Pharmacology Biochemistry & Behavior, Vol. 19, pp. 291–299, 1983. © Ankho International Inc. Printed in the U.S.A.

Nicotine Dependence in Cigarette Smoking: An Empirically-Based, Multivariate Model[1]

OVIDE F. POMERLEAU,* JOANNE B. FERTIG
AND SANDRA O. SHANAHAN

**Behavioral Medicine Laboratory, Veterans Administration Medical Center and Department of Psychiatry, University of Connecticut School of Medicine, Newington, CT 06111*

Received 17 January 1983

POMERLEAU, O. F., J. B. FERTIG AND S. O. SHANAHAN. *Nicotine dependence in cigarette smoking: An empirically-based, multivariate model.* PHARMACOL BIOCHEM BEHAV **19**(2) 291–299, 1983.—Nicotine dependence implies a pattern of heavy smoking which is resistant to change, as well as nicotine tolerance, withdrawal, and regulation. The present study attempted to develop a coherent model of cigarette smoking by examining responses on several different measures of nicotine dependence. Twenty-seven habitual smokers filled out questionnaires before and after smoking research cigarettes differing in nicotine content in the laboratory. Plasma cotinine was used to estimate nicotine intake from usual brand cigarettes outside the laboratory. Subjects in the high cotinine quartile (heavy smokers) were found to be consistently more nicotine-dependent than subjects in the low cotinine quartile (light smokers). Taking all subjects into account, the six measures of nicotine dependence which exhibited significant correlations with plasma cotinine accounted for about half of the cotinine variance in a multivariate, linear-regression model. Multivariate approaches provide additional tools for assessing biobehavioral mechanisms in substance abuse and may lead to the development of more-comprehensive and sufficient explanations of smoking than are currently available.

Cigarette smoking Nicotine addiction Nicotine tolerance Nicotine withdrawal Nicotine regulation
Heavy versus light smokers Multiple linear-regression Models of substance abuse Plasma cotinine
Plasma nicotine

NICOTINE dependence implies a pattern of heavy smoking which is resistant to change, as well as nicotine tolerance and withdrawal and the regulation of nicotine-intake within relatively narrow limits. Jarvik [15], Russell [27], and Schachter [33] have conceptualized smoking as primarily an escape/avoidance response to withdrawal from nicotine in an addictive cycle. An internal regulatory mechanism, a "nicostat," is postulated by which the level of nicotine is monitored and characteristic upper limits (toxic boundary) and lower limits (withdrawal boundary) [16,28] are maintained by changing the frequency, duration, and intensity of tobacco-smoke inhalation.

The scientific demonstration of these deceptively simple constructs has been fraught with difficulty. One problem has been that the importance of standardizing the smoking environment and of minimizing external, extraneous cues for smoking has been underestimated [9,24]. As a result, though numerous studies have examined various indicators of nicotine dependence, singly or in conjunction with one another, there have been many conflicting results and ambiguous findings [19,23]. Another problem has involved the quantitation of plasma nicotine and its major metabolite, cotinine. While sensitive gas-chromatography techniques have been in use for some time [5], the procedure is arduous and has numerous pitfalls. The wider availability of radioimmunoassay procedures for nicotine and cotinine [21] now makes possible high sample-capacity without sacrificing accuracy [10]. Inferences about nicotine regulation and nicotine dependence based on indirect measures of nicotine intake, e.g., measures such as heart-rate boost [18], or self-reported number of cigarettes [33], can now be re-examined and either substantiated or refuted.

The present study is an attempt to answer the question of whether heavy smokers simply smoke more than light smokers or whether, in addition, they are more dependent on nicotine. The experiment addressed several unresolved issues in the literature. In a recent study in which nicotine dosage was manipulated by changing the length of usual cigarettes, Russell *et al.* [30] observed that five of the ten subjects increased their smoking intake (as measured by carboxyhemoglobin levels) to compensate for decreased nicotine availability, while the other five did not; surprisingly, Russell *et al.* found no significant differences between compensators and non-compensators with respect to usual cigarette consumption, nicotine content of usual cigarettes, or carboxyhemoglobin and plasma nicotine levels, and there seemed to be no association between lack of compensation and withdrawal symptoms. Similar observations were made by Sutton *et al.* [36] in a study in which the nicotine yield of usual cigarettes was reduced by means of ventilated cigarette holders.

By classifying the usual level of nicotine intake using

[1]This research was supported by Veterans Administration Research Advisory Group and Merit Review Grants.

plasma cotinine as an objective indicator, by having subjects smoke research cigarettes which differed in nicotine but not in tar content, and by examining plasma nicotine and other relevant measures of nicotine dependence in the laboratory in a larger sample of smokers, it was hoped that the variables accounting for differences between heavy and light smokers could be identified. Of particular concern was Schachter's contention [33] that all regular smokers are dependent on nicotine and that the main difference between heavy and light smokers is that the latter are "dieters" who, by restricting their intake, subject themselves to chronic withdrawal symptoms. An additional consideration was that precise regulation of nicotine in the body has been assumed to characterize nicotine dependence [28]. But, as Kozlowski [16] has pointed out, the ability to regulate plasma nicotine perfectly does not necessarily define dependence, and other substances believed to be addictive such as alcohol and heroin do not show especially sensitive titration [14]. Finally, our general intent was to develop a cohesive model of cigarette smoking, one which was empirically derived and based on a relatively small number of measures of nicotine dependence.

METHOD

Subjects

Thirty male smokers in good general health, not taking psychotropic drugs or other medications, were recruited from the local community; they were paid $25 for participating in the study. Three subjects were subsequently deleted from the analysis because post-session plasma nicotine values could not be determined. For the remaining 27 subjects, mean (±SEM) age was 32.3 (±2.2) years (range 20 to 56); they had been smoking 16.7 (±2.2) years (range 4 to 45). Smoking rate was distributed fairly widely, with the range for the number of usual-brand cigarettes per day (self-report) going from 10 to 80, the nicotine content of the usual cigarettes going from 0.6 to 1.4 mg of nicotine, calculated nicotine exposure (number of cigarettes per day × mg nicotine per cigarette) going from 8 to 60 mg, and plasma cotinine prior to the first session going from 0.4 to 470 ng/ml.

Experimental Apparatus and Biological Sampling

Subjects sat in an easy chair and watched the "Sound of Music" on video cassette during testing. Temperature was maintained at 21±1°C and humidity at 50±5%. Subjects were observed through a one-way mirror, and there were no interactions between subjects and experimenter once the session was underway. Subjects were seated approximately 30 cm from a console that served to signal (by light and sound) the beginning and end of smoking trials, to dispense cigarettes under the subject's control during smoking trials, and to provide sensors for the measurement of behavioral, subjective, and physiological responses. The general setup was similar to that described by Henningfield and Griffiths [9]. Electrocardiographic (ECG) and peripheral (digit) skin temperature signals were amplified and recorded using Med Associates Modules (E. Fairfield, VT). Experimental sequences and data acquisition were fully automated using a minicomputer. Prior to experimental sessions, 20 cc urine samples were taken and pH determinations were made using a pH meter (Corning Company, Corning, NY; Model 125). Standard low and high nicotine research-cigarettes (Tobacco and Health Research Institute, University of Kentucky) were used to allow more accurate specification and control of nicotine and tar content than is possible in commercial brands and to avoid pre-existing brand preferences; according to the specifications of the manufacturer, the low nicotine cigarette (2A1) delivers 0.48 mg nicotine and 36.4 mg tar when smoked to 23 mm butt length, while the high nicotine cigarette (1A4) delivers 2.87 mg nicotine and 35.0 mg tar.

Blood samples were drawn from the median antecubital vein of the subject's left arm using an indwelling needle and a 1 m infusion-extension tubing with heparin. During the 55-minute smoking-test session, twelve 10 ml samples were taken at five minute intervals for nicotine analysis. Cotinine analysis was based on plasma samples obtained before the first smoking-test session. Blood was collected in heparinized plastic tubes, immediately stored in ice water, and then centrifuged at 4°C; plasma was kept frozen at −20°C.

Plasma nicotine and cotinine were quantitated by radioimmunoassay (RIA) as described by Langone *et al.* [21]. These analyses were conducted at the American Health Foundation (Valhalla, NY), using antisera produced by injection into rabbits of trans-4-carboxycotinine and trans-3-succinylmethylnicotine bound to albumin. The use of tritiated rather than iodinated cotinine and greater antibody specificity than previously available made possible inter-assay and intra-assay coefficients of variation of 6% and a lower limit of detectability of 0.4 ng/ml for nicotine and cotinine [10].

Experimental Procedure

At an initial interview (baseline), subjects completed a demographic questionnaire (which included the Fagerström Questionnaire) and filled out a Shiffman Withdrawal Scale to provide an assessment of subjective states during unrestricted smoking of usual-brand cigarettes. Subjects were then given a supply of the research cigarettes to be smoked on a given test session and instructed to smoke only those cigarettes on the day before (to get used to them). They were told they should eat breakfast (standardized for each subject) on the test days; they were asked to abstain from cigarettes starting at 10 p.m. the night before the test session (requested overnight deprivation of approximately 12 hours).

On a test day, subjects were scheduled for 90 min starting at 10 a.m. Prior to the 55-minute test session, a half-hour acclimation period was provided, during which sensors, etc. were attached. Sessions consisted of 5 five-minute smoking trials, preceded or followed by five-minute non-smoking intervals. On each trial, cigarette availability was signaled by a light and a brief sound (Sonalert); the subject could obtain a cigarette (and a match) by pressing a manipulandum, for a maximum of five cigarettes per session. Two sessions were provided over consecutive days, one with high (2.87 mg) nicotine and one with low (0.48 mg) nicotine research cigarettes; nicotine conditions were counterbalanced across subjects to minimize order effects.

Measurements

Self-reported number of cigarettes per day and calculated nicotine exposure (number of cigarettes per day × mg of nicotine in usual cigarette) have been used as indicators of smoking intensity; their accuracy, however, is open to question [23]. Cotinine is a unique nicotine metabolite with an approximate half-life of 30 hours and is relatively insensitive to the immediate effects of smoking [22], making it especially

TABLE 1

RELATIONSHIP BETWEEN USUAL SMOKING (SELF-REPORT) AND PLASMA COTININE

Variable	Mean (±SEM) Low cotinine quartile (N=7)	Mean (±SEM) High cotinine quartile (N=7)	Mean (±SEM) All subjects (N=27)
Plasma cotinine prior to first session (ng/ml) (dependent measure)	72.1 (±13.4)	374.0 (±22.9)	213.4 (±23.2)
Number of cigarettes per day	19.9 (± 3.2)	30.3 (± 2.9)	29.0 (± 2.8)
Nicotine content of usual cigarette (mg)	1.0 (± 0.10)	1.2 (± 0.08)	1.0 (± 0.04)
Calculated nicotine exposure (number of cigarettes per day × mg nicotine per cigarette)	19.7 (± 3.7)	35.7 (± 4.2)	29.2 (± 2.5)

suitable for estimating overall levels of chronic smoking [21]. For this reason, plasma cotinine, sampled prior to the first smoking session, was selected as the principal indicator of usual nicotine intake outside the laboratory, providing an objective measure for differentiating heavier from lighter smokers.

Several indicators of nicotine addiction were examined. They were classified into categories reflecting pattern and intake, tolerance, withdrawal, and regulation. Chief among the manifestations of nicotine addiction, Fagerström [2] identified frequent smoking, high intake of nicotine, smoking more or sooner in the morning, and difficulty refraining from smoking. These variables were assessed retrospectively using the eight-item questionnaire and scoring method developed by Fagerström [2]. Scores on this inventory can vary from 0 to 11, with higher numbers interpreted as indicating greater nicotine addiction. Since highly addicted smokers were presumed to have greater difficulty restricting their smoking, plasma nicotine prior to the test session was selected as an objective indicator of compliance with the instruction not to smoke after 10 p.m. the night before (requested overnight deprivation). The sum of the plasma nicotine levels after smoking in high and low nicotine sessions was used as an objective measure of nicotine intake in the laboratory [12,31].

Tolerance, withdrawal, and plasma nicotine regulation have also been held to characterize nicotine dependence [15, 27, 33]. Heart rate is known to increase and peripheral skin temperature to decrease with the administration of nicotine [35]. Fagerström [2] reported an inverse relationship between heart-rate boost and his addiction questionnaire, suggesting that more-dependent smokers were more tolerant to nicotine. In the present study, tolerance was based on changes in both heart rate and skin temperature (from samples taken in 30-second intervals before and after smoking) per plasma nicotine increment. Greater tolerance was defined as diminished physiological reactivity per unit nicotine.

The smoking withdrawal syndrome has been difficult to define and to measure [19]. Though subjective reports of craving for tobacco, or irritability, restlessness, dullness, amnesia, and anxiety as well as impairment of concentration, judgement, and motor performance are supposed to characterize the syndrome [13], the symptoms vary considerably in intensity in individual smokers and from smoker to smoker. The Shiffman Withdrawal Scale [34] has been validated with 40 smokers abstaining for two weeks and constitutes the current standard for the subjective assessment of withdrawal. In the present study, the 25-item questionnaire was given as an entity and scored as described by Shiffman and Jarvik [34]. The questionnaire was administered at the initial interview (baseline) and before and after each smoking session. Three subscales of five questions each were selected to focus on particular aspects of the withdrawal experience. The craving subscale consisted of: 1. Would you like a cigarette, 10. Thinking of a cigarette, 14. Refuse a cigarette,* 17. Miss a cigarette, and 20. Urge to smoke. The Perception of Physical Signs Subscale consisted of 2. Heart beating faster, 6. Wakeful,* 12. Fluttery feelings in chest, 13. Hungry, and 23. Hands shaky. The Discomfort Subscale consisted of 3. Calm,* 4. Concentration,* 7. Content,* 16. Tense, and 21. Irritable. Absolute scores could vary from 5 (a rating of 1 on each of the five questions) to 35 (a rating of 7). Higher scores were interpreted as signifying greater withdrawal (*identifies questions in which negative answers were scored higher). For difference scores (e.g., pre-session scores minus baseline scores), −30 indicates the greatest decrease, 0 is no change, and +30 indicates the greatest increase.

The measurement of nicotine regulation in the present study was based on the following assumptions: Since the subjects in the present experiment usually smoked cigarettes containing an average of 1 mg of nicotine, the guidelines proposed by Kozlowski [16] suggested that the high (2.87 mg) nicotine research cigarette should bring smokers near the upper (toxic) boundary for plasma nicotine with relatively little smoking (i.e., the high nicotine cigarette would require downward compensation); on the other hand, the low (0.48 mg) nicotine cigarette was expected to bring the smokers up to the lower (withdrawal) boundary for plasma

TABLE 2

MEASURES OF NICOTINE DEPENDENCE FOR LOW AND HIGH PLASMA COTININE SUBJECTS

Variable	Mean (±SEM) Low cotinine quartile (N=7)	Mean (±SEM) High cotinine quartile (N=7)	Mann-Whitney U-test; one-tailed *$p<0.05$, †$p<0.025$, ‡$p<0.01$
Plasma cotinine (ng/ml) prior to first session (dependent measure)	72.1 ± 13.4	374.0 ± 22.8	—
Pattern and Intake			
Fagerström Questionnaire	5.3 (±1.0)	8.6 (± 0.8)	†
Pre-smoking plasma nicotine mean (high and low nicotine sessions; ng/ml)	0.4 (±0.1)	5.3 (± 1.5)	‡
Post-smoking plasma nicotine sum (high and low nicotine sessions; ng/ml)	23.5 (±6.6)	67.9 (±13.5)	‡
Tolerance			
Change in heart rate per plasma nicotine increment (before and after smoking, high nicotine session; bpm/ng/ml)	+1.39 (±0.58)	+0.34 (± 0.09)	
Change in skin temperature per plasma nicotine increment (before and after smoking, high nicotine session; °C/ng/ml)	−0.23 (±0.07)	−0.08 (± 0.03)	*
Withdrawal			
Shiffman Withdrawal Scale (difference between baseline and the mean of high and low sessions)			
Craving Subscale	+1.6 (±2.1)	+8.9 (± 2.3)	*
Perception of Physical Signs Subscale	−0.1 (±1.2)	+3.6 (± 1.7)	*
Regulation			
Post-smoking plasma nicotine difference (high and low nicotine sessions; ng/ml)	15.0 (±4.7)	37.8 (±10.3)	*
Post-smoking plasma nicotine difference divided by sum (high and low nicotine sessions)	0.70 (±0.09)	0.53 (± 0.05)	*

nicotine only with considerably more smoking (upward compensation). It was hoped that this procedure would provide a provocative test of nicotine regulation for smokers who smoked regularly but varied considerably from one another in nicotine intake. Greater responsivity to changes in plasma nicotine (nicotine regulation) was expected to be manifested by small differences in plasma nicotine levels following smoking high and low nicotine cigarettes. In the analyses that follow, plasma cotinine served as the principal dependent variable and the several measures of nicotine addiction as independent variables.

RESULTS

The relationship between self-reported indicators of usual smoking and plasma cotinine is presented in Table 1. The 7 subjects with the lowest plasma cotinine levels and the 7 with the highest levels were significantly different from one another (Mann Whitney U-test; one-tailed) with respect to number of usual-brand cigarettes per day ($p<0.05$), nicotine content of usual brand ($p<0.05$), and calculated nicotine exposure ($p<0.01$). The low and high cotinine quartiles thus define the light and heavy smoker in the present sample. The subjects were not significantly different (Mann Whitney U-test; two-tailed) from one another with respect to chronological

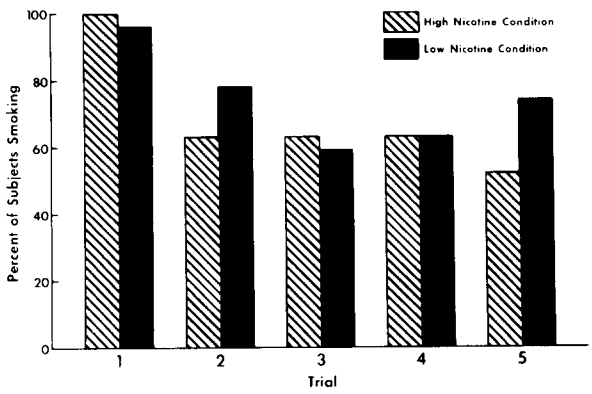

FIG. 1. Percent of subjects smoking in each five-minute trial (N=27).

age (24.4±2.2 versus 32.1±3.4 years, respectively). (Two-tailed statistical tests were used in those situations where the absence of a relationship was predicted or where the direction of the relationship between variables could not be predicted due to a lack of previous research.) Pearson Product-Moment Correlations for all 27 subjects were posi-

TABLE 3
MEASURES OF NICOTINE DEPENDENCE FOR ALL SUBJECTS

Variable	Mean (±SEM) (N=27)	Pearson correlation with plasma cotinine; *t*-test one-tailed *$p<0.025$, †$p<0.01$, ‡$p<0.001$
Plasma cotinine (ng/ml) prior to first session (dependent measure)	213.4 (±23.2)	—
Pattern and Intake		
Fagerström Questionnaire	7.3 (± 0.5)	+0.459†
Pre-smoking plasma nicotine mean (high and low nicotine sessions; ng/ml)	3.6 (± 0.8)	+0.389*
Post-smoking plasma nicotine sum (high and low nicotine sessions; ng/ml)	44.4 (± 5.8)	+0.595‡
Tolerance		
Change in heart rate per plasma nicotine increment (before and after smoking, high nicotine session; bpm/ng/ml)	+0.63 (± 0.20)	−0.470†
Change in skin temperature per plasma nicotine increment (before and after smoking, high nicotine session (°C/ng/ml)	−0.15 (± 0.05)	+0.234
Withdrawal		
Shiffman Withdrawal Scale (difference between baseline and the mean of high and low pre-sessions)		
Craving Subscale	+4.9 (± 1.1)	+0.405*
Perception of Physical Signs Subscale	+1.8 (± 0.7)	+0.281
Regulation		
Post-smoking plasma nicotine difference (high and low nicotine sessions; ng/ml)	26.1 (± 4.3)	+0.407*
Post-smoking plasma nicotine difference divided by sum (high and low nicotine sessions)	0.58 (± 0.05)	−0.290

tive though not statistically significant (*t*-test; one-tailed) between plasma cotinine and number of cigarettes (r=+0.231) or nicotine content (r=+0.290); the correlation between plasma cotinine and calculated nicotine exposure (r=+0.386) was statistically significant ($p<0.025$). The correlation between cotinine and age (r=+0.228) was not significant (*t*-test; two-tailed). These data are entirely consistent with previous reports indicating that plasma cotinine reflects the level of usual smoking [21,22].

During high-nicotine smoking sessions, the 27 subjects smoked a mean (±SEM) of 3.4 (±0.4) cigarettes and reached a mean plasma nicotine level of 35.2 (±5.0) ng/ml; in the low nicotine condition, they smoked a mean of 3.7 (±0.2) cigarettes and reached a mean plasma nicotine level of 9.2 (±1.3) ng/ml. As can be seen in Fig. 1, in the five minute period when cigarettes were first made available (trial 1), nearly all subjects smoked. On subsequent trials for both high and low nicotine conditions, the number of subjects who smoked decreased, with fewer subjects smoking at the end of the high nicotine sessions than at the end of the low nicotine sessions. The subjective effects of smoking research cigarettes were evaluated using as a baseline measurements taken when subjects were smoking their usual brand of cigarettes without restriction. Mean (±SEM) Shiffman Craving Subscale scores were significantly lower (Difference *t*-test; two-tailed, $p<0.01$) after smoking high or low nicotine research cigarettes (17.1±0.8 and 17.8±1.2, respectively), compared with baseline (22.2±1.1). Scores for Perception of Physical Signs were significantly higher ($p<0.0001$) after smoking high nicotine cigarettes (16.7±5.2) but scores were not significantly different after low nicotine cigarettes (14.9±5.2), compared with baseline (13.1±0.7). Scores for the Discomfort Subscale were not significantly different after smoking high nicotine (16.5±0.6) or low nicotine cigarettes (15.4±0.9), compared with baseline (15.3±0.6).

Table 2 compares light and heavy smokers (low and high plasma-cotinine quartile extremes) on the several measures of nicotine dependence. As can be seen, light smokers were significantly lower than heavy smokers on the variables in the Pattern and Intake Category. Light smokers were also significantly less tolerant with respect to skin temperature than heavy smokers; though light smokers were also less tolerant than heavy smokers with respect to heart rate, the difference was not statistically significant. (Similar findings for tolerance were obtained in both the high and the low nicotine conditions; since physiological data were not available for all low nicotine sessions, the tolerance category was based on the high nicotine condition.) There were no statistically significant differences (Mann Whitney U-test; two-tailed) in pre-session skin temperature for light and heavy smokers (33.4±0.9 versus 33.1±1.1°C, respectively); post-smoking skin temperatures were also not significantly differ-

ent (30.4±1.6 versus 30.4±1.3°C), despite a nearly threefold plasma nicotine increment for heavy smokers (+19.3±5.5 versus +47.6±11.4 ng/ml). Similarly, there were no statistically significant differences in pre-session heart rate for light and heavy smokers (69.3±4.9 versus 68.5±4.9 bpm, respectively); post-smoking heart rates were also not significantly different (82.1±2.7 versus 85.3±6.0 bpm). Light smokers exhibited significantly less change (smaller difference scores) on Shiffman Craving and Perception of Physical Signs Subscales after overnight deprivation than heavy smokers. Though concordant, the difference scores for light and heavy smokers were not statistically significant on the Discomfort Subscale.

Both indices of nicotine regulation differentiated light from heavy smokers significantly, though in opposite directions. Expressed as the simple difference in plasma nicotine after smoking in the high and low nicotine conditions, light smokers were shown to maintain plasma nicotine between absolute values that were less than half those for heavy smokers. When the difference was expressed in proportion to the total nicotine taken in, however, light smokers exhibited less nicotine regulation relative to intake (a larger ratio) than heavy smokers. The appropriateness of the latter as the better indicator of regulation is supported by some additional data: The ratio of the nicotine content of the high (2.87 mg) nicotine cigarette to the low (0.48 mg) nicotine cigarette was 5.98. For light smokers, mean plasma nicotine levels after smoking high nicotine cigarettes was 19.8 ng/ml and after smoking low nicotine cigarettes was 3.7 ng/ml, yielding a ratio of 5.4; for heavy smokers, mean plasma nicotine after smoking high nicotine cigarettes was 52.8 ng/ml and after smoking low nicotine cigarettes was 15.0 ng/ml, yielding a ratio of 3.5. Thus, plasma nicotine levels for light smokers reflected more closely the actual nicotine content of the cigarettes, suggesting lower relative responsivity to changes in plasma nicotine in this group. With respect to urine pH prior to the experimental sessions, there were no significant differences (Mann Whitney U-test; two-tailed) between light and heavy smokers (5.7±0.2 versus 5.8±0.2).

Table 3 examines the several measures of nicotine dependence for all 27 subjects. As can be seen, mean values for all subjects were in between values for light and heavy smokers (low and high cotinine quartile extremes). The Pearson Product-Moment Correlations for plasma cotinine were entirely concordant with the findings for the light and heavy smokers shown in Table 2. Correlations between plasma cotinine with skin temperature, Shiffman Perception of Physical Signs and Discomfort Subscales, and plasma nicotine difference divided by sum, however, were not statistically significant. A subscale analysis of the Fagerström Questionnaire revealed that the pattern questions were significantly correlated with plasma cotinine ($r=+0.342$; $p<0.05$; one-tailed) as were the intake questions ($r=+0.568$; $p<0.001$; one-tailed). There were no significant correlations (t-test; two-tailed) between pre-smoking heart rate or post-smoking heart rate with heart-rate boost; similarly, there were no significant correlations between pre-smoking skin temperature or post-smoking skin temperature with temperature decrement. There was also no significant correlation between plasma cotinine and pre-session urine pH.

The nine measures of nicotine dependence listed in Tables 2 and 3 either significantly differentiated light and heavy smokers (cotinine quartile extremes) or were significantly correlated with plasma cotinine or both. The requirement of a linear relationship between a given variable and plasma cotinine constitutes a more stringent test of the nicotine dependence hypothesis, and, for this reason, only the six variables which exhibited statistically significant correlations in Table 3 were selected for further analysis. The General Linear Model Procedure [32] was used to examine the relationship between the six variables as a group and the intensity of usual smoking as indicated by plasma cotinine. The order of each variable in the multiple regression analysis was prioritized by the strength of its correlation with plasma cotinine. The six variables accounted for 59% of the variance in plasma cotinine, $F(6,18)=4.27$, $p<0.008$. Correcting for possible overestimation caused by a relatively large ratio of independent variables to sample size [1] reduces the variance accounted for to 45%.

In order to evaluate the suggestion that light smokers experience chronic withdrawal because they restrict intake, their scores on the Shiffman Subscale prior to the experiment were compared with those of heavier smokers. Light smokers (subjects with low plasma cotinine levels) were not statistically different (Mann Whitney U-test; one-tailed) from heavy smokers at baseline (when they were smoking their usual cigarettes without restriction) with respect to Shiffman Craving (21.1±1.4 versus 19.0±2.4), Perception of Physical Signs (12.9±1.2 versus 12.1±1.0), or Discomfort Subscales (15.9±1.0 versus 14.7±1.3). For all smokers, there were no significant correlations (t-test; one-tailed) between plasma cotinine and baseline Craving, Physical Signs, or Discomfort scores.

DISCUSSION

The percentage of subjects who smoked was lower at the end of experimental sessions than at the beginning. After smoking as many as five research cigarettes for nearly an hour, the subjects indicated significantly lower Shiffman Craving Subscale scores than during baseline (when they were smoking their usual cigarettes without restriction). These findings, taken in conjunction with the observed increases in plasma nicotine after the subjects smoked high or low nicotine research-cigarettes, indicate that the research cigarettes provided some degree of satiation. The findings of significantly higher Shiffman Physical Signs scores after smoking high nicotine research-cigarettes suggests that this subscale did not differentiate nicotine toxicity from withdrawal effects. It also suggests that the upper boundary of regulation may have been approached in the high nicotine condition. In the condition with low nicotine research-cigarettes, on the other hand, Physical Signs scores were not significantly different from baseline; moreover, the percentage of subjects smoking per trial decreased in the middle of the session, then increased again at the end, suggesting that the amount of nicotine taken in from the first few low nicotine cigarettes was sufficiently close to the lower boundary of regulation to require additional smoking for maintenance of satisfactory plasma nicotine levels. These findings provide further evidence for the concept of upper and lower plasma-nicotine boundaries proposed by Kozlowski [16] and by Russell [28,29].

With respect to pattern and intake heavier smokers (subjects with higher cotinine levels) indicated significantly less ability to refrain as well as greater nicotine intake in their usual environment (Fagerström Questionnaire); they also exhibited significantly greater nicotine intake from research cigarettes in the laboratory. Pre-session plasma nicotine

levels were used as an objective indicator of the ability to follow the instruction to abstain from smoking overnight; though only one of the 27 subjects came to the laboratory with a nicotine level (14.9 ng/ml) that indicated flagrant noncompliance, and though average values were quite low for all subjects, mean pre-session plasma nicotine varied more than tenfold between cotinine quartile extremes (0.4 for the light smokers versus 5.3 ng/ml for the heavy smokers). This finding cannot be attributed simply to greater nicotine retention due to more basic pH [6], as urine pH was not significantly different between light and heavy smokers. While some pre-session elevation may have come from higher nicotine intake by heavy smokers the day before [12,26], the ratio of pre-session plasma nicotines for heavy and light smokers exceeds the ratio of their plasma nicotines or plasma cotinines, suggesting that heavy smokers did have greater difficulty abstaining completely. These results are entirely consistent with those of Fagerström [3] who reported significant positive relationships between smoking pattern, nicotine intake, and daily consumption of cigarettes (based on self-recording).

Heavier smokers were less reactive physiologically than lighter smokers, despite higher nicotine increments. In the comparison of cotinine quartile extremes, heavy smokers exhibited significantly greater tolerance than light smokers with respect to skin temperature; heart rate showed similar trends but did not differentiate heavy and light smokers significantly. When the analysis was extended to all subjects, the correlations were concordant, but only the relationship for heart-rate was significant. Since pre-smoking and post-smoking physiological measures were not significantly correlated with the amount of physiological change after smoking, neither the Law of Initial Value nor a ceiling effect [20,37] can explain the discrepancies. In the main, however, the findings are in agreement with those of Fagerström [2], who reported a positive relationship between nicotine addiction using the Fagerström Questionnaire and tolerance using heart rate as an indicator.

Subjects in the high cotinine quartile (heavy smokers) exhibited significantly greater increases on the Craving and Perception of Physical Signs Subscales, comparing unrestricted smoking of their usual cigarettes in baseline with overnight deprivation prior to the smoking sessions. Correlations for these measures with plasma cotinine were concordant, but the relationship for Physical Signs was not statistically significant. Though changes in the Discomfort Subscale corresponded to those for the other subscales, they failed to reach statistical significance, suggesting that these questions were either not pertinent or not as sensitive to the experimental manipulations. The findings are in general agreement with those of Fagerström [2] who showed a positive relationship between the Fagerström Questionnaire and an objective measure of withdrawal, decreased oral temperature, two days after the termination of smoking. Gritz and Jarvik [8] and Shiffman and Jarvik [34], however, found no consistent differences in withdrawal symptoms among various smokers and speculated that the range of nicotine intake for the subjects in their studies may have been insufficient. While the use of plasma cotinine in the present study seems to have provided a more sensitive, objective means of differentiating lighter from heavier smokers, the measures of withdrawal used here could be improved upon. Objective indicators of nicotine deprivation, such as decreased oral temperature [2] or increased Masseter EMG potentials [11], might provide a useful method for validating responses to subjective inventories such as the Shiffman Withdrawal Scale.

Two indicators of nicotine regulation were used: (a) the difference in plasma nicotine after smoking high versus low nicotine research-cigarettes and (b) the difference relative to the sum of the plasma nicotines. Both significantly differentiated plasma cotinine quartile extremes (light from heavy smokers); correlations for these measures with plasma cotinine were concordant, though only the relationship for the former was significant. Examination of the ratios for plasma nicotine after smoking research-cigarettes showed that subjects in the low cotinine quartile allowed plasma nicotine to reflect more closely the actual nicotine content of the research cigarettes, whereas heavy smokers adjusted their nicotine intake to maintain closer plasma nicotine levels. For this reason, (b) seems preferable as an indicator for expressing differential regulation in light and heavy smokers. The present experiment shows that degree of nicotine regulation varies widely in regular, presumably dependent smokers. While the data indicate that heavier smokers maintained plasma nicotine within narrower limits relative to total intake, regulation was far from perfect, in keeping with theoretical predictions by Kozlowski [16].

In the study on regulation closest to the present design in which plasma nicotine was measured following smoking, Russell *et al.* [29] reported that they observed nearly identical plasma nicotine values when smokers were shifted from their usual (1.34 mg nicotine) cigarette to a high (3.20 mg) nicotine cigarette; plasma nicotine fell precipitously, however, when low (0.14 mg) nicotine cigarettes were introduced. Russell *et al.* explained the latter finding by suggesting that the low nicotine cigarette probably contained too little nicotine to permit adequate regulation. In a well-controlled study, Godlfarb *et al.* [7] attempted to manipulate the tar and nicotine content of cigarettes independently; though they did not measure plasma nicotine directly, Goldfarb *et al.* did demonstrate that while number of cigarettes smoked per day was unaffected by tar content, number of cigarettes smoked increased significantly as nicotine content was decreased. Neither of the above studies differentiated light from heavy smokers, nor did they establish whether there were differences in nicotine dependence among subjects. Two recent studies by Fagerström [3,4] claim to have demonstrated a positive relationship between nicotine dependence and nicotine titration in regular smokers, but the specification of the relationship is limited by the absence of plasma nicotine data. As was mentioned above, in two studies in which nicotine dosage was manipulated by changing the length of usual cigarettes [30,36], the investigators were unable to demonstrate a relationship between compensation for decreased nicotine availability and usual smoking intake; the fact that usual smoking was not specified by an objective indicator such as cotinine and that the number of subjects in the studies was relatively small, however, may have obscured critical inter-relationships. The present findings suggest that, in addition to general difficulties posed by the susceptibility of smokers to extraneous cues for smoking and by the quantitation of nicotine and its metabolites in biological fluids [25], a major obstacle to the adequate demonstration of nicotine regulation in the past may have come from attempting to base conclusions on calculations of nicotine titration and compensation which combined data from smokers who varied in degree of dependence.

As a whole, the present study supports the hypothesis

that not only do heavier smokers smoke more, but they are also more nicotine-dependent than lighter smokers. The six variables that showed significant linear relationships with plasma cotinine accounted for about half of the plasma cotinine variance in the General Linear Model. The implication is not that these variables constitute unique indicators of nicotine dependence, but rather that they are representative measures of pattern, intake, tolerance, withdrawal, and regulation.

The present findings do not support Schachter's proposal [33] that light smokers, who are presumed to be actively restricting their nicotine intake, manifest greater withdrawal symptoms as a consequence. There were no significant relationships between plasma cotinine (intensity of usual smoking) and any of the Shiffman Withdrawal Subscales at baseline when subjects were smoking their usual-brand cigarettes. Furthermore, if light smokers were "dieters" who are as nicotine dependent as heavy smokers, they should not have exhibited significantly less tolerance and regulation along with a greater ability to refrain from smoking in the experimental setting. The present findings are entirely consistent with the conclusions of a recent study by Kozlowski *et al.* [17], also contradicting Schachter's hypothesis: Smokers who delayed the first cigarette of the day were found to be avoiding a cigarette they found noxious rather than simply postponing the smoking of a desired cigarette; moreover, smokers who delayed were found to be significantly less dependent on tobacco than early-morning smokers, using success in quitting in a smoking-cessation program as a criterion.

The research described here constitutes an attempt to construct a model of smoking which incorporates various measures of nicotine dependence previously studied in isolation. Though the model appears coherent and parsimonious, replication is desirable and several refinements and extensions might be made. Multiple sessions in a repeated measures design with cigarette nicotine-content as a parameter could provide more accurate specification of key variables in nicotine dependence. Similarly, longer sessions and the use of several different nicotine conditions should help establish the upper and lower boundaries of nicotine regulation more precisely. Finally, though multivariate and multiassay procedures are prohibitively expensive as the number of measures and samples increases, the use of plasma cotinine and/or the Fagerström Questionnaire could provide a relatively inexpensive solution to the problem of characterizing critical aspects of smoking in larger populations.

REFERENCES

1. Cohen, J. and P. Cohen. Shrinkage of the multiple correlation and cross-validation. *Applied Multiple Regression/Correlation Analyses for the Behavioral Sciences*. New York: John Wiley, 1975, pp. 282–283.
2. Fagerström, K. Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. *Addict Behav* **3:** 235–241, 1978.
3. Fagerström, K. Effects of a nicotine enriched cigarette on nicotine titration, daily cigarette consumption, and levels of carbon monoxide, cotinine, and nicotine. *Psychopharmacology (Berlin)* **77:** 164–167, 1982.
4. Fagerström, K. and S. Bates. Compensation and effective smoking by different nicotine dependent smokers. *Addict Behav* **6:** 331–336, 1981.
5. Feyerabend, C., T. Levitt and M. Russell. A rapid gas-liquid chromatographic estimation of nicotine in biological fluids. *J Pharm Pharmacol* **27:** 434–436, 1975.
6. Feyerabend, C. and M. Russell. Effect of urinary pH and nicotine excretion rate on plasma nicotine during cigarette smoking and chewing nicotine gum. *Br J Clin Pharmacol* **5:** 293–297, 1978.
7. Goldfarb, T., E. Gritz, M. Jarvik and I. Stolerman. Reactions to cigarettes as a function of nicotine and "tar." *Clin Pharmacol Ther* **19:** 767–772, 1976.
8. Gritz, E. and M. Jarvik. Preliminary study: Forty-eight hours of abstinence from smoking. *Proc Am Psychol Assoc* **1:** 1039–1040, 1973.
9. Henningfield, J. and R. Griffiths. A preparation for the experimental analysis of human cigarette smoking behavior. *Behav Res Methods Instr* **11:** 538–544, 1979.
10. Hill, P., N. Haley and E. Wynder. Cigarette smoking as a risk for cardiovascular disease: 1. Biochemical analyses carboxyhemoglobin, plasma nicotine, cotinine, and thiocyanate versus self-report smoking data. *J Chronic Dis*, in press, 1983.
11. Hutchison, R. and G. Emley. Effects of nicotine on avoidance, conditioned suppression and aggression response measures in animals and man. In: *Smoking Behavior: Motives and Incentives*, edited by W. Dunn. Washington: Winston, 1973, pp. 171–196.
12. Isaac, P. and M. Rand. Cigarette smoking and plasma levels of nicotine. *Nature* **236:** 308–310, 1972.
13. Jaffe, J. and M. Jarvik. Tobacco use and the tobacco use disorder. In: *Psychopharmacology: A Generation of Progress*, edited by M. Lipton, A. DiMascio and K. Killam. New York: Raven Press, 1978.
14. Jaffe, J. and M. Kanzler. Smoking as an addictive disorder. In: *Cigarette Smoking as Dependence Process*, (NIDA Monograph 23), edited by N. Krasnegor. Rockville, MD: National Institute on Drug Abuse, 1979, pp. 4–23.
15. Jarvik, M. Biological factors underlying the smoking habit. In: *Research on Smoking Behavior*, (NIDA Research Monograph 17), edited by M. Jarvik, J. Cullen, E. Gritz, T. Vogt and L. West. Rockville, MD: National Institute on Drug Abuse, 1977, pp. 122–148.
16. Kozlowski, L. The role of nicotine in the maintained use of cigarettes. *Drug Merchandizing* (Canada), January: 36–43, 1980.
17. Kozlowski, L., J. Director and M. Harford. Tobacco dependence, restraint and time to the first cigarette of the day. *Addict Behav* **6:** 307–312, 1981.
18. Kumar, R., E. Cooke, M. Lader and M. Russell. Is nicotine important in tobacco smoking? *Clin Pharmacol Ther* **21:** 520–529, 1977.
19. Kumar, R. and M. Lader. Nicotine and smoking. In: *Current Developments in Psychopharmacology*, vol 6, edited by W. Essman and L. Valzelli. New York: Spectrum, 1981, pp. 127–163.
20. Lacey, J. The evaluation of autonomic response: Toward a general solution. *Ann NY Acad Sci* **67:** 123–164, 1956.
21. Langone, J., H. Gjika and H. Van Vunakis. Nicotine and its metabolites: Radioimmunoassays for nicotine and cotinine. *Biochemistry* **24:** 5025–5030, 1973.
22. Matsukura, S., N. Sakamoto, Y. Seino, T. Tamada, H. Matsuyama and H. Muranaka. Cotinine excretion and daily cigarette smoking in habituated smokers. *Clin Pharmacol Ther* **25:** 555–561, 1979.
23. Moss, R. and D. Prue. Research on nicotine regulation. *Behav Ther* **13:** 31–46, 1982.
24. Pomerleau, O. Why people smoke: Current psychobiological models. In: *Behavioral Medicine: Changing Health Lifestyles*, edited by P. Davidson. New York: Pergamon Press, 1980.

25. Pomerleau, O. Underlying mechanisms in substance abuse: Examples from research on smoking. *Addict Behav* **6:** 187–196, 1981.
26. Rosenburg, J., N. Benowitz, P. Jacob and M. Wilson. Disposition kinetics and effects of intravenous nicotine. *Clin Pharmacol Ther* **28:** 517–522, 1980.
27. Russell, M. Smoking problems: An overview. In: *Research on Smoking Behavior*, (NIDA Monograph 17), edited by M. Jarvik, J. Cullen, E. Gritz, T. Vogt and L. West. Rockville, MD: National Institute on Drug Abuse, 1977, pp. 13–34.
28. Russell, M. Self-regulation of nicotine intake by smokers. In: *Behavioral Effects of Nicotine*, edited by K. Bättig. Basel, Switzerland: S. Karger, 1978, pp. 108–122.
29. Russell, M., S. Sutton, C. Feyerabend and P. Cole. Addiction Research Unit: Nicotine titration studies. In: *Smoking Behavior*, edited by R. Thornton. London: Churchill Livingstone, 1978, pp. 336–348.
30. Russell, M., S. Sutton, C. Feyerabend and Y. Saloojee. Smokers' response to shortened cigarettes: Dose reduction without dilution of tobacco smoke. *Clin Pharmacol Ther* **27:** 210–218, 1980.
31. Russell, M., C. Wilson, V. Patel, P. Cole and C. Feyerabend. Plasma nicotine levels after smoking cigarettes with high, medium and low nicotine yields. *Br Med J* **2:** 414–416, 1975.
32. SAS Institute, *SAS User's Guide, 1982 edition*. Cary, NC: SAS Institute, 1982, pp. 139–199.
33. Schachter, S. Pharmacological and psychological determinants of smoking. *Ann Inter Med* **88:** 104–114, 1978.
34. Shiffman, S. and M. Jarvik. Smoking withdrawal symptoms in two weeks of abstinence. *Psychopharmologia* **50:** 35–39, 1976.
35. Stephens, R. Psychophysiological variables in cigarette smoking and reinforcing effects of nicotine. *Addict Behav* **2:** 1–7, 1977.
36. Sutton, S., M. Russell, C. Feyerabend and Y. Saloojee. Smokers' response to dilution of smoke by ventilated cigarette holders. In: *Smoking Behavior*, edited by R. Thornton. London: Churchill Livingstone, 1978, pp. 330–335.
37. Wilder, J. The Law of Initial Value in neurology and psychiatry. *J Nerv Ment Dis* **125:** 73–86, 1957.