T- mixture was 50 g and was the same as that used by Young et al. (1989): L-alanine, 2.75 g; L-arginine, 2.45 g; L-cysteine, 1.35 g; glycine, 1.6 g; L-histidine, 1.6 g; L-isoleucine, 4.0 g; L-leucine, 6.75 g; L-lysine monohydrochloride, 5.5 g; L-methionine, 1.5 g; L-phenylalanine, 2.85 g; L-proline, 6.1 g; L-serine, 3.45 g; L-threonine, 3.25 g; L-tyrosine, 3.45 g; L-valine, 4.45 g. The B mixture contained the same AAs plus 1.15 g L-tryptophan. The B mixture

Table 2
Test session timeline

Time (h:min)	Activity
08:00 a.m.	EEG hook-up and ad lib smoking
08:30 a.m.	Baseline test battery assessment (TB1[a])
08:50 a.m.	Initiate smoking abstinence
09:00 a.m.	Administer amino acid mixture
09:10 a.m.	Initiate amino acid absorption period (5 h)
02:10 p.m.	Postmixture test battery assessment (TB2[a])
02:30 p.m.	Sham smoking
02:40 p.m.	Postsham smoking test battery assessment (TB3)
03:00 p.m.	Cigarette smoking
03:10 p.m.	Postcigarette smoking test battery assessment (TB4)
03:30 p.m.	Administer protein snack and Tryptan tablet
03:45 p.m.	Departure

[a] TB1 and TB2 included blood sampling for assaying total plasma tryptophan levels.

contained approximately the same amount of AAs as 250 g of steak and were in the same proportion as in milk, except that aspartic acid and glutamic acid were omitted because of concern about their toxicity at high doses (Olney, 1984).

The mixture was prepared within a few minutes of oral administration by mixing the powdered AAs with 150 ml of water, 50 ml of chocolate syrup and one half packet of saccharin. Because of the unpleasant taste of L-methionine, L-cysteine and L-arginine, these AAs were put into capsules and were administered while the subjects were ingesting the liquid mixture. Subjects were asked to swallow the suspension in as short a time as possible because of its somewhat unpalatable taste. A nose plug was also used during ingestion to reduce olfactory and gustatory cues and, after ingestion, before removing the plug, they chewed on a half stick of cinnamon gum to help cleanse the mouth of AA residue.

2.5. Sham/cigarette smoking

Sham smoking required subjects to take 10 puffs (inhalations) on a nonlighted cigarette (own brand), puffing once every 60 s (as cued by a stopwatch), while cigarette smoking required subjects to puff at the same rate on one of their own lighted cigarettes.

2.6. Withdrawal ratings

Withdrawal symptoms were examined with the self-rated Smoking Withdrawal Symptom (SWS) checklist (Hughes and Hatsukami, 1981), which is based on the DSM-IV criteria for nicotine withdrawal (American Psychiatric Association, 1994). This scale consists of rating eight withdrawal symptoms (irritable, frustrated or angry, difficulty concentrating, restless, anxious, hunger, depressed mood, sad or feeling blue, and craving or urge to smoke) on a four-point scale (0 = *not present*, 1 = *mild*, 2 = *moderate* and 3 = *severe*), with a total withdrawal score being calculated by the summing of all ratings. This measure has evidenced elevated scores following acute tobacco abstinence (Hughes and Hatsukami, 1981; Hughes et al., 1984, 1987), in smokers abstaining from smoking in their natural environment and in controlled clinical trials (Hughes, 1992; Hughes and Hatsukami, 1981) and the derived symptoms have been confirmed by independent observer ratings (Hatsukami et al., 1987; Hughes and Hatsukami, 1981).

2.7. Mood ratings

Affective responses were examined with the bipolar Profile of Mood States (POMS) (McNair et al., 1988; Lorr et al., 1982). Subjects rated themselves (using a five-point scale: *not at all*, *a little*, *moderately*, *quite a bit*, *extremely*) on 65 adjectives; the results of which were then converted to six bipolar mood dimensions: tension–anxiety (T–A), depression–dejection (D–D), anger–hostility (A–H), vigor–activity (V–A), fatigue–inertia (F–I) and confusion–bewilderment (C–B). As well, a total mood disturbance (TMD) score was derived by summing the five negative mood dimension scores minus the V–A score. POMS scores are highly sensitive to nonclinical changes in mood states and have shown alterations following TD (Knott et al., 1999b; Smith et al., 1987; Young et al., 1989), following acute nicotine administration (Knott et al., 1999a,b) and during tobacco abstinence (Hatsukami et al., 1984, 1985).

2.8. EEG measurement

Subjects sat in a reclining chair for a 5-min, eyes closed rest period while electrical activity was recorded from six scalp sites utilizing a monopolar (linked ears reference) double-banana montage with electrodes positioned at homologous sites on left (F3, C3, O1) and right (F4, C4, O2) hemisphere regions. Additional electrodes were placed around the orbital ridges and external canthi to monitor vertical and horizontal electrooculographic (EOG) activity. Electrode impedances were kept below 5 K and all electrical activity was recorded with bandpass filter settings at 0.1–40.0 Hz. EEG was digitized at 256 Hz and a minimum of thirty artifact-free 2 s epochs were processed by a high-pass autoregressive filter, weighted by a 5% cosine taper, and were then subjected to a conventional fast Fourier transform algorithm for computation of absolute and relative amplitude in six frequency bands including: δ (0.75–3.75 Hz), θ (3.75–7.75 Hz), α_1 (7.75–10.75 Hz), α_2 (10.75–13.75 Hz) and β (13.75–29.75 Hz). As with previous studies in our laboratory (Knott et al., 1999a), absolute values were log-transformed (log[x]) to achieve normal distributions prior to statistical analysis .

2.9. Plasma tryptophan

A venous blood sample (5 ml) was collected in a heparinised tube at each of the two sample times. The samples were centrifuged, plasma aliquoted into plastic tubes and stored at -20 °C until assayed. Measurement of plasma tryptophan was carried out by high-performance liquid chromatography on a Waters μBondapak C_{18} reverse phase column with fluorometric detection, as described by Anderson et al. (1979).

2.10. Carbon monoxide

Expired alveolar CO levels (parts per million: ppm) were assessed on tidal-breath samples using an Ecolyser model 2000.

2.11. Data analysis

Each dependent measure was subjected to repeated measures analysis of variance (ANOVA) procedures. Individual POMS and SWS item scores, and CO were each analyzed

with 2 (treatment: T and B mixtures) × 4 (time: test battery assessments 1–4) ANOVAs, while plasma tryptophan levels, assessed only at baseline and 5-h postmixture, were assessed with 2 × 2 ANOVAs. Amplitude in each EEG band was analyzed with separate 2 (treatment) × 4 (time) × 2 (hemisphere: right, left) × 3 (region: frontal [F3, F4], central [C3, C4], occipital [O1, O2]) ANOVAs. Greenhouse–Geisser corrections were applied and Bonferroni-adjusted significance levels were utilized in all follow-up *t*-tests. Although the primary intent of follow-up tests focused on measures exhibiting significant interactions involving treatment, significant effects of time (i.e., test battery number) were also followed-up if they were significant on their own, or in the case of EEG measures, if they interacted with treatment, region and/or hemisphere. Follow-up analyses of main effects of time were specifically carried out to determine: (a) whether smoking abstinence resulted in significant withdrawal effects, as assessed by comparison of TB1 values vs. TB2 values; (b) whether abstinence associated alterations were altered by sham smoking and by the smoking of a single cigarette, as assessed by TB3 vs. TB4 comparisons; and (c) if altered by cigarette smoking, whether the alterations were partially or fully reversed by the smoking of a single cigarette, as assessed by TB1 vs. TB4 comparisons. Follow-up of significant time effects not involving a treatment interaction was carried out with averaged treatment values.

3. Results

3.1. Tryptophan depletion

Significant condition ($F = 152.1$, $df = 1, 17$, $P < .00001$) and Condition × Time ($F = 160.6$, $df = 1, 17$, $P < .00001$) effects were observed. As shown in Fig. 1, follow-up analysis within the T- condition (TB1 vs. TB2) showed a marked 71% decrease in total plasma tryptophan levels over 5 h ($t = 15.9$, $df = 17$, $P < .00001$), whereas analysis within the B condition found stable tryptophan levels over time, with total plasma tryptophan increasing by only 4% over the 5-h period.

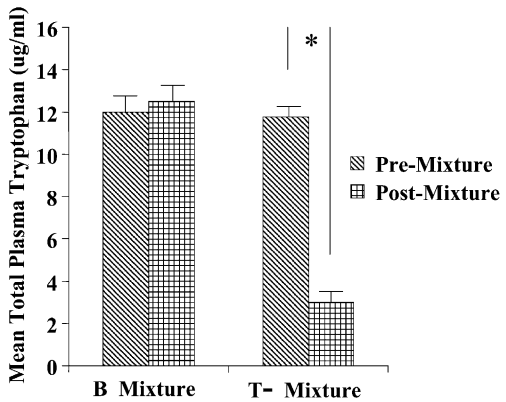

Fig. 1. Mean (±S.E.) total plasma tryptophan levels at baseline and 5 h following ingestion of B and T mixtures.

Table 3
Mean (±S.E.) expired breath carbon monoxide (CO) levels (ppm)

Test battery assessment period	B mixture	T- mixture
TB1	16.3 (6.0)	15.6 (5.1)
TB2	8.2 (3.8)	7.0 (3.7)
TB3	8.2 (3.4)	7.4 (3.4)
TB4	14.9 (4.9)	13.9 (4.3)

TB1 = baseline premixture/presmoking abstinence assessment; TB2 = postmixture/smoking abstinence assessment; TB3 = postsham smoking assessment; TB4 = postcigarette smoking assessment.

3.2. Carbon monoxide

A significant time effect was found in the analysis of CO levels ($F = 136.92$, $df = 3, 51$, $P < .001$) and as shown in Table 3; this was characterized with follow-up tests (Bonferroni corrected at .008 α level), by a decrease from baseline (TB1) to TB2 ($t = 3.6$, $df = 17$, $P < .007$). Postcigarette smoking levels (TB4) were significantly increased beyond those seen with postsham smoking ($t = 3.1$, $df = 17$, $P < .008$) and, although slightly lower, were not significantly different from baseline levels.

3.3. Withdrawal ratings

Table 4 displays the mean (±S.E.) individual and total withdrawal item scores for each treatment condition at each assessment time. No significant treatment effects were observed but significant time effects were observed for seven of the eight items, including 'irritable/frustrated/anger' ($F = 7.6$, $df = 1, 17$, $P < .001$), 'restless' ($F = 6.4$, $df = 1, 17$, $P < .0003$), 'anxious' ($F = 7.9$, $df = 1, 17$, $P < .001$), 'hunger' ($F = 19.3$, $df = 1, 17$, $P < .0001$), 'depressed/sad/feeling blue' ($F = 6.6$, $df = 1, 17$, $P < .001$), 'craving/urge to smoke' ($F = 20.8$, $df = 1, 17$, $P < .0001$) and total withdrawal discomfort ($F = 32.0$, $df = 1, 17$, $P < .00001$). The only item not evidencing time effects was "difficulty concentrating." As shown in Table 4, of the seven items exhibiting time effects, Bonferroni corrected (at .008 α level) follow-up tests found all increased from baseline (TB1) to 5 h after the initiation of smoking abstinence (TB2).

Follow-up tests (with Bonferroni corrected at .008 α level) found only 'craving/urge to smoke' and total withdrawal discomfort ratings were affected by smoking, craving being reduced ($t = 4.3$, $df = 17$, $P < .0005$) postcigarette smoking (TB4) compared to postsham smoking (TB3) and total withdrawal discomfort being reduced ($t = 3.3$, $df = 17$, $P < .004$) in the same manner. Comparison of these two postcigarette smoking values with baseline values found craving to be similar at both times but, the lowered total withdrawal discomfort ratings found postcigarette smoking were still significantly higher than values assessed at baseline ($t = 4.5$, $df = 17$, $P < .0003$).

Table 4
Mean (±S.E.) ratings on the withdrawal symptom checklist

Variable	Test battery (TB)	B mixture	T mixture
Irritable/frustrated/angry	TB1	0.22 (0.10)	0.16 (0.09)
	TB2[a]	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[a]	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[a]	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[a]	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[a]	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[a]	0.55 (0.14)	0.72 (0.19)
	TB3	0.44 (0.12)	0.72 (0.19)
	TB4[b]	0.27 (0.10)	0.55 (0.17)
Craving/urge to smoke	TB1	1.22 (0.15)	0.88 (0.22)
	TB2[a]	1.94 (0.15)	2.17 (0.15)
	TB3	2.06 (0.17)	2.11 (0.19)
	TB4[b]	1.17 (0.18)	1.33 (0.84)
Total withdrawal discomfort	TB1	4.72 (0.53)	4.27 (0.68)
	TB2[a]	8.44 (0.63)	8.89 (0.57)
	TB3	8.56 (0.66)	8.83 (0.63)
	TB4[b,c]	6.67 (0.74)	7.11 (0.81)

TB1 = baseline premixture, presmoking abstinence assessment; TB2 = postmixture, smoking abstinence assessment; TB3 = postsham smoking assessment; TB4 = postcigarette smoking assessment.

[a] Items showing significant (see text) increases at TB2 compared to TB1.

[b] Items showing significant (see text) decreases at TB4 compared to TB2.

[c] Items showing significant (see text) decreases at TB4 compared to TB2 but still exhibit values significantly greater than at TB1.

3.4. Mood ratings

No significant treatment effects were obtained for mood subscales or for TMD scores. Significant time effects were observed for TMD ($F = 12.9$, $df = 1, 17$, $P < .00001$) and, except for 'fatigue–inertia,' for five of the six subscales, including 'tension anxiety' ($F = 6.1$, $df = 1, 17$, $P < .007$), 'depression–dejection' ($F = 6.9$, $df = 1, 17$, $P < .002$), 'anger–hostility' ($F = 4.7$, $df = 1, 17$, $P < .01$), 'vigor–activity' ($F = 5.8$, $df = 1, 17$, $P < .003$) and 'confusion–bewilderment' ($F = 3.4$, $df = 1, 17$, $P < .02$). As shown in Fig. 2, all of these significant effects were in the direction of increased negative mood following 5 h of smoking abstinence (TB1 vs. TB2).

Although there was a trend for both sham and cigarette smoking to alter most mood scale scores, follow-up tests (with Bonferroni corrected at .008 α level) revealed that cigarette smoking effects were found only for 'depression–dejection' and total mood discomfort, each being decreased after cigarette smoking (TB4) compared to after sham smoking (TB3). Comparison of these postcigarette smoking values with baseline (TB1) values found no significant differences, indicating that elevations in 'depression–dejection' and total mood discomfort induced by smoking abstinence were reversed with the smoking of a single cigarette.

3.5. EEG measures

There was a general trend for all frequency bands to exhibit smaller amplitude values in the T- condition compared to the B condition, but a significant treatment effect emerged only with α_2 ($F = 4.7$, $df = 1, 17$, $P < .04$), mean amplitude being 95.9 (S.E. ±13.49) for T- and 105.3 (S.E. ±12.42) for B. Significant time effects were observed for all but one (α_1) of the five bands including δ ($F = 10.3$, $df = 3, 48$, $P < .00001$), θ ($F = 4.4$, $df = 3, 48$, $P < .02$), α_2 ($F = 42.9$, $df = 3, 48$, $P < .00001$) and β ($F = 3.8$, $df = 3, 48$, $P < .03$).

Although abstinence appeared to alter activity in all bands (Fig. 3) follow-up analyses (using *t*-tests with Bonferroni-corrected significance values at .008 α level)

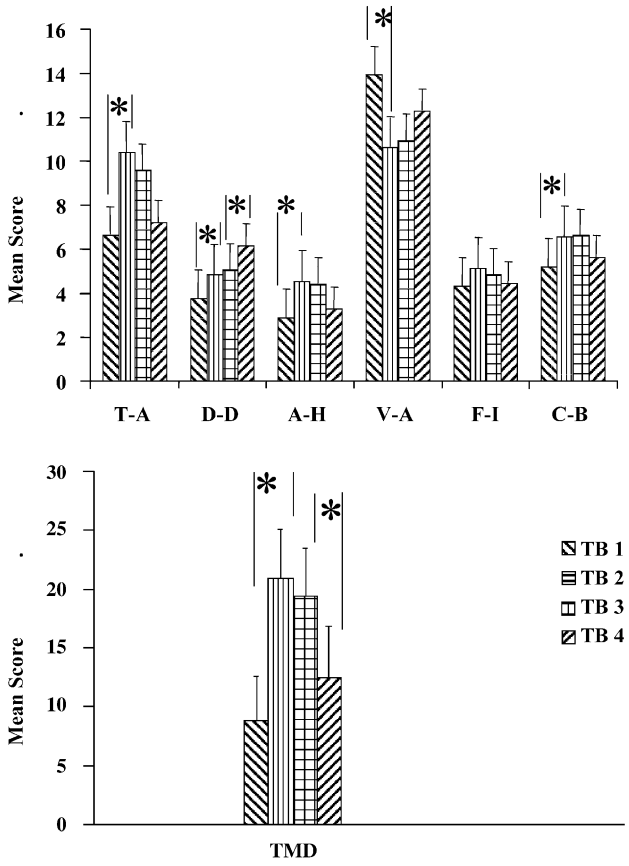

Fig. 2. Mean (±S.E.) POMS scores assessed at TB1, TB2, TB3 and TB4.

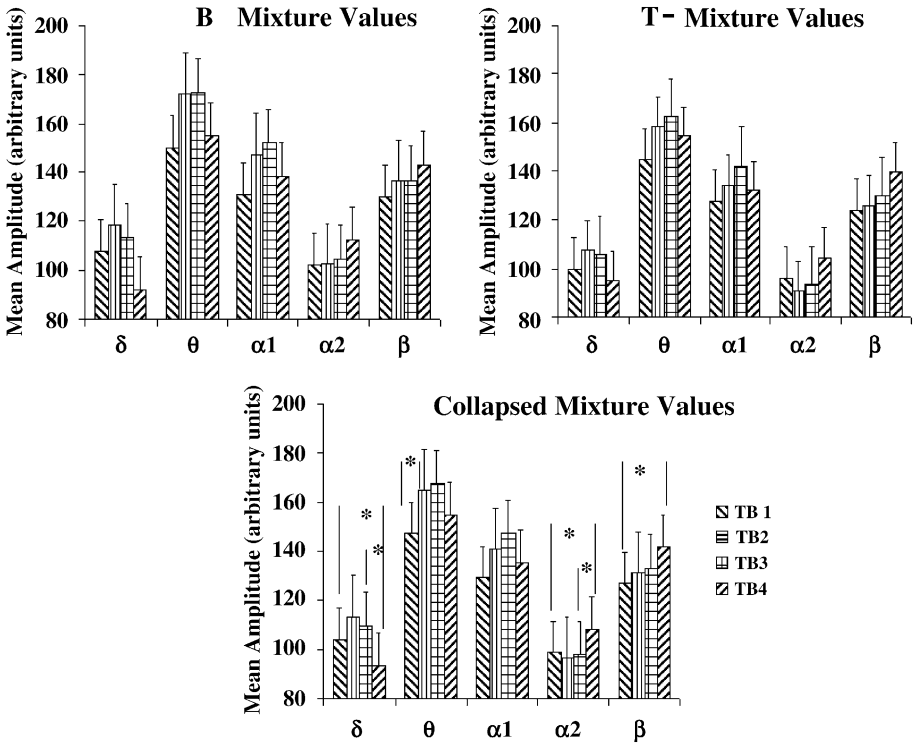

Fig. 3. Mean (±S.E.) EEG δ, θ, α_1, α_2 and β amplitude values for B and T mixture sessions and for the average (collapsed) of the two sessions.

revealed that significant EEG alterations with 5 h of smoking abstinence were evident only with θ, this band exhibiting increases from baseline (TB1) to TB2 ($t=3.4$, $df=17$, $P<.005$). Comparison of sham (TB3) and cigarette smoking (TB4) effects showed that the smoking of a single cigarette resulted in significant decreases in δ ($t=3.30$, $df=17$, $P<.004$) and increases in α_2 ($t=3.2$, $df=17$, $P<.002$). Postcigarette smoking EEG (TB4) was also shown to be significantly different from baseline EEG (TB1), δ amplitude being smaller ($t=3.71$, $df=17$, $P<.001$) and α_2 ($t=3.30$, $df=17$, $P<.004$) and β ($t=3.54$, $df=17$, $P<.002$) amplitudes being larger at post-smoking assessment than at baseline assessment.

4. Discussion

The general aim of this study was to elucidate the role of serotonergic neurotransmission in the generation of electrocortical and affective responses to acute smoking abstinence and acute cigarette smoking. Interpretation of study findings is dependent on the experimental paradigm from which the observations were derived and it is clear that the present study design was limited with respect to several procedures including: (1) use of a relatively young adult smoker sample consisting only of males and the failure to include smoker subtypes, e.g., heavy vs. light smokers, etc.; (2) nonrandomized comparison of sham vs. cigarette smoking effects in each session; (3) use of a subject's preferred cigarette with no attempt to standardize cigarette tar/nicotine yield across subjects and no blood sampling to assess nicotine bioavailability; (4) use of a single TD dose to reduce plasma tryptophan and a failure to examine time–response relationships. Despite these study limitations, the derived observations yield some preliminary insight into the pharmacologic basis of smoking behavior, which may be useful in formulating testable hypothesis regarding the neurochemistry underlying neuroelectric and affective alterations accompanying smoking and smoking withdrawal.

Five hours of smoking abstinence produced clear withdrawal symptoms and mood disturbances similar to those experienced previously by smokers undergoing morning- (Knott and Harr, 1998) and day-long (Knott, 1995) smoking deprivation in laboratory settings and by smokers who are monitored over sustained (31 days) smoking cessation periods (Gilbert et al., 1999). This cluster of symptoms, which have been characterized as being similar to subclinical depression and anxiety (Covey et al., 1990; Gilbert et al., 1998), was paralleled by the appearance of a cortical hypo-arousal state as evidenced by increases in slow frequency (θ) amplitude activity, a spectral EEG pattern shift (i.e., from fast frequencies to slow frequencies) which has been frequently observed with mild sedatives (Saletu, 1989), and has characterized both brief (2–24 h) and prolonged (3–31 days) smoking abstinent periods (Pickworth et al., 1989; Gilbert et al., 1999).

Although withdrawal discomfort postcigarette smoking was still significantly greater than that observed at baseline, the smoking of a single cigarette attenuated discomfort and cigarette cravings and reversed to baseline the elevated

TMD and depressive affect scores associated with cigarette abstinence. Coincident with these effects were changes in EEG, with smoking acting not only to reverse the spectral shift accompanying abstinence, but to push cortical arousal beyond that seen at baseline, as evidenced by reduced slow (δ) and increased fast (α_2, β) wave amplitude alterations. This acute EEG activation response, although consistently seen with smoking (Knott, 2001), nicotine administration (Knott et al., 1999a,b) and with plasma nicotine levels exceeding 10 ng/ml (Kadoya et al., 1994), was not expressed asymmetrically across the two hemispheres in these healthy smokers, thus, adding support to the suggestion that smoking and smoking abstinence may impact on lateralized neural networks mediating affect processing/regulation only in individuals who are experiencing substantial negative affect and/or who have trait vulnerabilities for negative affective states (Gilbert, 1995, 1996; Gilbert et al., 1997).

Although the smokers in this study clearly demonstrated both negative and positive reinforcing consequences of smoking abstinence and smoking, respectively, these effects appeared not to be affected by a transient reduction in 5-HT function. Exposing smokers to a marked (71%) TD did not exacerbate abstinence-related withdrawal, mood or electrophysiologic symptoms, nor did it blunt the reversal of these symptoms with acute smoking. Moreover, the TD strategy itself failed to alter mood or induce changes in EEG recordings. Early studies in healthy volunteers convincingly demonstrated mood lowering (although never amounting to clinical depression) effects following TD but subsequent investigations, however, have tended to not support these TD effects on depressive mood or have observed these effects only in individuals who are more serotonergically vulnerable to depression as evidenced by higher (i.e., upper end of normal range) baseline depression scores or the presence of a positive family history of depression (see Bell et al., 2001; Van der Does, 2001 for reviews). In this current study, smokers were excluded if they reported a psychiatric history or family history (in first-degree relatives) of mood disorder, and all the included smokers exhibited normal range POMS scores. Further studies may consider investigating TD in normal populations of smokers who are at high risk for affective disorders (i.e., exhibit a positive family history), score in the upper end of normal range of psychometrically assessed depression scales and/or who smoke primarily to manage negative affect.

Despite the absence of a mood effect with TD in this study, a recent investigation with normal subjects in our laboratory did observe lowered mood and EEG hypoarousal shifts with TD but all of the subjects were nonsmokers (Knott et al., 1999a,b). Although differences between smokers and nonsmokers in response to acute TD may be related to constitutional make-up (Pomerleau, 1995) or differences in AA absorption and/or metabolism, chronic long-term nicotine exposure in smokers is known to induce neuroadaptive changes, resulting not only in the upregulation of brain nAchRs (Benwell et al., 1988) but also an increase in the number of 5-HT_{1A} receptors (Benwell et al., 1990) as well as a selective decrease of hippocampal 5-HT (Benwell and Balfour, 1979). nAchR desensitization combined with upregulated postsynaptic 5-HT_{1A} receptors may be hypothesized to contribute to a blunting of response to exogenous 5-HT manipulations (Watkins et al., 2000) or to centrally acting drugs in general. Nonsmokers and smokers have been reported to be differentially responsive to sedating agents (Bashir et al., 1981), including commonly used psychotropic substances such as alcohol (Knott and Venables, 1979). Additional investigations examining the unique role of serotonin in smoking behavior should attempt to compare smokers and nonsmokers with respect to responsivity to a range of 5-HT agonist and antagonist agents, taking care to assess response profiles in smokers during abstinence and nonabstinence states.

The use of male smokers in this study is somewhat problematic in explaining the absence of TD effects as gender comparisons have indicated that females may be more sensitive to the dysphoric-inducing properties of TD than males (Bell et al., 2001). This is of particular interest in that female smokers exhibit different EEG and subjective response profiles to smoking abstinence and smoking than male smokers (Gilbert et al., 1994) and females, in general, as evidenced by positron emission tomography (PET) imaging, exhibit greater biochemical effects (i.e., 5-HT synthesis) with TD than males (Nishizawa et al., 1997). A review of the Nishizawa et al.'s (1997) study and a dog PET study (Diksic et al., 1991) suggested that these sex differences in 5-HT synthesis rates could be mostly attributed to differences in plasma free tryptophan, with concentrations for women being half that of men before TD and five-fold lower following TD (Shoaf et al., 1999). Additional studies comparing acute TD in male and female smokers and nonsmokers, with and without a family history of affective disorder, are required to fully understand the influence of transient disturbances in 5-HT on responses to smoking abstinence and acute smoking.

Although in vivo measurement with PET has shown that a 100-g TD dose reduces the rate of synthesis by up to 90% of baseline values (Nishizawa et al., 1997), the failure of the 50-g TD dose to instigate any mood or electrophysiologic changes may imply that the plasma tryptophan reductions were not necessarily associated with a consequent reduction in brain 5-HT synthesis and release. Also, there is some apparent discrepancy with respect to the time course of central and peripheral responses to TD as alterations in 5-HT appear somewhat delayed with respect to plasma changes (peak reductions at 5–7 h), with the nadir for CSF tryptophan and 5-HIAA being 7–10 and 12–14 h, respectively, after TD (Carpenter et al., 1998; Williams et al., 1999). It should be noted however that 5-H1AA in CSF has a relatively slow turnover so that there is a delay in the time for changes in brain 5-HT synthesis to be reflected in CSF 5-H1AA (Shoaf et al., 1998, 1999). The Nishizawa et

al.'s (1997) study was carried out in the 5–7 h time period and showed marked reductions in serotonin synthesis at this time. Of course, it may well be that neither smoking abstinence nor acute smoking effects are dependent on the short term availability of the 5-HT precursor tryptophan, but additional TD investigations will require longer monitoring periods and perhaps the induction of a more protracted (>5 h) depletion of tryptophan. It is also possible that TD is only of importance for smoking withdrawal after prolonged smoking abstinence, and is of significance for acute smoking impact only when examined in smokers who are specifically smoking for control and modulation of affect. TD investigational strategies which incorporate individual differences (e.g., personality and race), serotonergic vulnerability (e.g., male and female smokers with and without positive family history of affective disorder), affective state manipulations (via cognitive challenges, transient physical and/or psychological stressors) and time–response measurements (which include not only plasma tryptophan but also plasma nicotine and, ideally, CSF 5-H1AA as an integrative measure of 5-HT neurometabolism) will provide a more accurate view of the effects of TD on smoking withdrawal and on the acute response to cigarette smoking.

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