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Acute Phenylalanine/Tyrosine Depletion Reduces Motivation to Smoke Cigarettes Across Stages of Addiction

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The neurobiology of tobacco use is poorly understood, possibly in part because the relevant mechanisms might differ depending on past nicotine exposure and degree of addiction. In the present study we investigated whether these factors might affect the role of dopamine (DA). Using the acute phenylalanine/tyrosine depletion method (APTD), DA synthesis was transiently decreased in three groups of abstinent smokers ($n = 47$): (1) early low-frequency smokers, who had smoked a maximum of five cigarettes per day for less than one year, (2) stable low-frequency smokers smoking at the same level as early low-frequency smokers for at least 3 years, and (3) stable high-frequency smokers, who smoked a minimum of 10 or more cigarettes per day for at least 5 years. Motivation to obtain tobacco was measured using a progressive ratio breakpoint schedule for nicotine-containing and de-nicotinized cigarettes. Compared with a nutritionally balanced control mixture, APTD decreased the self-administration of nicotine-containing cigarettes, and this occurred in all three groups of smokers. The results suggest that DA influenced the willingness to sustain effort for nicotine reward, and this was seen in participants at all three levels of cigarette addiction. In the transition from sporadic to addicted use, the role of DA in the motivation to seek drug may change less than previously proposed.
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INTRODUCTION

The neurobiology of tobacco use at varying stages of addiction is not well understood. Studies in laboratory animals, however, implicate a role for nicotine-induced dopamine (DA) release (Clarke, 1990; Rose and Corrigall, 1997; Sorge and Clarke, 2009). Following acute administration, nicotine binds to nicotinic cholinoceptors on DA cell bodies in the ventral tegmental area of the midbrain (Clarke *et al*, 1988) and elicits DA release in the ventral striatum (Di Chiara and Imperato, 1988). Repeated nicotine exposure can lead to progressive increases in its rewarding and behaviorally activating effects (Vezina *et al*, 2007). For example, initial intermittent exposures to nicotine (three to six times) result in progressively greater effects on locomotor activity (Clarke and Kumar, 1983; Louis and Clarke, 1998), enhance the acquisition of self-administration (Shoaib *et al*, 1997), increase conditioned place preferences (Shoaib *et al*, 1994), and precipitate greater DA overflow in the nucleus accumbens (Balfour *et al*, 1998; Benwell and Balfour, 1992; Domino and Tsukada, 2009; Shim *et al*, 2001). Preventing this DA response disrupts nicotine self-administration and nicotine-related-conditioned place preferences (Corrigall and Coen, 1991; Liu *et al*, 2010; Shoaib *et al*, 1994; Sorge and Clarke, 2009; Spina *et al*, 2006; Tang and Dani, 2009). The contribution of DA following more extensive nicotine exposure, though, remains largely unexplored.

Attempts to translate these findings to humans have been equivocal. Functional neuroimaging studies have demonstrated DA release in response to cigarette smoking (Barrett *et al*, 2004; Brody, 2006; Brody *et al*, 2004; Scott *et al*, 2007), but attempts to alter smoking behavior by decreasing DA transmission have provided contradictory results. In these studies, smoking patterns were seen to increase (Caskey *et al*, 1999; Caskey *et al*, 2002; Dawe *et al*, 1995; Hitsman *et al*, 2008), decrease (Brauer *et al*, 2001), and remain unchanged (Casey *et al*, 2006; Hutchison *et al*, 2004). This variability might reflect differences in nicotine self-administration paradigms, the use of nonspecific DA receptor antagonists, or a change in the role of DA, as smokers transition from occasional to dependent cigarette use.

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In the present study, we investigated the effect of decreasing DA transmission on the motivation to smoke and self-reports of cigarette pleasure and craving at three different stages of tobacco addiction: (1) low-frequency early smokers (LFES), who have smoked less than 1 year and smoke no more than five cigarettes per day and not every day, (2) low-frequency stable smokers (LFSS), who have smoked for at least 3 years, smoking no more than five cigarettes a day, not necessarily every day, and (3) high-frequency stable smokers (HFSS) who have smoked for more than 5 years and smoke 10 or more cigarettes per day, every day. Motivation to obtain cigarettes was measured using a progressive ratio (PR) breakpoint paradigm adapted for humans (Barrett *et al*, 2006). DA transmission was decreased using the acute phenylalanine/tyrosine depletion method (APTD) (Leyton *et al*, 2000b).

MATERIALS AND METHODS

Subjects

Forty-seven smokers, aged from 18 to 25 years old (20.4 ± 2.5 years; mean $\pm$ SD), were recruited from the community through advertisements placed on the McGill University classified website and in local Montreal newspapers. All subjects underwent a routine medical exam and standard blood work, and were deemed fit to participate by a physician. All subjects were evaluated using the semi-structured clinical interview using DSM-IV criteria (First, 1995). This study was approved by the Research Ethics Board of the Royal Victoria Hospital, McGill University Health Center. Written, informed consent was obtained from all participants. All participants were free of all current Axis I disorders, including major depressive disorder, bipolar disorder, psychosis, psychoactive substance use disorder, excluding nicotine and caffeine, panic disorder, generalized anxiety disorder, and obsessive compulsive disorder.

Group Assignment

Subjects who met our eligibility criteria were divided into HFSS, LFSS, and LFES groups, based on cigarettes smoked per day, number of years smoking, and on the degree of nicotine dependence as determined by the Fagerström test for nicotine dependence (FTND) (Fagerstrom, 1978; Fagerstrom and Schneider, 1989; Heatherton *et al*, 1991) and the hooked on nicotine checklist (HONC) (DiFranza *et al*, 2002). HFSS had a combined FTND and HONC score of 10 or higher. Both groups of low-frequency smokers had a combined score of 9 or less.

Study Design

Two test sessions were conducted at minimum of 3 days apart. On the morning of each test day, subjects ingested a drink deficient in phenylalanine and tyrosine (APTD), or a nutritionally balanced control mixture (BAL). The order of conditions was randomly assigned and counterbalanced across groups. Both subjects and experimenters were blind to condition. The composition, preparation, and administration of the APTD mixture were based on a balanced amino acid (AA) mixture, with phenylalanine and tyrosine withheld (Leyton *et al*, 2000b). The AA mixtures were adapted for women by reducing the amount of protein by 17% to account for lower body weight. On the day before testing, subjects ate a low-protein diet supplied by the investigators. They were asked to refrain from alcohol use on this day and to restrict themselves to three caffeinated drinks. Subjects were asked to fast and remain abstinent from cigarettes from midnight before the test session.

On test days, subjects arrived at the laboratory at 0930 h. At this time, they provided a urine sample, which was tested for drugs (Triage Panel for Drugs of Abuse, sensitive to cocaine, amphetamines, barbiturates, benzodiazepines, Δ^9-tetrahydrocanabinol, opiates, and phencyclidine; Biosite Diagnostics, San Diego, CA, USA). They also provided a breath carbon monoxide measure (cutoff for abstinence <10 p.p.m., Vitalograph-Breath CO Monitor) to confirm smoking abstinence. All female participants were given a urine pregnancy screen and were tested while in the follicular phase of their menstrual cycle. Subjects who tested positive for any drug, or had carbon monoxide levels in excess of 10 p.p.m. on a testing day were not tested on that date. Following confirmation of a negative toxicology and pregnancy screening, blood samples were then drawn to assess baseline plasma AA levels. All samples were immediately centrifuged and the plasma was fractioned-off and stored at $-80\,^{\circ}$C until analysis. Following the baseline blood sample draw and the administration of self-report scales, subjects were asked to ingest the AA mixture. Four hours following ingesting of the AA mixture, a second blood draw was performed to assess any changes in plasma AA levels compared with baseline.

Plasma Amino Acids

Plasma concentrations of phenylalanine, tyrosine, and other large neutral AAs (LNAAs) were measured by HPLC with precolumn derivatization and fluorometric detection. Plasma samples were missing from 3 of the 94 test days.

Subjective States

Subjects were administered 14 visual analog scales (VAS) at three time points over the course of the test day: immediately before ingesting the AA mixture, 4 h after ingesting the AA mixture, and at the end of the test session. The items were: alert, anxious, bored, edgy, energetic, euphoria, excited, hungry, intend to smoke, interested, like cigarette, mind-racing, rush, and want cigarette. Items were rated on a 10-cm line labeled with the integers 1–10 and anchored with the words 'least' and 'most'. Subjective cigarette craving was measured with the questionnaire on smoking urges (QSU) (Tiffany and Drobes, 1991); administered at the same time points as the VAS. This self-report measure yields a total craving score, and scores for two factors ranging from 1 to 7, with 4 representing a neutral midpoint. The total score represents overall instantaneous cigarette craving. Factor 1 measure aspects of smoking related to positive affect such as the pleasantness or satisfaction derived from smoking a cigarette. Factor 2 emphasizes craving for cigarettes to relieve withdrawal or negative emotions related to cigarettes. Clinical and

sub-clinical changes in mood state were measured with the Beck depression inventory (BDI) (Beck, 1961) and the bipolar profile of mood states (POMS) (Lorr *et al*, 1982), each of which was administered at the beginning and end of the test days.

Cue-Induced Cigarette Craving

Before each cigarette self-administration session, participants were comfortably seated in a chair in front of a computer. Participants were instructed to handle a neutral cue (ie, a pen) and following that to complete VAS and QSU scales. Participants were then instructed to hold a cigarette (lit by the experimenter) for 1 min, but were not allowed to smoke the cigarette for 1 min. Again participants were asked to complete VAS and QSU scales after having held the lit cigarette.

Cigarette Self-Administration Task

Four hours following AA ingestion, participants were offered the opportunity to earn up to 10 mini-cigarettes (one-tenth of a regular sized cigarette) on a PR schedule, an objective behavioral measure of the motivation to smoke. This time point was chosen to coincide with peak biochemical and behavioral effects observed in previous APTD studies (Leyton *et al*, 2005; Leyton *et al*, 2004). Participants had the choice to earn and smoke nicotine-containing cigarettes (0.6 mg/entire cigarette) or de-nicotinized Quest 1 tobacco cigarettes (Vector Tobacco, USA). Before the task, however, subjects were informed only that they could earn mini-cigarettes from two different cigarette brands. To earn each mini-cigarette, participants were required to repeatedly press the letters 'w' and 'a' for a predetermined number of times to earn a nicotine-containing cigarette, whereas de-nicotinized cigarettes were similarly earned using the letters 'd' and 'r'. Participants were blind to the outcome of the key presses, that is, which key press combination would earn nicotine-containing or de-nicotinized mini-cigarettes. For each type of cigarette, the first earned mini-cigarette required 50 button presses and the number of presses required to earn each subsequent mini-cigarette of either type doubled (PR ratio: 50, 100, 200, 400, 800, 1600, 3200, 6400, 12 800, and 25 600). Each session lasted until the maximum number of mini-cigarettes was earned or to a maximum of 2 h, whichever came first. Participants were not required to earn mini-cigarettes during the sessions, but were required to remain seated in the testing room until each session was completed. Upon completion of the self-administration task, participants were brought a meal and then sent home by taxi and were paid at the end of the experiment.

Statistical Methods

All data were analyzed using Statview, version 5.0.1. The primary outcome variables in this study were the number of button presses during the PR task to earn mini-cigarettes in each AA session. The PR (button press) data were log-transformed to permit parametric analyses. These data were then analyzed using a $2 \times 2 \times 3$ repeated measures ANOVA using AA condition (BAL, APTD) and cigarette type (nicotine-containing and de-nicotinized) as within-subject factors and group (LFES, LFSS, and HFSS) as between-subject factor. Familywise Bonferroni corrections were applied when related analyses were conducted on several variables. Analyses of the VAS and QSU data were subjected to repeated measures ANOVAs, with AA mixture (BAL, APTD), and Time (pre-session, t + 4, and post-session) as within subject factors and group (LFES, LFSS, HFSS) as between-subject factor. VAS and QSU data following cue exposure were analyzed using $2 \times 2 \times 3$ repeated measures ANOVA with cue (neutral, cigarette) and AA mixture (BAL, APTD) as within-subject factors and group (LFES, LFSS, HFSS) as a between-subject factor.

RESULTS

Participants

Forty-seven participants completed the study protocol. Four additional participants discontinued following their first AA session; three withdrew because of nausea following ingestion of the AA mixture, while the fourth did not complete the study because of relocation.

As expected, the three groups differed significantly on variables related to their level of tobacco use. The three groups did not differ, though, on demographic features, including age, sex, gender, and education. LFES consumed significantly more alcoholic drinks per week than HFSS [$F(2, 45) = 3.7$, $p = 0.04$] (Table 1).

Amino Acid Depletion

On the APTD test session, plasma concentrations of tyrosine and phenylalanine decreased significantly, as reflected by AA Mixture × Time interactions: tyrosine $F(2, 1) = 228.3$ $p < 0.0001$; phenylalanine $F(2, 1) = 203.74$ $p < 0.0001$.

Table 1 Subject Characteristics

Group	N	Age (years)	Sex (M/F)	Education (years)	FTND	HONC	Cigarettes/day	Drinks/week
HFSS	16	21.0 (1.8)	10/6	14.4 (1.5)	5.3 (1.4)***	8.4 (1.2)***	12.6(4.4)***	6.1 (4.8)*
LFSS	16	21.8 (2.4)	6/10	15.0 (1.0)	3.1 (1.5)	5.0 (1.9)**	4.9 (3.2)	8.9 (7.0)
LFES	15	19.9 (2.3)	6/9	14.0 (1.5)	2.5 (1.7)	3.4 (2.2)	2.7 (1.7)	10.9 (7.0)

Abbreviations: FTND, Fagerström test for nicotine dependence; HFSS, high-frequency stable smokers; HONC, hooked on nicotine checklist; LFES, low-frequency early smokers; LFSS, low-frequency stable smokers. See text for details. Mean given with SD in parentheses.
***$p < 0.0001$.
**$p < 0.005$.
*$p < 0.05$ (in comparison with LFES).

Four hours after ingesting the mixture, the APTD treatment decreased tyrosine and phenylalanine levels to 22.9% and 15.1%, respectively, of morning baseline levels, whereas the BAL mixture increased plasma tyrosine and phenylalanine levels by 240% and 236%, respectively. Ingestion of both Mixtures also significantly decreased the ratios of plasma tyrosine and phenylalanine to other LNAA (tyrosine, phenylalanine, tryptophan, leucine, isoleucine, valine, and methionine) ($p<0.001$). However, AA Mixture × Time interactions revealed that these reductions were significantly more pronounced in the APTD relative to the BAL condition: tyrosine (94.8% *vs* 32.5%), $F(2, 1)=35.2$, $p<0.0001$; phenylalanine (96% *vs* 33.7%), $F(2, 1)=66.1$, $p<0.0001$ (Table 2).

Cigarette Self-Administration Progressive Ratio Breakpoints

As depicted in Figure 1, all three groups worked more for nicotine-containing cigarettes than for de-nicotinized ones [$F(1, 39)=84.4$, $p<0.0001$]. A Group × Cigarette-type interaction [$F(2, 39)=6.3$, $p<0.01$] indicated that high-frequency smokers worked more than low-frequency smokers for nicotine-containing mini-cigarettes ($p<0.01$) but not for de-nicotinized ones ($p=0.43$). Finally, APTD, compared with BAL, decreased the number of mini-cigarettes worked for by all three groups of smokers [$F(1, 39)=4.4$, $p<0.05$)], and this effect was the same in all three groups as indicated by the absence of Group × AA Mixture [$F(1, 39)=0.52$, $p=0.59$] and Group × Cigarette-type × AA Mixture interactions [$F(2, 39)=0.86$, $p=0.43$]. The AA Mixture × Cigarette-type interaction was not significant [$F(1, 39)=0.99$, $p=0.32$], but inspection of the data suggested that the main effect of APTD was primarily driven by decreased self-administration of the nicotine-containing cigarettes ($p<0.05$), but not denicotinized ones ($p=0.50$).

Effects Of APTD And Time On VAS Scores

Repeated measures ANOVA (Group × AA Mixture × Time) showed main effects of Group [$F(2, 39)=5.2$, $p<0.05$] and Time [$F(4, 39)=12.3$, $p<0.001$)] on ratings of VAS items Like Cigarette and Want Cigarette. *Post-hoc* analyses revealed that HFSS and LFSS rated Like Cigarette and Want Cigarette higher compared with LFES at all time points ($p<0.01$) (Table 3). Following the first cigarette, ratings were significantly lower than at previous time points ($p<0.001$).

Repeated measures ANOVA (Group × AA Mixture × Time) revealed main effects of Group [$F(2,39)=4.9$, $p<0.05$] and Time [$F(4,39)=6.7$, $p<0.01$) on ratings of VAS item 'Euphoria' and 'High'. *Post-hoc* analyses revealed that ratings of 'Euphoria' and 'High' were higher in HFSS than in LFES and LFSS ($p<0.05$). Ratings of 'Euphoria' and 'High' were significantly different at time points 'Cig cue' and 'Post-Cig' compared with all three previous time points ($p<0.01$) (Table 3). An effect of Time and Group differences was observed on several VAS items (Supplementary Table S1). However, APTD had no effect on the rating of any VAS items at different time points.

The Effects Of APTD And Time On Self-Reported Craving

Group differences on Factor 2 ratings of the QSU were observed with HFSS and LFSS scoring significantly higher than LFES ($p<0.01$). No group differences were observed on Factor 1 ratings. Effects of AA Mixture or Cue on QSU Factor 1 and Factor 2 ratings were not observed (Table 4).

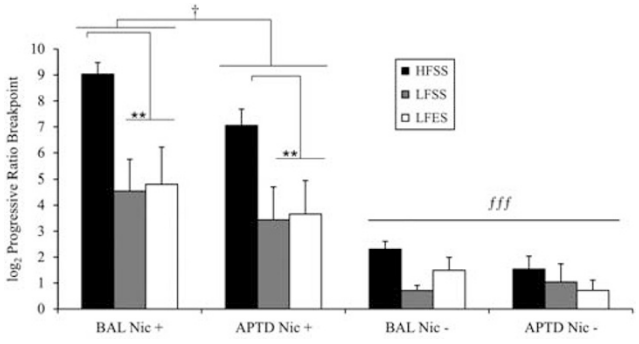

Figure 1 Effect of acute phenylalanine and tyrosine depletion (APTD) on progressive ratio breakpoints for nicotine-containing (Nic +) and de-nicotinized cigarettes (Nic −). HFSS, LFSS, and LFES refer to high-frequency stable smokers, low-frequency stable smokers, and low-frequency early smokers, respectively. All three groups of smokers worked for more Nic + cigarettes compared with Nic − ones ($p<0.0001$, (*fff*)). HFSS worked for more Nic + compared with both groups of low-frequency smokers ($p<0.01$), (**). In all three groups, Nic + self-administration was significantly attenuated in the APTD condition with relative to BAL ($p<0.05$, (†)).

Table 2 Plasma Levels of Tyrosine and Phenylalanine, and Ratios of Tyrosine and Phenylalanine to Large Neutral Amino Acids at a.m. Baseline and 4 h Following Amino Acid Ingestion

Amino acid	BAL (a.m.)	BAL (p.m.)	% Difference	APTD (a.m.)	APTD (p.m.)	% Difference
Tyrosine	56.7 (13.3)	136.7 (43.6)**	241.1	54.5 (8.9)	12.6 (2.9)**	−76.9
Phenylalanine	50.5 (9.0)	119.1 (50.9)**	235.8	49.1 (6.9)	7.4 (1.9)**	−84.9
Tyrosine:LNAA	0.117 (0.05)	0.079 (0.01)*	−32.5	0.12 (0.05)	0.007 (0.003)**	−94.2
Phe:LNAA	0.104 (0.04)	0.069 (0.02)*	−33.7	0.10 (0.03)	0.004 (0.002)**	−96.0

Abbreviations: APTD, acute phenylalanine tyrosine depletion; BAL, balanced; LNAA, large neutral amino acid; Phe, phenylalanine.
Biochemical data are presented as mean μmol/l (SD).
*$p<0.001$.
**$p<0.0001$.

Table 3 Visual Analog Scores as a Function of Time and Cue Presentation

Group			HFSS					LFSS					LFES				
VAS item	**Effect**	**AA Mixture**	**Baseline**	**t+4**	**Neu Cue**	**Cig Cue**	**Post-Cig**	**Baseline**	**t+4**	**Neu Cue**	**Cig Cue**	**Post-Cig**	**Baseline**	**t+4**	**Neu Cue**	**Cig Cue**	**Post-Cig**
Euphoria	a, bb	BAL	3.9 (2.2)	3.6 (1.8)	3.9 (2.1)	5.1 (2.2)	4.6 (1.9)	2.4 (1.6)	2.6 (1.6)	2.5 (1.4)	3.1 (1.9)	4.1 (2.6)	2.8 (1.3)	1.9 (1.1)	2.3 (1.4)	2.9 (1.5)	2.4 (1.6)
		APTD	3.3 (1.1)	2.6 (1.4)	3.3 (1.8)	4.2 (2.3)	3.9 (2.2)	2.4 (1.1)	2.0 (0.8)	2.3 (1.3)	2.7 (1.5)	4.4 (2.4)	2.1 (1.1)	1.9 (1.1)	2.0 (1.2)	2.3 (1.1)	2.8 (1.6)
High	a, bb	BAL	3.0 (2.0)	3.1 (1.3)	3.6 (1.9)	4.4 (2.0)	4.5 (2.1)	2.5 (1.6)	2.3 (1.2)	2.1 (1.4)	2.9 (2.0)	4.1 (2.2)	2.6 (1.5)	1.9 (0.8)	1.9 (0.8)	2.2 (1.2)	2.0 (0.7)
		APTD	2.2 (1.3)	2.3 (1.3)	2.8 (1.5)	3.4 (1.7)	4.3 (2.6)	2.2 (1.1)	2.5 (1.6)	2.2 (1.1)	2.8 (1.5)	4.7 (2.1)	1.8 (0.8)	1.6 (0.5)	1.4 (0.5)	1.8 (1.0)	2.4 (1.7)
Like Cigarette	a, bb	BAL	6.2 (2.3)	6.4 (2.6)	7.3 (1.8)	7.8 (2.0)	5.6 (2.2)	5.9 (1.7)	6.8 (1.5)	7.3 (1.6)	6.1 (1.9)	5.3 (2.6)	5.1 (2.1)	4.8 (1.4)	5.2 (1.6)	5.3 (2.9)	3.8 (1.5)
		APTD	5.7 (2.5)	6.5 (1.8)	6.4 (2.6)	7.3 (2.2)	5.4 (2.8)	5.5 (3.5)	6.1 (3.2)	6.0 (3.2)	6.8 (2.5)	5.8 (2.0)	4.2 (1.9)	4.6 (1.7)	4.3 (2.1)	5.3 (1.9)	4.7 (2.2)
Want Cigarette	a, bb	BAL	6.8 (2.3)	6.6 (2.4)	7.6 (1.7)	8.0 (2.2)	5.4 (2.3)	5.9 (2.2)	6.3 (2.0)	7.1 (2.0)	7.3 (1.8)	4.5 (2.5)	4.5 (2.3)	4.8 (1.9)	5.1 (2.0)	6.0 (2.7)	3.3 (1.6)
		APTD	5.7 (2.8)	6.7 (2.2)	6.8 (2.4)	7.5 (2.5)	5.0 (3.0)	5.9 (3.7)	6.4 (3.4)	7.0 (2.7)	6.4 (3.3)	5.0 (2.4)	3.9 (2.0)	4.5 (2.1)	4.5 (1.5)	5.5 (2.1)	3.9 (1.7)

VAS data are presented as mean (SEM).
Results: 'a', Indicate group differences in ratings by HFSS compared with LFSS and LFES, $p<0.05$.
'bb', Refers to differences in ratings at Cig cue and Post-Cig time points across smoker groups, $p<0.01$.
Rating of VAS items 'Euphoria', 'High', 'Like Cigarette' and 'Want Cigarette' at five different time points following ingestion of a nutritionally balanced AA Mixture (BAL) compared with an AA Mixture deficient in phenylalanine and tyrosine depletion (APTD). HFSS, LFSS, and LFES refer to high-frequency stable smokers, low-frequency stable smokers and low-frequency early smokers, respectively. Neu Cue and Cig Cue refer to Neutral Cue and Cigarette Cue. Values are reported as mean+SEM.

Table 4 Questionnaire of Smoking Urges Scores as a Function of Time and Cue Presentation

AA Mixture	Effect	QSU	HFSS					LFSS					LFES				
			Baseline	**t+4**	**Neu Cue**	**Cig Cue**	**Post-Cig**	**Baseline**	**t+4**	**Neu Cue**	**Cig Cue**	**Post-Cig**	**Baseline**	**t+4**	**Cig Cue**	**Neu Cue**	**Post-Cig**
BAL	bb	Factor 1	5.6 (0.8)	5.4 (1.1)	6.0 (1.0)	5.8 (1.4)	4.8 (1.9)	4.8 (1.7)	5.3 (1.0)	5.7 (1.2)	5.6 (1.4)	4.1 (1.6)	4.4 (1.3)	4.6 (0.8)	5.1 (1.3)	4.3 (1.4)	4.2 (1.5)
	a, b	Factor 2	4.6 (0.8)	4.2 (1.2)	4.8 (1.7)	4.7 (1.0)	2.9 (1.6)	3.8 (0.8)	4.1 (1.0)	4.4 (1.3)	4.5 (1.6)	3.2 (1.7)	2.5 (0.8)	2.9 (0.9)	3.0 (1.2)	2.7 (1.1)	2.6 (0.9)
APTD	bb	Factor 1	5.6 (0.7)	5.3 (1.5)	5.9 (1.0)	5.5 (1.3)	4.9 (1.8)	5.1 (1.7)	5.2 (1.6)	5.4 (1.6)	5.7 (1.4)	3.6 (1.6)	4.4 (1.2)	4.5 (1.2)	5.0 (1.0)	5.1 (1.0)	3.9 (1.0)
	a, b	Factor 2	4.2 (1.1)	4.4 (1.3)	4.9 (1.4)	4.2 (1.4)	3.8 (1.7)	3.7 (1.2)	4.3 (1.2)	4.5 (1.6)	4.8 (1.7)	3.0 (1.6)	2.4 (0.9)	2.5 (1.0)	3.1 (1.0)	3.1 (1.2)	2.5 (1.1)

Abbreviations: APTD, AA Mixture deficient in phenylalanine and tyrosine depletion; BAL, nutritionally balanced AA Mixture; Cig Cue, Cigarette Cue; HFSS, high-frequency stable smokers; LFES, low-frequency early smokers; LFSS, low-frequency stable smokers; Neu Cue, Neutral Cue; QSU, questionnaire of smoking urge.
QSU data are presented as mean (SEM).
Results: a, main effect of group, $p<0.01$.
b, bb, Main effect of time, $p<0.01$, $p<0.05$, respectively.
QSU Factor 1 and Factor 2 scores at five different time points following ingestion of BAL compared with an APTD. Values are reported as mean+SEM. 'aa', Indicate differences in Factor 2 scores in HFSS and LFSS compared with LFES, $p<0.01$. 'bb' Refers to differences in Factor 1 and Factor 2 scores at the Post-Cig time point across smoker groups, $p<0.01$.

Effect Of APTD On Self-Reported Mood

Analyses did not reveal main effects of Group, AA Mixture, or of Time ($p > 0.4$) on BDI or POMS scores, or significant interactions ($p = 0.58$).

DISCUSSION

The present study provides the first investigation of the role of DA in low-frequency smokers. As seen previously in non-dependent users of other substances, reducing DA transmission decreased self-administration behavior (Barrett *et al*, 2008; Brauer *et al*, 2001; Enggasser and de Wit, 2001; Leyton *et al*, 2000a; although see Leyton *et al* (2005)). More importantly, the same reduction was seen in high-frequency, nicotine-dependent smokers. Together, these findings suggest that DA affects motivation to obtain drug reward across degrees of addiction, at least in the case of tobacco.

In previous research, APTD has been demonstrated to decrease striatal DA release induced by *d*-amphetamine (Leyton *et al*, 2004). In the present study, APTD likely decreased nicotine-induced DA release, an effect proposed to diminish the ability to sustain motivation to obtain multiple mini-cigarettes. More generally, the results add to the evidence that the motivation to seek drugs is promoted by increases in DA transmission rather than decreases (Stewart, 2008).

The measure of motivation to obtain cigarettes was the PR breakpoint (Roberts *et al*, 1989). Reductions in breakpoints could occur for multiple reasons, including decreases in craving or pleasurable effects related to reward. In the present study, PR breakpoints were reduced in the absence of changes to craving or pleasure. This observation is supported by growing evidence that mesolimbic DA transmission influences incentive salience but that this is not because of conscious processing or altered hedonic experience (Berridge and Robinson, 1998; Leyton, 2009). Consistent with this interpretation, neither APTD (Casey *et al*, 2006; Hitsman *et al*, 2008; Munafo *et al*, 2007) nor DA receptor antagonists (Brauer *et al*, 2001; Dawe *et al*, 1995; Mahler and de Wit, 2005) diminished self-reported craving or pleasure in previous studies conducted in dependent smokers.

As expected, HFSS worked for more nicotine-containing cigarettes than either group of low-frequency smokers. Unexpected, though, was that both groups of long-term smokers (LFSS, HFSS), regardless of smoking frequency, rated cigarette 'Liking', 'Wanting', and the negative aspects of craving (eg, negative mood, irritability) significantly higher than those who had been smoking for less than a year. These observations suggest that subjective symptoms of dependence can precede additional increases in smoking behavior (DiFranza *et al*, 2007; Gervais *et al*, 2006).

The observation that low- and high-frequency smokers all worked more for nicotine-containing cigarettes than de-nicotinized ones adds to a literature that has been surprisingly equivocal. Although smokers consistently prefer nicotine-containing cigarettes when given a choice (Barrett, 2010; Shahan *et al*, 1999), de-nicotinized cigarettes mimic the sensory properties of smoking (Donny and Jones, 2009), reduce smoking urges, elicit positive emotional states (Barrett, 2010), and have been reported to maintain similar rates of self-administration as nicotine-containing cigarettes, as measured with a PR breakpoint task (Donny *et al*, 2007; Rusted *et al*, 1998; Shahan *et al*, 1999). The discrepancy between the results obtained in the present study and those reported previously is likely due to methodological differences. First, in those studies, smokers were minimally deprived of nicotine (less than 1 h) (Rusted *et al*, 1998; Shahan *et al*, 1999), likely dampening their motivation to work for nicotine-containing puffs. In the present study, all smokers were nicotine abstinent for at least 12 h. Second, in the previous studies the nicotine-containing and de-nicotinized cigarettes were self-administered by separate groups of subjects, making conclusions about relative reinforcing efficacy difficult because individual subjects were not given a choice between the two cigarette types. Preference in a choice situation is generally a more sensitive measure of differences in reinforcer efficacy (Shahan *et al*, 1999).

The results of the present study should be interpreted in light of the following considerations. First, the sample sizes were modest ($n = 15$–16 per group); however, previous studies have demonstrated that 8 to 14 subjects are sufficient to see effects of APTD on PR self-administration breakpoints for alcohol and the ability to preferentially respond to reward-paired cues (Barrett *et al*, 2008; Leyton *et al*, 2007; Leyton *et al*, 2005). Although we might have missed group differences in the magnitude of this effect, all three groups exhibited a 20–25% decrease in PR breakpoints, and the Group × AA Mixture interaction effects were far from the trend level, $\alpha = 0.59$ and 0.43. Second, in previous studies that tested the effect of decreasing DA transmission on cigarette self-administration in dependent smokers, the effects have been inconsistent, possibly reflecting differences between *ad lib* self-administration regimens *vs* PR breakpoints, length of nicotine abstinence, or other methodological differences. Third, we did not measure plasma levels of nicotine or cotinine. This noted, previous studies indicate that low- and high-frequency smokers do not differ in smoking topography, nicotine metabolism, or blood nicotine levels following smoking (Arcavi *et al*, 1994; Shiffman *et al*, 1992). In comparison, the primary focus of the present study was whether lowering DA synthesis would alter the motivation to obtain cigarettes. Notably, APTD does not alter the inhalation topography of smoking puffs (Casey *et al*, 2006). Finally, we recruited smokers aged 18–25 years, university educated, of normal weight, and without significant mental health issues. We selected this population to ensure that the LFSS and HFSS groups would be similar in terms of the number of years smoking and to avoid demographic confounds. Nevertheless, we were able to detect group differences in PR responding and self-reported craving, indicating that the experimental design could capture the targeted differences.

In summary, two main novel findings were obtained in this study. First, APTD diminished the willingness to work for nicotine-containing cigarettes without changing self-reported craving or pleasure. This suggests that the motivation to smoke *vs* conscious craving and pleasure might be mediated by distinct neurochemical systems, with DA having greater importance for the first. Second, motivation

to earn cigarettes remained DA-sensitive across varying levels of addiction as the magnitude of APTD's effect was the same in all three smoking groups. Although smoking might induce long-lasting changes to the DA system, such as tolerance or sensitization, our results suggest that the motivational drive to obtain nicotine-containing cigarettes remains under the transmitter's influence.

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DISCLOSURE

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