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Clonidine pre-treatment fails to block acute smoking-induced EEG arousal/mood in cigarette smokers

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Abstract

Given the arousal eliciting actions of smoking and nicotine, and the contributing role of noradrenaline in brain arousal systems, this study examined the neuroelectric and affective correlates of cigarette smoking following acute pre-treatment with the alpha 2-noradrenergic autoreceptor agonist, clonidine. In a double-blind placebo-controlled crossover design, quantitative electroencephalography (EEG), mood, and smoking withdrawal symptoms were assessed in 12 overnight smoking abstinence smokers, before and after sham and cigarette smoking. Orally administered clonidine (0.1 mg) failed to alter overnight smoking abstinence symptoms or the EEG arousal and mood-elevating response seen with the smoking of a single cigarette. The results are discussed in relation to neural mechanisms underlying the acute reinforcement maintaining nicotine use.

Keywords: Clonidine; Electroencephalography; Cigarette smoking

1. Introduction

It is generally accepted that the neural mechanisms contributing to the maintenance of tobacco smoking involve the addictive properties of nicotine, which include its ability to serve as a positive reinforcer of drug-seeking behaviour, a mood elevator, an arousal modulator, a locomotor stimulant, and a cognitive enhancer (Watkins et al., 2000). Nicotine also evokes a marked abstinence syndrome when it is abruptly withdrawn following a period of chronic treatment (Kenny and Markou, 2001).

The arousing actions of nicotine have been most consistently observed with electrophysiological recordings carried out both in animal and human subjects (Clarke et al., 1990). Quantitative electroencephalography (EEG), via spectral analysis, is an established methodology for pursuing pharmacodynamic profiles of psychoactive agents (Knott, 2000) and has been shown to be reliably sensitive to the central actions of nicotine, smoking, and smoking withdrawal (Houlihan et al., 1996; Knott, 2001). Frequently accompanied by self-reports of increased arousal and vigor, spectral EEGs have typically portrayed a desynchronized, electrically activated brain pattern following the acute smoking of one or two cigarettes, a pattern which is characterized by an acceleration of the dominant frequency, and a shift in spectrum activity involving voltage suppression in slow wave (delta, theta) and voltage augmentation in fast wave (alpha, beta) frequency bands. Nicotine polacrilex (Pickworth et al., 1980) and transdermal nicotine (Knott et al., 1999) have produced similar neuroelectric patterns, and non-smokers have also exhibited EEG arousal with nicotine administration (Foulds et al., 1994). In contrast to the acute

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effects of smoking and nicotine, smoking abstinence is associated with EEG deactivation and mood lowering, with spectrums evidencing increased slow frequency activity, and with affective states being characterized by reduced alertness and increased dysphoria (Gilbert et al., 1999; Knott et al., 1998a,b; Knott and Venables, 1977).

Arousal increments are purported to play a significant role in the mood-modulating and cognitive-enhancing actions of smoking (Gilbert and Gilbert, 1998), but the neurochemical mechanisms mediating these nicotine-induced brain/behaviour alterations are unclear. To date, only a limited number of studies have attempted to examine the pharmacological basis of the smoking-associated arousal/mood profile and conflicting findings have been observed with respect to the role of acetylcholinergic (ACh) influences. Whereas initial reports did show that pre-treatment with a nicotinic receptor (nAChR) antagonist (mecamylamine) blocked alpha frequency and alpha power increments induced with nicotine gum (Pickworth et al., 1988), but failed to block either slow wave (theta) voltage suppression or fast wave (alpha) voltage augmentation associated with cigarette smoking (Knott et al., 1995), subsequent findings showed nAChR, but not muscarinic receptor (mAChR) blockade (via scopolamine), abolished both the slow wave (delta, theta) voltage reductions and the subjective increases in alertness seen with the smoking of a cigarette (Knott et al., 1998a,b). Acute pre-treatment with the dopamine (DA) antagonist haloperidol did not impact on smoking-associated reductions in slow wave (delta, theta, alpha$_1$) frequencies, increases in fast alpha (alpha$_2$), or subjective reported alertness, but it did inhibit voltage increments in the fast (beta) frequency band (Walker et al., 2001). Manipulation of the serotonergic (5-HT) system via an acute tryptophan depletion manouevre, a procedure which rapidly diminishes brain 5-HT synthesis, failed to alter either the EEG/mood activation seen with cigarette smoking, or the withdrawal symptoms associated with brief (5 h) smoking abstinence (Perugini et al., 2003).

Infrahuman investigations involving brain lesions, pharmacological manipulations and electrophysiological monitoring have shown the coeruleo-cortical noradrenergic system, which includes the noradrenaline (NA)-enriched locus coeruleus (LC) and its massively divergent efferent projections, to be intimately involved in arousal modulation (Robbins, 1997). This neuronal system plays a central role in initiating and maintaining one or more stages of the sleep–wake arousal cycle, and LC neurons have exhibited enhanced discharge rates just before and during periods of arousal as indicated by behavioural and forebrain EEG indices (Ashton-Jones et al., 1991; Berridge and Foote, 1991; Coull, 1998; Foote et al., 1991). As animal studies have found that nicotine increases, in a dose-dependent manner, the LC firing rate and the release of NA from LC neurons (Mitchell et al., 1993; Svenson and Engerg, 1980), it is reasonable to speculate that pharmacological manipulations of central NA activity may alter the smoking-induced EEG/mood activation response profile. Clonidine is an anti-hypertensive medication with noradrenergic agonist actions, mimicking the effects of NA at α_2 auto-receptors in the LC and resulting in a reduction of LC neuronal firing rate and inhibition of NA release in cortical terminal fields (Robbins, 1997). Given the pivotal role of NA in electrocortical arousal, the primary objective of the present study was to examine the effects of clonidine pre-treatment on EEG arousal and mood alterations induced with acute cigarette smoking following overnight smoking abstinence. A secondary objective of the study was to determine whether clonidine pre-treatment alters smoking withdrawal symptoms following overnight smoking abstinence and/or their attenuation with acute smoking.

2. Method

2.1. Experimental subjects

Twelve right-handed cigarette smokers (10 males) voluntarily participated in this study following written consent to the investigation which was approved by the hospital research ethics board. This sample size was utilized as it closely approximates that typically used in pharmaco-EEG Phase I investigations with healthy volunteers (Saletu et al., 1987), and it is also similar to that used in our own studies which have evidenced significant smoking- and drug-related EEG alterations with these sample numbers. For study inclusion, subjects had to be smoking a minimum of 10 cigarettes per day for the past 5 years and were required to be free of medications (except birth control) and any medical, neurological (including seizures and recent [<6 months] head trauma), psychiatric or alcohol/drug abuse disorders. All participants were required to exhibit normal diastolic (DBP) and systolic blood pressure (SBP) readings. Subject characteristics, including scores on the Fagerstrom Tolerance Questionnaire (Fagerstrom and Schneider, 1989), an indicator of nicotine dependence, are shown in Table 1.

2.2. Study design

Subjects attended the laboratory for two test sessions in which they received, under randomized double-blind

Table 1
Subject characteristics

	Mean	±S.D.
Age (years)	22.9	3.6
Years smoking	8.2	2.3
Cigarettes/day	15.3	3.9
FTQ[a]	4.0	2.0
CO (ppm)	17.2	2.2
Nicotine (mg)	0.6	0.4
Tar (mg)	16.8	3.8

CO, nicotine, and tar values reflect yields of the preferred cigarettes used by the volunteers on a daily basis.

[a] FTQ=Fagerstrom Tolerance Questionnaire.

conditions, placebo or clonidine treatment. Test sessions were separated by a minimum 2-day interval and the treatments were administered in a counterbalanced order such that half of the subjects (randomly selected) were assigned to receive placebo on the first test session and clonidine on the second session, while the remaining subjects received placebo and clonidine treatments in the reverse order. EEG and self-report assessments were collected prior to placebo/clonidine administration and three more times, in the following sequence: after drug absorption, after sham smoking, and again after cigarette smoking. Although the non-randomization of sham and cigarette smoking confounds a time effect with a smoking effect within each test session day, the consecutive assessments of sham and cigarette smoking effects were chosen in order to relate to findings with our previous cholinergic, dopaminergic, and serotonergic pre-treatment studies which also utilized a non-randomized sham vs. cigarette smoking assessment sequence (Knott et al., 1998a,b; Perugini et al., 2003; Walker et al., 2001).

2.3. Study procedure

Test sessions were initiated in the morning, beginning at 8:00 a.m. Subjects were required to abstain from caffeine (including chocolate), alcohol, drugs, and food, confirmed by self-report, and smoking for 12 h (overnight) prior to morning sessions. Smoking, and alcohol/drug abstinence was verified through self-report, and expired carbon monoxide (CO) levels (parts per million [ppm]) assessed on tidal-breath samples (using an Ecolyzer 2000) was also used to verify compliance with smoking abstinence instructions. CO levels below 13 ppm were considered as being compliant with abstinence instructions. Each session followed an identical timetable. Upon arrival at the laboratory, an EEG hook-up was initiated, and baseline pre-drug EEG recordings and self-report ratings were collected. The pre-drug testing was followed by the administration of placebo or clonidine and a subsequent 2-h drug absorption period during which subjects viewed a selection of emotionally neutral content videos. Measures collected during baseline were acquired again immediately after drug absorption (120 min) and then after sham smoking (160 min) and cigarette smoking (200 min).

2.4. Drug administration

A tablet of clonidine (Catapres®, Boehringer Ingelheim), at the lowest recommended dose of 0.1 mg, was crushed and packaged in capsule form. Placebo consisted of cornstarch in an identical capsule. Clonidine plasma levels are purported to peak at approximately 3–5 h but the maximal physiologic (blood pressure) response is typically evidenced within 2–4 h post-dosing. The plasma elimination half-life of clonidine ranges from 12 to 16 h (CPS, 1997).

2.5. Cigarette/sham smoking

Cigarette smoking involved eight puff inhalations (one puff per 60 s) of their own preferred brand cigarette, while sham smoking required the same rate of inhalation of a non-lighted cigarette from their preferred brand. This paced puffing has been used previously in our laboratory to examine smoking-induced EEG alterations (Knott, 1988, 1989a,b).

2.6. EEG acquisition/quantification

EEGs were acquired in an electrically shielded, sound-attenuated, dimly lit chamber. Subjects sat in a reclining chair for a 2-min, eyes-closed rest period while electrical activity was recorded from eight scalp sites utilizing a monopolar (linked-ears reference) doubled-banana montage with tin electrodes positioned at homologous frontal, central, parietal and occipital sites on left (F_3, C_3, P_3, O_1) and right (F_4, C_4, P_4, O_2) hemisphere scalp regions. Electrodes were placed on each hemisphere as previous studies have reported frontal hemisphere alpha voltage asymmetry (right>left) accompanying smoking abstinence, and normalization of frontal asymmetries with nicotine administration (Gilbert et al., 2000, 1994; Gilbert and Gilbert, 1998). An additional electrode was placed on the mid-forehead site to serve as ground, and electro-oculographic (EOG) activity associated with horizontal and vertical eye-movements/blinks were recorded in a bipolar fashion from electrodes placed on the left supra-orbital ridge and external canthus. Electrode impedances were kept below 5 kΩ and all electrical activity was recorded with amplifier bandpass filter settings at 0.1–30.0 Hz. EEG was digitized at 256 Hz, and a minimum of thirty 2-s duration, artifact-free 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 amplitude in six frequency bands including: delta (1.5–3.5 Hz), theta (3.5–7.5 Hz), $alpha_1$ (7.5–10.0 Hz), $alpha_2$ (10.0–12.5 Hz), and beta (12.5–25.0 Hz). Since amplitude value distributions were not markedly different from normal, they were not log-transformed as is typically recommended. Spectrum shift was also indexed by an amplitude ratio index defined as summed amplitude of the two slowest frequencies over the summed amplitude of the two highest frequencies (delta+theta/$alpha_2$+beta), and by peak alpha frequency (PAF), defined as the spectral bin (Hz) within the 7.5–12.5 Hz band range exhibiting the maximum voltage. Ratios were derived for each recording site but PAF was only derived from bilateral occipital (O_1, O_2) and parietal (P_3, P_4) sites, where alpha activity is most prominent.

2.7. Self-report ratings

Mood was assessed using the Bond and Lader self-report scale (Bond and Lader, 1974) which consists of 16 bipolar visual analogue scales for various mood dimensions (e.g.

alert–drowsy, attentive–dreaming, interested–bored) that, when analyzed and grouped, result in three mood factors: alertness (average score from 9 scales), calmness (average score from 2 scales), and contentedness (average score of 5 scales). Previous research has shown this instrument to be sensitive to acute and chronic administration of a wide variety of psychotropics and is also impacted by cigarette smoking (Knott et al., 1998a,b; Walker et al., 2001).

A euphoria questionnaire (the Morphine–Benzedrine Group [MBG] from the Addiction Research Centre Inventory) was also administered (Haertzen and Hickey, 1987). This questionnaire consisted of 17 true–false statements (e.g. I feel high) and the number of true items were totaled to yield a single score.

Smoking withdrawal symptoms were examined with the self-rated Smoking Withdrawal Symptom (SWS) checklist (American Psychiatric Association, 1994; Hughes and Hatsukami, 1981). The scale involves the rating of 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, 3=severe), with a total withdrawal score being calculated by the summing of all ratings. The SWS has shown elevated scores following acute tobacco abstinence (Perugini et al., 2003) in smokers abstaining from smoking in their natural environment, and as well as controlled clinical trials (Hatsukami et al., 1987; Hughes and Hatsukami, 1981; Hughes et al., 1984, 1987).

2.8. Carbon monoxide

As an indicator of smoke exposure, expired alveolar carbon monoxide (CO) levels (parts per million [ppm]) were

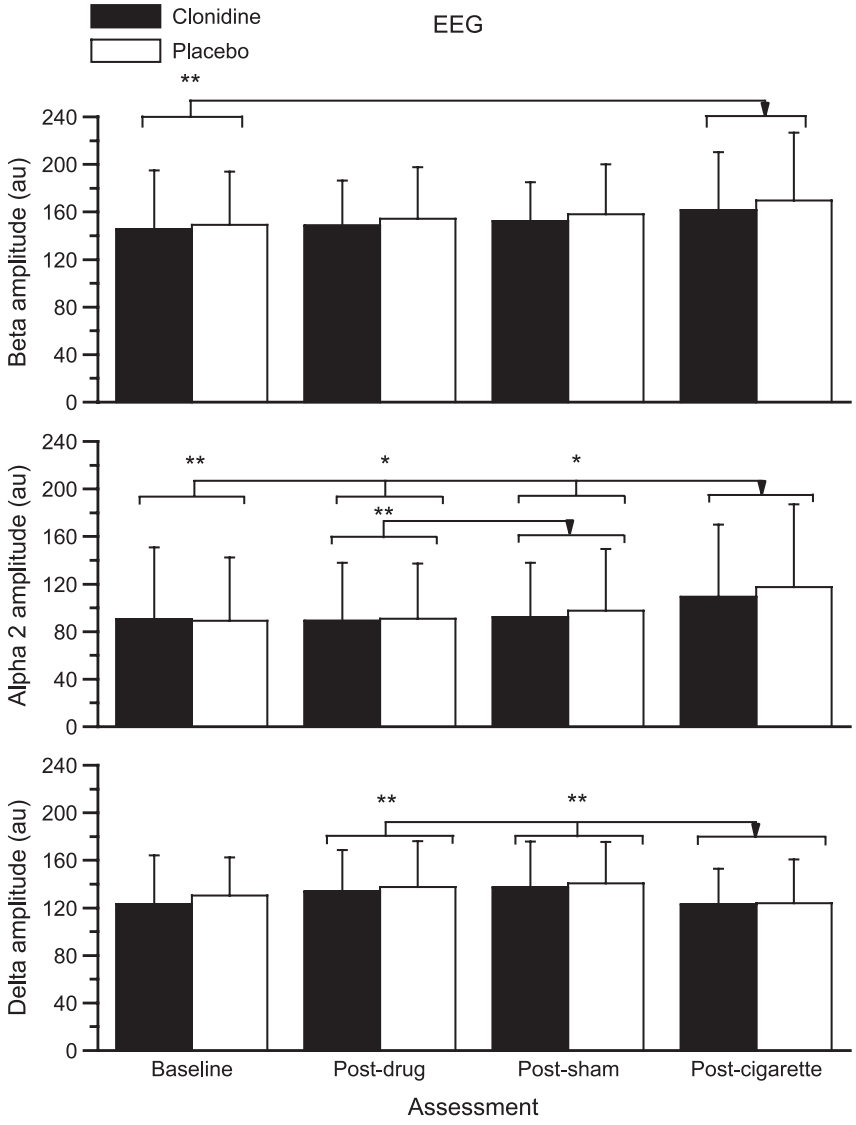

Fig. 1. Mean (±S.D.) EEG absolute amplitude in arbitrary units (au), averaged across all sites in the delta, alpha 2, and beta bands for each of the four assessments in the clonidine and placebo sessions. * and ** indicate significance at the $p<0.05$ and $p<0.01$ levels, respectively.

assessed on tidal-breath samples (using an Ecolyzer 2000) collected at baseline and post-sham/cigarette smoking.

2.9. Vital signs

Seated systolic (SBP) and diastolic (DBP) blood pressure (millimeters of mercury [mm Hg]) as well as heart rate (beats per minute [bpm]) were assessed at baseline, post-drug absorption, and post-cigarette smoking.

2.10. Data analysis

EEG values in each frequency band, as well as ratio values, were subjected to separate 2 (drug: placebo, clonidine)×4 (assessment time: baseline, post-drug, post-sham smoking, post-cigarette smoking)×2 (hemisphere: left, right)×4 (region: frontal, central, parietal, occipital) repeated-measures analysis of variance (ANOVA) procedures. PAF was subjected to the same ANOVA but with two levels of region (occipital, parietal). Self-report ratings were analyzed with 2 (drug)×4 (time) ANOVAs. CO levels and vital signs were subjected to separate 2 (drug)×3 (time) ANOVAs. Huynh and Feldt's (1976) corrections were applied where appropriate to compensate for violations of sphericity assumptions (i.e. equal covariance among all pairs of levels of repeated measures). For convenience, the Huynh–Feldt probability levels, but not the associated degrees of freedom, are presented in the results. Significant ($p<0.05$) main and interaction effects were followed up using pairwise comparisons with Bonferroni-adjusted p-values being reported to reduce the chance of Type I statistical errors associated with multiple tests. The primary

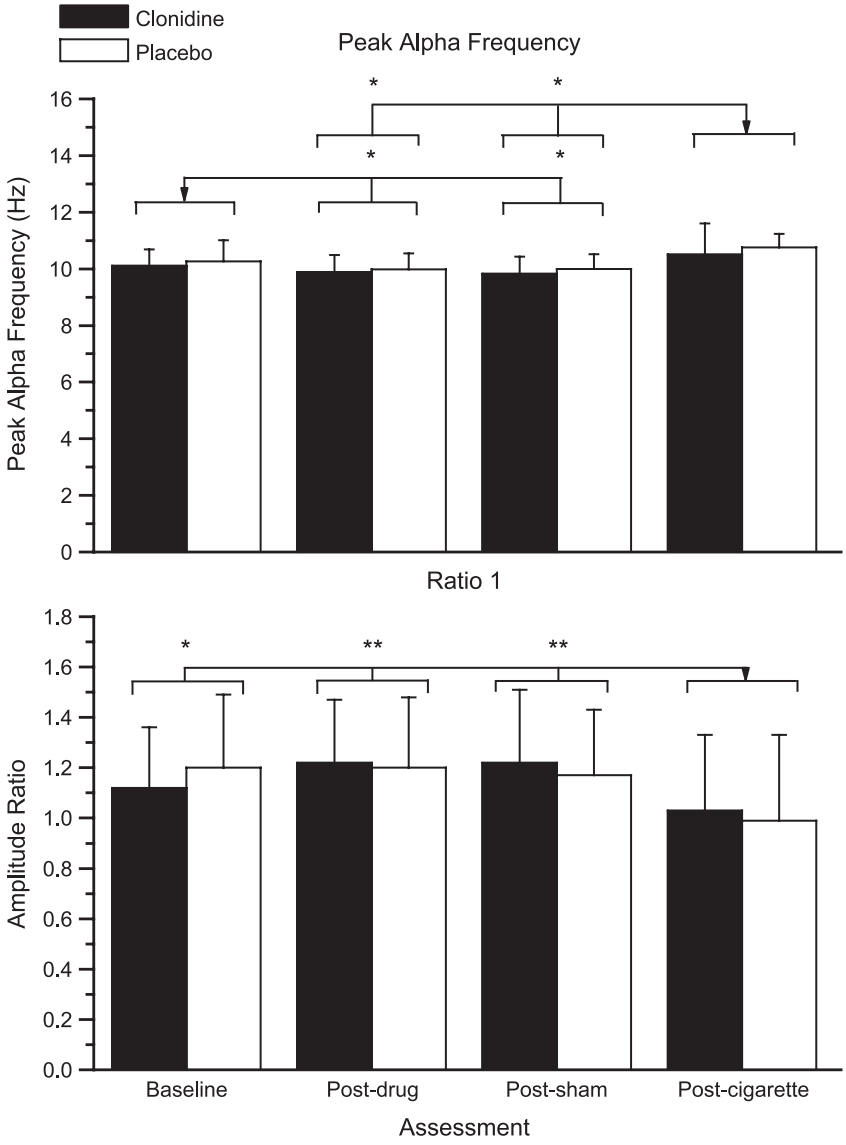

Fig. 2. Mean (±S.D.) peak alpha frequency (top panel) and amplitude ratios (lower panels) across the four assessments in the clonidine and placebo sessions. * and ** indicate significance at the $p<0.05$ and $p<0.01$ levels, respectively.

comparisons were post-cigarette vs. post-sham, post-drug, and baseline, while secondary comparisons involved: baseline vs. post-drug, to determine if measures (e.g. withdrawal symptoms) changed with an additional 2-h smoking abstinence; and post-sham vs. baseline and post-drug, to determine if the behavioural act of smoking alone influenced study measurements.

3. Results

Due to technical problems related to EEG acquisition, data from two of the participants was not used in the analysis of EEG measures, thus leaving 10 subjects. One subject also failed to complete all the items on the three self-reports, leaving 11 subjects for the analysis of these self-ratings.

3.1. EEG

No significant drug or interaction effects were observed for any of the EEG measurements. Significant time effects were found for delta (F=5.4, df=3/27, p<0.01), alpha$_2$ (F=6.6, df=3/27, p<0.01), beta (F=3.2, df=3/27, p<0.06), ratio (F=8.4, df=3/27, p<0.001), and PAF (F=7.3, df=3/27, p<0.009). As shown in Fig. 1, delta activity after cigarette smoking was significantly reduced compared to activity seen post-drug (p<0.009) and post-sham smoking (p<0.002). Alpha$_2$ significantly decreased from baseline to post drug (p<0.02) and although sham smoking tended to increase alpha$_2$ relative to that assessed post-drug (p<0.06), alpha$_2$ was greatest with cigarette smoking, with post-cigarette smoking activity in this band being significantly larger than that seen post-sham (p<0.05), post-drug (p<0.02), and pre-drug (p<0.002). Follow-up analysis of

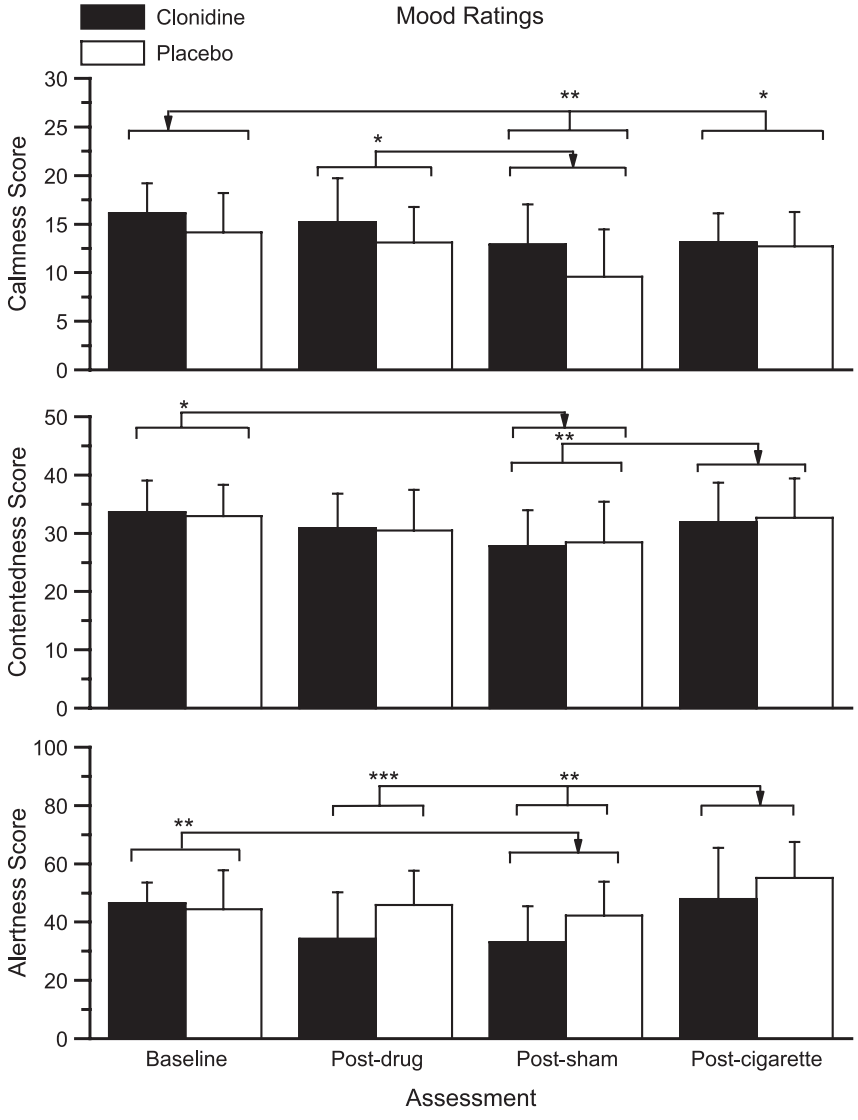

Fig. 3. Mean (±S.E.M.) mood scores from the Bond and Lader across the four assessments in the clonidine and placebo sessions. *, **, and *** indicate significance at the p<0.05, p<0.01, and p<0.001 levels, respectively.

significant time observations for beta found activity in this band to be greater post-cigarette smoking than post-baseline ($p<0.006$). The ratio index (Fig. 2) was altered only by cigarette smoking, with values being lower post-cigarette smoking compared to post-sham smoking ($p<0.006$), post-drug ($p<0.004$), and baseline ($p<0.01$) values.

PAF was slower at post-drug ($p<0.04$) and post-sham smoking ($p<0.04$) recordings than at baseline recordings and post-cigarette smoking PAF was faster than post-sham ($p<0.04$) and post-drug ($p<0.04$) PAF.

Significant site effects were found for alpha$_1$ ($F=9.5$, $df=3/27$, $p<0.008$) and alpha$_2$ ($F=17.0$, $df=3/27$, $p<0.001$) as well as for the ratio index ($F=55.3$, $df=3/27$, $p<0.0001$), with the three measures evidencing expected site differences, being larger at frontal/central sites than parietal/occipital sites. Alpha$_1$ exhibited significant hemispheric differences at frontal recordings, with alpha$_1$ at F_3 being smaller than alpha$_1$ at F_4 ($p<0.03$). Similar asymmetry was seen with alpha$_2$, it being higher at F_4 than at F_3 ($p<0.03$).

3.2. Self-reports

Although in general, clonidine-related calmness ratings (i.e. pre- and post-clonidine) were lower than placebo-related calmness ratings ($F=5.2$, $df=1/10$, $p<0.05$), drug administration did not affect ratings of alertness, contentedness, or euphoria, nor did it interact with smoking in influencing these mood ratings. Significant time effects were observed for alertness ($F=7.8$, $df=3/30$, $p<0.002$), contentedness ($F=4.5$, $df=3/30$, $p<0.02$), and calmness ($F=6.6$, $df=3/30$, $p<0.001$). As shown in Fig. 3, all ratings significantly ($p<0.01$) declined from baseline to post-sham evaluations, and they were elevated with cigarette smoking relative to sham smoking ($p<0.002$). In the case of

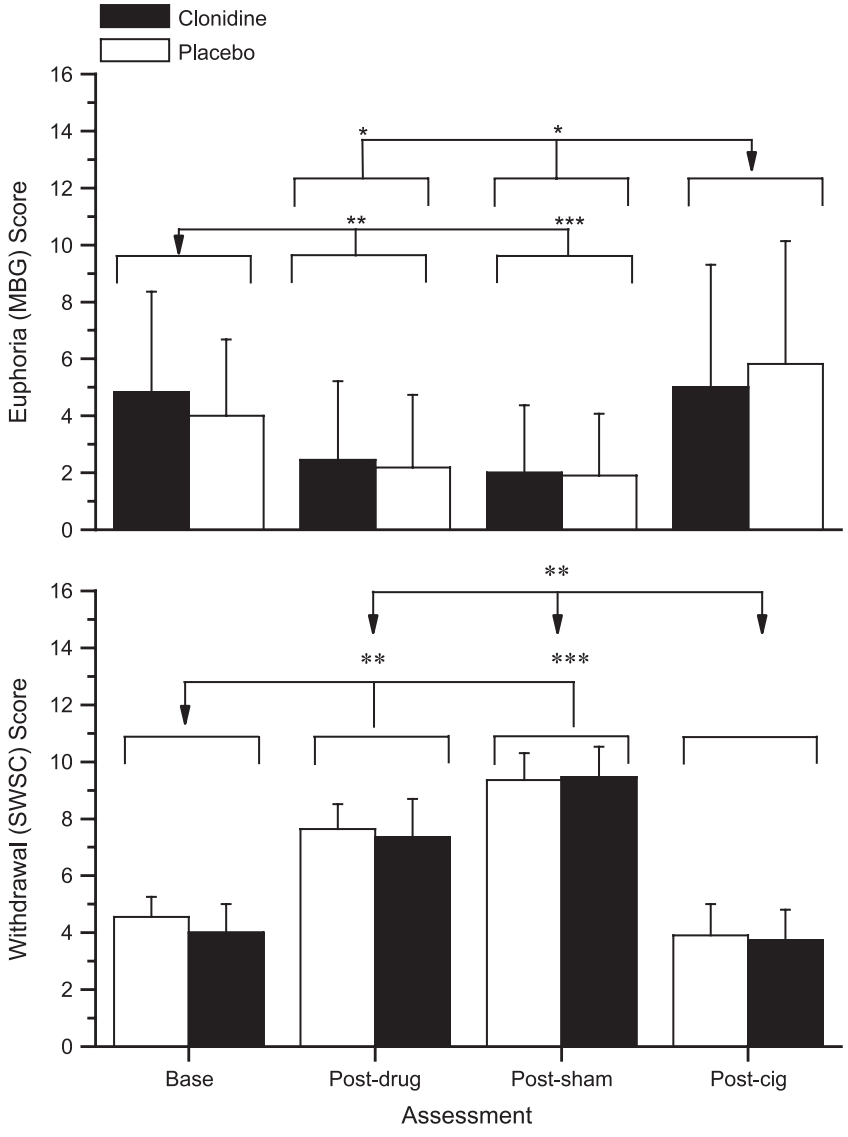

Fig. 4. Mean (±S.E.M.) euphoria ratings (MBG scores) (top panel) and withdrawal (SWSC) scores (lower panel) across the four assessments in the clonidine and placebo session. *, **, and *** indicate significance at the $p<0.05$, $p<0.01$, and $p<0.001$ levels, respectively.

contentedness and alertness, these elevations reached a level similar to that seen at baseline.

Euphoria ratings, shown in Fig. 4, declined from baseline to post-drug ($p<0.002$) and post-sham smoking ($p<0.001$). Ratings were increased with cigarette smoking, primarily in the placebo session, beyond ratings seen at post-drug ($p<0.03$) and post-sham evaluations, and were equivalent with ratings seen at baseline.

Analyses of withdrawal symptoms assessed with SWS yielded a significant time ($F=25.3$, $df=3/10$, $p<0.001$) effect. As shown in Fig. 4, SWS scores progressively increased from baseline to post-drug (placebo) to post-sham ($p<0.008$) assessments. Relative to the post-sham ($p<0.008$) and post-drug assessment ($p<0.009$), SWS scores were significantly lower post-cigarette and were equivalent to baseline SWS scores.

3.3. Carbon monoxide

Follow-up of a significant time effect ($F=31.6$, $df=2/30$, $p<0.001$) found post-sham levels ($M=6.5$ ppm, S.E.=±4.7) to be lower ($p<0.05$) than baseline levels ($M=7.8$ ppm, S.E.=±3.4), and showed post-cigarette CO ($M=12.9$ ppm, S.E.=±5.2) to be higher than both baseline ($p<0.001$) and post-sham ($p<0.001$) CO.

3.4. Vital signs

Although a significant drug effect was observed with DBP ($F=7.2$, $df=1/11$, $p<0.03$), with pressure being lower in the clonidine (pre- and post-treatment) session ($M=67.6$ mm Hg, S.E.=±9.0) than in the placebo session ($M=72.3$ mm Hg, S.E.=±6.9), it did not interact with time to modulate DBP, SBP, or heart rate. Significant time effects were observed for DBP ($F=4.8$, $df=2/22$, $p<0.02$), SBP ($F=4.0$, $df=2/22$, $p<0.04$), and heart rate ($F=4.0$, $df=2/30$, $p<0.02$). Although SBP declined ($p<0.04$) from baseline ($M=112$ mm Hg, S.E.=±8.3) to post-drug assessment ($M=107.2$ mm Hg, S.E.=±6.9), and a similar decline ($p<0.03$) was seen with DBP assessed at baseline ($M=71.1$ mm Hg, S.E.=±7.2) and post-drug ($M=68.1$ mm Hg, S.E.=±7.3), only DBP was significantly ($p<0.01$) increased ($M=70.6$ mm Hg, S.E.=±6.9) by cigarette smoking relative to baseline. Cigarette smoking also significantly ($p<0.02$) increased heart rate ($M=69.6$ bpm, S.E.=±9.5) relative to post-drug heart rate ($M=61.6$ bpm, S.E.=±12.3).

4. Discussion

The general aim of this study was to further explore the neurochemistry underlying the generation of electrocortical and affective responses to acute cigarette smoking. The role of noradrenergic neurotransmission was specifically targeted as nicotine is known to augment central NA release, and selective NA reuptake inhibitors block nicotine self-administration (Mitchell et al., 1993; Svenson and Engerg, 1980; Rauhut et al., 2002). Interpretation of the study findings depends to a great extent on the experimental design and procedures from which the observations were derived and clearly the present study was lacking on several fronts including: (1) use of a limited sample size consisting of relatively young, moderately dependent smokers, and the failure to include smoker subtypes, e.g. heavy vs. light smokers, etc.; (2) no inclusion of a control de-nicotinized cigarette, and 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 and/or puffing (e.g. volume, duration) parameters across subjects and sessions: (4) use of a single clonidine dose, and failure to assess dose– or time–response relationships or nicotine/drug bioavailability with blood level measurements. Despite these limitations, the derived observations provide some preliminary insight into the pharmacologic and physiologic basis of the acute smoking response which may be useful in formulating testable neurochemical hypothesis regarding the motivational significance of neuroelectric and affective arousal in the initiation, maintenance and cessation of smoking behaviour.

Similar to previous spectral EEG studies of acute cigarette smoking in our laboratory, the smoking of a single cigarette following overnight smoking abstinence was characterized by voltage reductions in slow (delta) frequencies and voltage increments in fast (alpha$_2$, beta) frequencies, the voltage shift also being evident with an accelerated PAF with cigarette (vs. sham) smoking. In line with topographic analyses, the scalp distribution of these effects were relatively diffuse with all recording regions exhibiting equivalent EEG alterations with smoking, a pattern also evident with our previous investigations of cigarette smoking (Domino and Matsuoka, 1991, 1994; Domino et al., 1992) and transdermally applied nicotine. The fact that this same neuroelectric response profile is not induced with de-nicotinized cigarettes (Pickworth et al., 1999; Robinson et al., 1992) is evident only with smoking-related plasma nicotine increments exceeding 10 ng/ml (Kadoya et al., 1994), and appears to be dose- (nicotine-yield) and time-(puff) dependent (Knott, 1988, 1989a,b; Lindgren et al., 1999), suggests that smoking may be motivated in part by the desire to experience psychological states associated with nicotine-generated fast electrocerebral frequencies. As the fast low amplitude asynchronous beta and several forms of "functional alpha" have been consistently observed during sensory, cognitive, and motor processes (Basar et al., 1997a,b, 2001), it may be suggested that smoke-inhaled nicotine states are synonymous with brain-behavioural activation and alertness. Subjective alertness and a stimulant-like euphoria were clearly evident with cigarette smoking, and they are consistent with previous reported linear relationships between nicotine dose and measures of

drug high in nicotine-deprived smokers (Kalman, 2002), However, as this study did not include non-abstaining smokers or non-smokers, it is ambiguous as to whether the arousing profile is a reversal of abstinence-related sedation or an "absolute" arousal increase (Knott and Harr, 1995). As these mood and EEG alterations were accompanied by self-reported withdrawal reductions and increments in calmness and contentedness, the latter emotional states usually being absent during smoking withdrawal (West, 1984), it would appear that the findings in these studies reflect an abstinence reversal effect of smoking. However, given that nicotine-induced augmentation of subjective alertness and PAF, but not alpha$_2$–beta power, is also evident in non-smokers (Foulds et al., 1994, 1997), it may be that the present findings reflect a combination of primary or "absolute" and abstinence reversal effects of nicotine (Lindgren et al., 1999). In general, mood responses to smoking, although evidencing reductions in negative affective states (i.e. withdrawal symptoms) as well as increases in positive affect states, were not different from mood assessed at baseline. Arousal modulation of affect via smoke-inhaled nicotine has been proposed as a significant motivation for smokers (Eysenck et al., 1973), and although the current findings do not support a role for NA tone in arousal/mood interactions during smoking, they reinforce the paradox that the central stimulating actions of nicotine can be concurrently experienced as both arousing (i.e. alerting) and calming (Gilbert, 1979).

Pre-treatment with clonidine did not exacerbate the apparent abstinence-related withdrawal, mood or electrophysiologic symptoms, nor did it blunt the reversal of these symptoms with acute smoking. Accordingly, these findings suggest that noradrenergic neurotransmission may not play a role in the EEG/mood accompaniments of smoking or smoking abstinence. It is possible that these negative findings may be due to the use of an inadequate dose of clonidine as neither EEG/mood nor blood pressure effects were evident with the study dose. Administration of the frequently used of 0.15–0.20 mg doses has typically resulted in spectral EEG slowing, with decreases in alpha and increases in theta (Bischoff et al., 1998, 2000; Itil and Itil, 1983; Yamadira et al., 1985), but as this is usually associated with marked soporific effects, the present study utilized a lower dose of 0.10 mg of clonidine. Although the absence of clonidine effects on the smoking-induced EEG/mood arousal profile may suggest sub-threshold dosing, it is also possible that they reflect altered LC noradrenergic receptor sensitivity in smokers. In a recent study of postmortem LCs of smokers and non-smokers, long-term smoke exposure was shown to affect noradrenergic proteins of the LC, reducing amounts of tyrosine hydroxylase immunoreactivity and radioligand binding to α_2-adrenoreceptors (Klimack et al., 2001), actions which are similar to those seen with chronic administration of mood-elevating antidepressant agents. The down-regulation of α_2-NA autoreceptors suggests that repeated central release of noradrenaline elicited with acute smoking may have resulted in neuroadaptive processes in feedback mechanisms that regulate this transmitter system. Future study designs pursuing noradrenergic mechanisms underlying the acute response may want to include clonidine pre-treatment in smokers and non-smokers administered nicotine, with clonidine and nicotine both being administered with multiple doses. Although clonidine treatment for smoking cessation has not consistently affected withdrawal symptoms or abstinence rates in smokers (Appel, 1987; Franks et al., 1989), antidepressants believed to be acting through noradrenergic mechanisms, such as bupropion and nortriptyline, have shown promising results in smoking cessation studies (Balfour, 2001; Da Costa et al., 2002; Hayford et al., 1999; Jorenby et al., 1999). Long-term EEG/mood monitoring of successful and relapsed smokers emerging from placebo-controlled smoking cessation treatment with these agents may provide useful and more meaningful insight into the relationship between abstinence-related EEG slowing and affective disturbances and NA neurotransmission.

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