

Marijuana Smoking: Effects of Varying Puff Volume and Breathhold Duration¹

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ABSTRACT

Two studies were conducted to quantify biological and behavioral effects resulting from exposure to controlled doses of marijuana smoke. In one study, puff volume (30, 60 and 90 ml) and in a second study, breathhold duration (0, 10 and 20 sec) were systematically varied while holding constant other smoking topography parameters (number of puffs = 10, interpuff interval = 60 sec and inhalation volume = 25% of vital capacity). Each study also varied levels of Δ^9 -tetrahydro-cannabinol marijuana cigarette content (1.75% and 3.55%). Regular marijuana users served as subjects ($n = 7$ in each experiment). Subjects smoked 10 puffs in each of six sessions; a seventh, nonsmoking session (all measures recorded at the same times as in active smoking sessions) served as a control. Variations in puff volume produced significant dose-related changes in

postsmoking plasma Δ^9 -tetrahydro-cannabinol levels, carbon monoxide boost and subjective effects (e.g., "high"). In contrast, breathholding for 10 or 20 sec versus 0 sec increased plasma Δ^9 -tetrahydro-cannabinol levels but not CO boost or subjective effects. Task performance measures were not reliably influenced by marijuana smoke exposure within the dosing ranges examined. These findings confirm the utility of the controlled smoking technology, support the notion that cumulative puff volume systematically influences biological exposure and subjective effects, but cast doubt on the common belief that prolonged breathholding of marijuana smoke enhances classical subjective effects associated with its reinforcing value in humans.

Marijuana smoking is currently the most prevalent form of illicit drug self-administration in the United States (Harris and Martin, 1991). The acute psychoactive effects of marijuana smoking (e.g., subjective euphoria, tachycardia, impaired performance) have been characterized in many studies (Mendelson, 1987), but precise control over dose delivery has rarely been attempted. Measuring the effects of inhaled drugs is technically challenging: dose of an inhaled substance can be controlled by extracting and administering the active ingredient, by having the smoker inhale a known premeasured volume and concentration of smoke or by controlling the relevant parameters of smoking behavior (Pomerleau *et al.*, 1989). Marijuana smoking involves a complex chain of behaviors that differs from tobacco smoking (Wu *et al.*, 1988) but, as with tobacco smoking, adjustments in smoking style (e.g., puff size) may be expected to produce variations in dose delivery. Precise determination of dose delivery requires

knowing the interactive effects of smoking parameters as well as cigarette THC content, and must be accompanied by some measure of biological exposure.

To control marijuana dose, it is necessary to know which parameters of smoking behavior are important for determining dose. Previous research with tobacco smoking has identified cumulative puff volume as an important determinant of exposure dose, but neither inhalation volume nor breathhold duration influenced postsmoking levels of nicotine exposure (Zacny *et al.*, 1987). Although partial control over marijuana smoking behavior has been attempted in paced smoking protocols (e.g., Barnett *et al.*, 1985; Marks and MacAvoy, 1989; Moskowitz and McGlothlin, 1974), only one previous study (Tashkin *et al.*, 1991a) has examined the effect of puff volume on marijuana exposure. Some altered subjective responses were observed, but the study varied total dose over a relatively limited range. Effects of breathhold time are of particular interest with marijuana because smokers engage in a stereotypical pattern of extended breathholding, presumably to maximize THC absorption. Three previous studies have examined breathhold duration effects. One study showed enhanced exposure with longer breathholds on measures of CO, HR and THC levels (Tashkin

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ABBREVIATIONS: THC, Δ^9 -tetrahydrocannabinol; CO, carbon monoxide; COHb, carboxyhemoglobin; ppm, parts per million; VC, vital capacity; HR, heart rate; ANOVA, analysis of variance.

et al., 1991a) whereas two other studies found no reliable effect on CO or HR indices of biological exposure (Zacny and Chait, 1989, 1991).

Recently, Azorlosa *et al.* (1992) reported a study of marijuana smoking in which all major puff and inhalation parameters were carefully controlled using a computerized feedback system (Zacny *et al.*, 1987). They varied numbers of puffs (4, 10, 25) and cigarette THC concentration (1.75% and 3.55%) while holding constant puff volume (60 ml), inhalation volume (25% of VC), breathhold duration (10 sec) and interpuff interval (60 sec) and established dose-response effects across a range of biological exposure and behavioral variables. In that study, clear dose-effects were observed on plasma THC levels with peak exposure of 268 ± 47 ng/ml in the highest dose condition (25 puffs from cigarettes containing 3.55% THC). In general, subjective reports were more sensitive to increasing marijuana smoke exposure than were measures of psychomotor performance.

The present two studies used this controlled smoking method to examine the effects of varying puff volume, breathhold duration and cigarette THC concentration on plasma THC levels, CO exposure, HR boost, subjective reports and psychomotor performance measures. In each study, the smoking method was identical: subjects smoked 10 puffs from marijuana cigarettes containing 1.75% or 3.55% THC. In study 1, subjects took different-sized puffs (30, 60 or 90 ml) and held their breath for 10 sec. In study 2, subjects took 60-ml puffs and held their breath for 0, 10 or 20 sec. Puff volumes were selected to span the range of values observed with *ad libitum* smoking in previous laboratory studies that have measured this parameter (Heishman *et al.*, 1989; Herning *et al.*, 1986; Wu *et al.*, 1988). Breathhold durations observed during *ad libitum* smoking have typically been 10 to 15 sec (Heishman *et al.*, 1989; Wu *et al.*, 1988). The two studies reported extend previous reports by varying puff volume and breathhold duration over a range typical of normal marijuana smoking, while simultaneously controlling other smoking and inhalation parameters, and measuring biological and behavioral effects resulting from varying these smoking behaviors.

General Methods

Subject recruitment and screening. Recreational drug users were recruited through newspaper advertisements and were paid \$10.00/h. Before the study, subjects were medically examined and interviewed about current and past psychoactive substance use. Only generally healthy, regular marijuana users were included. Subjects were excluded if they reported current illicit drug use at screening, but the majority had experience with other illicit drugs.

Experimental design and procedure. Before the study, subjects practiced a set of computerized psychomotor tasks for 1 to 2 h until stable performance was achieved. They were also trained to achieve the target puffing and inhalation behaviors using placebo marijuana cigarettes during one or two sessions of smoking practice. Subjects then participated in seven experimental sessions, including six marijuana controlled smoking sessions and one no smoking control session. During the control session, all measures were taken at the same times used in the active smoking sessions. Conditions were presented in a counterbalanced order according to a Latin square design. Subjects and staff were blind to THC cigarette content. Subjects were instructed not to drink alcohol for 24 h or smoke marijuana for 48 h before sessions, not to smoke tobacco for 1 h before sessions, and to abstain from other illicit drug use during the

study. To encourage compliance, subjects were given urine and breathalyzer tests before each session. Sessions were separated by at least 48 h.

At the start of each session, HR and respiratory monitoring equipment were attached to the subject and an i.v. catheter was inserted in an antecubital vein. The first blood sample was obtained, a computerized battery of subjective reports and performance measures was completed and presmoking HR and expired CO levels were recorded. Smoking then began and, shortly after the last puff, blood and expired CO samples were collected. Postsmoking HR was measured and the battery of subjective reports and performance measures was completed. This entire set of measurements was repeated at 15, 30 and 45 min postsmoking. Subjects remained in the laboratory under staff observation until they were no longer intoxicated.

Marijuana cigarettes. Marijuana cigarettes were supplied by the National Institute on Drug Abuse Research Technology Branch. These cigarettes were approximately 85 mm (length) \times 25 mm (circumference), weighed from 750 to 900 mg, and contained either 1.75% or 3.55% THC, as assayed by the National Institute on Drug Abuse Research Technology Branch. Cigarettes were stored in a -20°C freezer before use. At least 12 h before each smoking session, moisture content of the cigarettes was raised by placing them above a saturated NaCl solution in a closed humidifier at room temperature. In each session, the subject smoked from two cigarettes (five puffs each cigarette) that were lighted by the experimenter.

Smoking topography measures. The smoking topography system, originally developed for use with tobacco cigarettes, has been described in detail elsewhere (Zacny *et al.*, 1987). An Apple IIe microcomputer recorded the following puffing and respiratory parameters: interpuff interval, puff duration, puff volume, inhalation volume and inhalation duration. The cigarettes were inserted into a plastic mouthpiece modeled after an ADL dosimeter (Arthur D. Little, Inc., Cambridge, MA). This was connected to a pressure-sensitive switch that detected puff onset and offset. The mouthpiece was also connected to a pressure transducer that tracked rate of smoke flow through the mouthpiece. Voltage output of the pressure transducer was linearly related to flow rate ($r = 0.98$); smoke flow was integrated on-line over the duration of the puff, yielding puff volume. The system was calibrated daily by drawing 50 ml of air from an unlit tobacco cigarette into a syringe. If the measured puff volume differed by more than 3 ml, the system was adjusted.

Respiratory parameters were measured with a respiratory inductive plethysmograph (Respirace; Non-Invasive Monitoring Systems, Inc., Ardsley, NY). Elastic cloth bands with induction coils were placed around the subject's thorax and abdomen and connected to the Respirace. Breathing produced a changing electrical signal, which was digitized and used by the Apple IIe to determine inhalation volume. The relationship between chest movements produced by breathing and actual inhalation volumes was determined before each session by having the subject breathe in and out of an 800-ml plastic expandable bag. To control for different lung sizes, inhalation volumes used in the sessions were based on each subject's percent of VC, with VC determined by having subjects inhale as deeply as possible and exhale into a water spirometer (Vitalometer; Warren E. Collins, Inc., Boston, MA).

Control over puff and inhalation volumes and breathhold duration was accomplished by means of a feedback system. When the required puff volume was reached, the Apple IIe sounded a tone, which signaled the subject to stop the puff and start inhaling. When the required inhalation was reached, a second tone signaled the subject to stop inhaling and to start the breathhold. A third tone signaled the subject to exhale. In each smoking session, the subject took 10 puffs of specified volumes spaced at 60-sec intervals (timed by the experimenter), inhaling smoke to a depth of 25% of VC, and holding the smoke for a specified time before exhaling. Inhalation duration was measured as the time (in sec) from the start of inspiration to 25% VC. Lung exposure duration was calculated as the time (in sec) from the start of inspiration to end-tidal volume (*i.e.*, the sum of inhalation,

breathhold and exhalation durations). Although subjects were not specifically trained to control inhalation times, these were relatively constant across conditions within each study (see tables 1 and 3). Inhalation constituted the majority of lung exposure time other than breathholding (*i.e.*, exhalation times were negligible).

CO. Expired air CO samples were obtained by having subjects fully inhale, exhale, inhale again, hold their breath for 15 sec, and then exhale successively into two 1-l polyvinyl bags. The CO content of the second bag was measured in parts per million with an Ecolyzer 2000 (Energetics Science, Elmsford, NY). CO levels were measured before and immediately after smoking. CO boost was calculated as the presmoking to postsmoking change in expired breath CO levels. Alveolar CO boost is a standard method for measuring amount of smoke intake and consistent with many other studies in the literature. However, its reliability as an indicator of presmoking to postsmoking changes in COHb has been questioned when the measure is taken within 30 min after smoking (Guyatt *et al.*, 1988).

HR. HR was measured continuously using three silicon EKG electrodes (NDM Corp., Dayton, OH) placed on the right deltoid muscle and second and fifth intercostal space. The electrodes were attached to an EKG monitor and Schmitt trigger, which sent a pulse to the Apple IIe at the start of each R-wave. The computer timed the interval between pulses and computed an average rate each minute. HR data were reduced to 5-min averages beginning 10 min before smoking and from 0 to 55 min postsmoking. HR elevations remained stable over the first 40 min after smoking and then began to decline. Therefore, HR change was calculated by subtracting the average of the 5 min immediately before smoking from the mean across the first 40 min postsmoking.

Plasma THC. Five blood samples (5 ml each) were collected in each session—at presmoking baseline, and at 0, 15, 30 and 45 min postsmoking. After each session, plasma was separated and immediately frozen. All plasma samples were sent in a single batch to the Research Triangle Institute (Research Triangle Park, NC) for radioimmunoassay of Δ^9 -THC content in ng/ml (Cook *et al.*, 1982). Inter-assay reliability was within 5% when standards were run at 8.0 ng/ml and 30.0 ng/ml, respectively.

Subjective effects. Subjective ratings on 10 separate dimensions (high, stoned, drunk, impaired, energetic, clear-headed, anxious, sluggish, confused and relaxed) were measured using a 100-point visual analog scale on the video monitor of the Apple IIe computer. The 15-cm horizontal line was marked "Not at all" on the left and "Extremely" on the right, and subjects responded by moving a cursor along the line with a joystick. Immediately after each smoking session, subjects also rated on visual analog scales the taste ("terrible" to "great"), harshness ("not at all" to "extremely"), draw ("very hard" to "very easy"), and potency ("no drug effect" to "very strong drug effect") of the cigarettes. Finally, subjects compared the strength of their experimental drug effect with effects from naturalistic smoking on a 100-point scale (0 = "much weaker," 50 = the "same" and 100 = "much stronger"). These measures were taken at presmoking and at 0, 15, 30 and 45 min postsmoking. Subjective effects were stable across postsmoking assessment times; therefore, these data were averaged in the reporting of results. Peak subjective effects did not appreciably differ from the averaged data.

Psychomotor performance. Three computerized tasks were performed at presmoking baseline and at 0, 15, 30 and 45 min postsmoking: 1) Forward and Reverse Digit Span, a measure of memory recall; 2) Digit Symbol Substitution Test provided measures of encoding speed and accuracy; and 3) Divided Attention, which involves simultaneous motor tracking and visual detection. These measures have been described in detail elsewhere (Azorlosa *et al.*, 1992).

Data analysis. Smoking topography data were collected only during the six smoking sessions and were analyzed with a two-factor [puff volume (study 1) or breathhold duration (study 2) \times cigarette potency] repeated measures ANOVA. All smoking topography measures were averaged over the 10 puffs in each session. Presmoking

and postsmoking plasma THC, CO, HR, subjective report and psychomotor performance data were first analyzed using an overall 7 condition \times session time ANOVA (*i.e.*, all active smoking conditions and the no smoking control condition were included). This overall analysis determined whether there was a significant difference between the no smoking control condition and at least one of the marijuana smoking conditions. *Post hoc* Tukey tests from this analysis were used to compare individual condition means. A second two-factor, 3 [parameter: puff volume (study 1) or breathhold duration (study 2)] \times 2 (cigarette potency) ANOVA, which excluded the no smoking condition and session time factor, was conducted to determine whether there were dose-related effects of the main smoking parameters on biological and behavioral measures. Data for this analysis were the first postsmoking value obtained for plasma THC and CO boost; average session data were used for HR boost and subjective effects, because these measures remained elevated throughout the session. Huynh-Feldt adjusted significance levels are reported for the repeated measures analysis to correct for violations of sphericity. Statistical results were considered significant at $P < .05$.

Study 1: Puff Volume

Puffing is the first and probably the most important step in extracting active drug from the cigarette. Puff volume determines exactly how much smoke and (with a known concentration of THC) how much active drug is available for absorption into body tissue. Inasmuch as THC is highly lipophilic and thus readily absorbed, biological exposure should be directly related to cumulative puff volume. One previous study (Tashkin *et al.*, 1991a) examined the effect of puff volume on biological exposure and subjective reports. However, this study used a limited range of cumulative puff volumes, measured only a single subjective dimension (drug "high") and did not assess performance effects. Our purpose was to determine the effect of a wider range of puff volumes on biological and behavioral effects, using a more extensive assessment battery.

Methods

Subjects. The seven participants (five white, two African-American) were healthy males ranging in age from 19 to 37 yr (mean = 25.1, S.E. = 5.9) with educational levels ranging from 11 to 16 yr (mean = 14.1, SE = 0.6). Current reported use of marijuana ranged from 2 to 15 cigarettes/wk (mean = 6.9, SE = 1.8). All subjects reported current use of tobacco (averaging about one pack/day), alcohol (averaging about six beers/wk). All subjects reported past lifetime use of cocaine; two subjects had used cocaine within 6 months of the study. Three subjects reported past sporadic illicit use of opiates and barbiturates. Four subjects reported using LSD in the past (two subjects within 6 months of the study); two subjects reported past sporadic illicit use of diazepam, inhalants and amphetamine. No subject tested urine-positive for any illicit drug other than marijuana during the study.

Procedure. During each of the six active marijuana smoking sessions, subjects took 10 puffs of 30, 60 or 90 ml volume from cigarettes with 1.75% or 3.55% THC concentration (*i.e.*, one cigarette potency \times puff volume combination was tested in each session) and measures were taken before and after smoking as previously described.

Results

Controlled smoking. Smoking topography measures for each condition are presented in Table 1. Actual puff volumes

TABLE 1
Marijuana smoking topography: puff volume study^a

Topography Measure	Cigarette Potency					
	1.75% THC			3.55% THC		
	Puff volume (ml)			Puff volume (ml)		
	30	60	90	30	60	90
Puffing parameters						
Interpuff interval (sec)	60.3 (0.2)	60.0 (0.1)	60.2 (0.2)	60.2 (0.1)	60.1 (0.1)	60.1 (0.1)
Puff duration (sec)	1.5 (0.1)	2.9 (0.3)	5.0 (0.5)	1.7 (0.1)	3.1 (0.2)	4.6 (0.6)
Puff volume (ml)	30.5 (0.4)	60.4 (0.4)	90.4 (0.4)	30.6 (0.2)	60.4 (0.3)	90.4 (0.2)
Respiratory parameters						
Inhalation volume (% VC)	25.6 (0.6)	24.8 (0.6)	26.2 (0.9)	25.3 (0.5)	26.5 (0.9)	24.6 (0.7)
Inhalation duration (sec)	4.4 (0.8)	5.0 (1.0)	5.1 (1.1)	4.5 (0.5)	5.0 (0.9)	5.2 (0.6)
Lung exposure duration (sec)	14.3 (0.3)	14.0 (0.2)	14.1 (0.5)	14.2 (0.2)	14.3 (0.2)	14.3 (0.2)

^a Sample mean ($n = 7$, S.E. below in parentheses) topography measures for the six marijuana dosing conditions: 30, 60 and 90 ml puffs from cigarettes containing 1.75% and 3.55% THC.

closely approximated the designated 30-, 60- and 90-ml target conditions, thereby supporting the effectiveness of the manipulation, $F(2,12) = 17,129$. Puff duration (not controlled by the computer) increased linearly with puff volume, $F(2,12) = 46.33$. Other smoking parameters did not significantly differ across experimental conditions. Thus, the feedback procedures were effective in controlling subjects' smoking behavior.

CO boost. There was a significant mean CO boost immediately after marijuana smoking, relative to a small decrease in the no smoking session, Condition $F(6,36) = 3.05$ (fig 1, middle panel). *Post hoc* tests confirmed that both 90-ml puff conditions (high and low potency cigarettes) produced significantly greater CO boost than the 30-ml low THC condition. A separate ANOVA excluding the no smoking session revealed that magnitude of the CO boost was significantly related to puff volume, $F(2,12) = 11.36$, but not to cigarette potency, $F(1,6) = 1.53$ (N.S. interaction).

HR. After smoking marijuana, HR generally accelerated from session baseline, peaking at 15 to 20 min and remaining elevated until 40 min postsmoking; no significant elevations were observed in the no smoking session. Mean HR boost was significantly greater for marijuana smoking than no smoking sessions, Condition $F(6,36) = 13.42$, but there was no differential HR boost between marijuana conditions (data not shown). A separate ANOVA excluding the no smoking condition found that increases were not significantly related to either puff volume, $F(2,12) = 1.04$, cigarette potency, $F(1,6) = 1.23$, or the interaction, $F(2,12) = 2.34$. Inspection of the data revealed that the lack of orderly relationships for HR increase was due in large part to high baseline values for some subjects that suggested unreliable measurement.

Plasma THC. Table 2 shows mean plasma THC levels obtained at each sample collection time for each marijuana dose condition, whereas the first postsmoking values are depicted in figure 1 (top panel). Within each cigarette potency condition, plasma levels increased systematically as puff volume increased. In all marijuana conditions, THC levels were maximal immediately after smoking and decreased rapidly.

At 45 min postsmoking, levels declined to 20% of peak values. Analysis of THC levels immediately after smoking indicated a significant condition effect, $F(6,36) = 41.71$. Mean THC values ranged from 33 ng/ml (30 ml, low potency) to 164 ng/ml (90 ml, high potency). *Post hoc* analysis indicated that all but the 30-ml low potency condition yielded significantly greater plasma THC levels than the no smoking session. Highly similar plasma levels were observed in the 90-ml low potency and 60-ml high potency conditions (120.3 and 124.7 ng/ml, respectively), and in the 60-ml low potency and 30-ml high potency conditions (72.3 ng/ml and 64.9 ng/ml, respectively). Figure 1 (upper right) suggests that THC levels immediately postsmoking were clearly related to both puff volume and cigarette potency. A separate ANOVA, which excluded the no smoking condition, confirmed a significant puff volume effect, $F(2,12) = 47.43$, and potency effect, $F(1,6) = 52.19$ (N.S. interaction).

Subjective effects. Significant condition effects were obtained for 3 of the 10 analog scale items (high, stoned and impaired; $F_s(6,36) = 7.73, 7.91$ and 6.36 , respectively) in the seven condition ANOVA (condition \times time interactions, N.S.). Figure 1 (bottom panel) shows subjective ratings of "high." *Post hoc* analysis indicated that for subjective "high," all 3.55% cigarette potency conditions (30-, 60- and 90-ml puffs) and the 90-ml 1.75% potency condition produced increases that were significantly greater than the no smoking control. The 30-ml low potency condition did not differ from control for any subjective rating. In the supplemental six-condition ANOVA, "high" ratings were significantly related to both puff volume, $F(2,12) = 4.69$, and cigarette potency, $F(1,6) = 13.23$ (N.S. interaction). Subjective ratings of "stoned" and "impaired" showed similar patterns of means and statistical outcomes.

Subjects compared the strength of their experimental marijuana experience with their naturalistic experience (a rating of 50 means the same). This measure, which was obtained only in the smoking sessions, was dose-related to both cigarette potency, $F(1,6) = 7.32$, and puff volume, $F(2,12) = 13.66$ (N.S. interaction). *Post hoc* tests from this analysis indicated that the

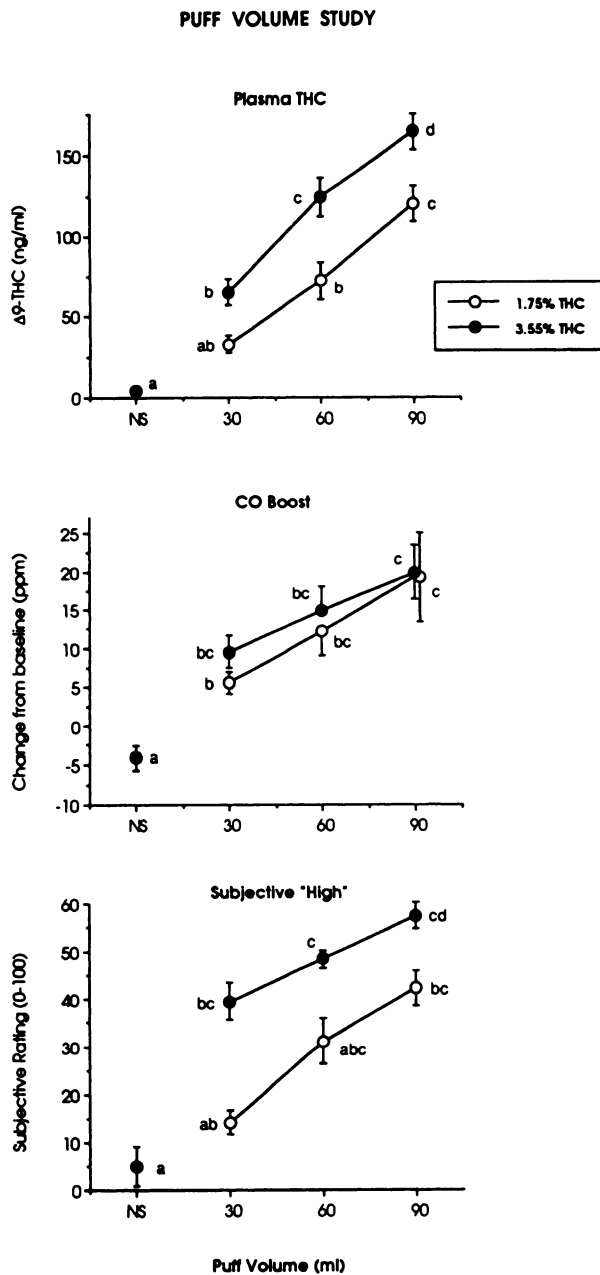


Fig. 1. Plasma THC level immediately after smoking (top), expired air CO increase from presmoking baseline to immediately after smoking (middle), and postsmoking subjective "high" (bottom) as a function of puff volume and cigarette THC content. NS on the x-axis indicates the no smoking control session. Each data point represents the mean \pm S.E. of seven subjects. If the S.E. is not visible, it was less than the radius of the symbol. Lower case letters next to data points indicate Tukey *post hoc* test results from the overall (7 condition) analysis. Data points not sharing a common letter are significantly different ($P < .05$); those with common letters are not significantly different.

90-ml high potency condition was rated as equal to subjects' naturalistic experience (mean = 51 ± 10). The 60-ml and 90-ml low potency and 60-ml high potency conditions were also rated as similar to naturalistic experience (mean = 40 ± 10 , 40 ± 9 and 39 ± 10 , respectively). In contrast, effects of 30-ml puffs were rated as significantly weaker than effects obtained during naturalistic smoking (mean = 29 ± 10 and 9 ± 4 for high and low potency cigarettes, respectively).

Psychomotor performance. There were no significant effects of the experimental conditions on the psychomotor performance measures.

Study 1: Summary

As expected, varying marijuana dose by manipulating puff volume produced linear changes in CO boost, plasma THC levels, and subjective reports. Inasmuch as puffing is the first step in smoking, and because other topographic variables (*i.e.*, inhalation volume and duration, breathhold duration and interpuff interval) were controlled in this study, these data support the conclusion that the cumulative puff volume inhaled is a central determinant of THC exposure from marijuana cigarettes. Tashkin *et al.* (1991a) varied cumulative puff volume and found significant effects only for subjective reports of "high." However, in that study, volumes were varied over a more limited range (270–450 ml) as compared to volumes in this study (300–900 ml). Therefore, our study provides a more definitive demonstration that systematic variations in marijuana cumulative puff volume produce orderly biological exposure and subjective effects.

Study 2: Breathhold Duration

Extended breathholding is a commonly described characteristic of marijuana smoking that is not shared with tobacco smoking (Perez-Reyes *et al.*, 1982; Wu *et al.*, 1988). This suggests that there may be functional value in breathholding that contributes to the absorption or behavioral (*e.g.*, subjective, reinforcing) effects of THC. Alternatively, if breathholding does not enhance the desired behavioral effects of marijuana, then these smokers may be needlessly increasing their health risk from toxic smoke and inhaled particulates (Wu *et al.*, 1988). The purpose of this study was to determine the biological and behavioral effects of different marijuana smoke breathholds.

Methods

Subjects. The seven participants (four white, three African-American) were healthy males ranging in age from 20 to 38 yr (mean = 28.9, S.E. = 7.6) with educational levels ranging from 12 to 14 yr (mean = 12.6, S.E. = 0.4). Current reported use of marijuana ranged from 2 to 14 cigarettes/wk (mean = 6.4, S.E. = 1.4). All subjects reported current use of tobacco (averaging about 12 cigarettes/day) and alcohol (averaging about 8 beers/wk). Five subjects reported past illicit use of cocaine (two subjects within the last 6 mo) and LSD (one subject within the last 6 mo). Four subjects reported past illicit amphetamine use, and two subjects reported having used diazepam and opiates illicitly (two others reported taking these drugs with a prescription). Two subjects reported past use of phencyclidine, and one subject reported past illicit use of barbiturates. Two subjects tested urine-positive for cocaine during the study (one and two times, respectively), and one subject tested positive for morphine once during the study. Data from these sessions were included in the analysis.

Procedure. During each of the six active marijuana smoking sessions, subjects took 10 puffs. After each puff, the subjects held their breath for either 0, 10 or 20 sec. Each of these breathhold duration conditions was tested in separate sessions with 1.75% or 3.55% THC concentration cigarettes. Measures were collected before and after smoking, as previously described.

TABLE 2
Plasma THC: puff volume study^a

Measurement Time Point	Cigarette Potency					
	1.75% THC			3.55% THC		
	Puff volume (ml)			Puff volume (ml)		
	30	60	90	30	60	90
Presmoking baseline	3.4 (1.1)	4.3 (1.3)	3.4 (1.5)	3.3 (1.0)	3.0 (1.1)	3.7 (1.2)
Postsmoking (min)						
0	32.8 (5.2)	72.3 (11.6)	120.3 (10.8)	64.9 (8.1)	124.7 (12.2)	164.4 (10.9)
15	11.1 (2.2)	24.9 (5.3)	41.3 (9.7)	25.4 (4.5)	46.2 (7.3)	61.5 (10.7)
30	8.1 (1.9)	17.6 (3.9)	30.6 (6.0)	17.2 (3.1)	31.0 (5.6)	41.6 (6.9)
45	5.7 (1.0)	14.6 (3.4)	22.9 (3.7)	12.8 (2.0)	25.9 (4.1)	30.5 (4.7)

^a Sample mean ($n = 7$, S.E. below in parentheses) plasma THC levels (ng/ml) for the six marijuana dosing conditions: 30, 60 and 90 ml puffs from cigarettes containing 1.75% and 3.55% THC.

Results

Controlled smoking. Smoking topography data are presented in table 3. Again, the computer feedback system yielded smoking parameter values consistent with those designated for this study. Breathhold durations of 0, 10 and 20 sec produced lung exposure times (sum of inhalation, breathhold and exhalation times) of about 5, 14, and 24 sec, yielding a highly significant effect, $F(2,12) = 359.20$. Other smoking parameters (interpuff interval, inhalation volume, puff duration) did not significantly differ across experimental conditions. Thus, our procedures were generally effective in controlling subjects' smoking behavior.

CO boost. Figure 2 (upper left) shows expired CO as a function of breathhold duration and cigarette potency. There was a significant CO boost immediately after smoking in each marijuana condition relative to the no smoking control session, condition $F(6,36) = 13.40$. *Post hoc* analysis indicated that smoking conditions did not significantly differ from each other in the degree of CO boost, but all signifi-

cantly differed from no smoking control. In the supplemental ANOVA, CO boost was not significantly related to breathhold time, $F(2,12) = 2.67$ ($P < .11$), cigarette potency, $F(1,6) = 1$, or their interaction.

HR. After marijuana smoking, HR generally accelerated from session baseline, peaking within 20 min and remaining elevated until 40 min postsmoking; no significant elevations were observed in the no smoking session. As shown in figure 2 (lower left), mean HR boost was significantly greater after smoking marijuana, relative to the no smoking control session, condition $F(6,36) = 13.42$. *Post hoc* tests revealed that for high potency cigarettes, HR boost was significantly greater with 10-sec than 0-sec breathholds but that HR boost in the 20-sec condition did not significantly differ from the other two breathhold times. For low potency cigarettes, HR boost did not significantly differ across breathhold times. HR boost in the supplemental ANOVA was significantly greater after smoking high than low potency cigarettes (means = 24.2 and 14.1 bpm, averaged across breathhold duration),

TABLE 3
Marijuana smoking topography: breathhold duration study^a

Topography Measure	Cigarette Potency					
	1.75% THC			3.55% THC		
	Breathhold (sec)			Breathhold (sec)		
	0	10	20	0	10	20
Puffing parameters						
Interpuff interval (sec)	59.3 (0.9)	60.1 (0.1)	60.0 (0.1)	59.3 (0.8)	58.4 (1.1)	59.2 (0.9)
Puff duration (sec)	3.9 (0.1)	3.9 (0.1)	3.9 (0.1)	3.9 (0.1)	4.1 (0.1)	4.0 (0.1)
Puff volume (ml)	60.2 (0.2)	60.5 (0.7)	60.0 (0.2)	59.8 (0.2)	61.1 (0.8)	60.1 (0.1)
Respiratory parameters						
Inhalation volume (% VC)	24.9 (0.3)	24.9 (0.5)	25.8 (0.4)	25.3 (0.6)	25.7 (0.9)	26.0 (0.8)
Inhalation duration (sec)	2.9 (0.5)	4.6 (1.1)	3.1 (0.3)	2.3 (0.2)	3.1 (0.5)	3.9 (0.8)
Lung exposure duration (sec)	5.3 (0.8)	14.6 (0.5)	22.5 (1.1)	5.8 (0.6)	14.3 (0.3)	24.6 (0.3)

^a Sample mean ($n = 7$, S.E. below in parentheses) topography measures for the six marijuana dosing conditions: 0, 10 and 20 sec breathhold times after inhalation of smoke from cigarettes containing 1.75% and 3.55% THC.

BREATHHOLD DURATION STUDY

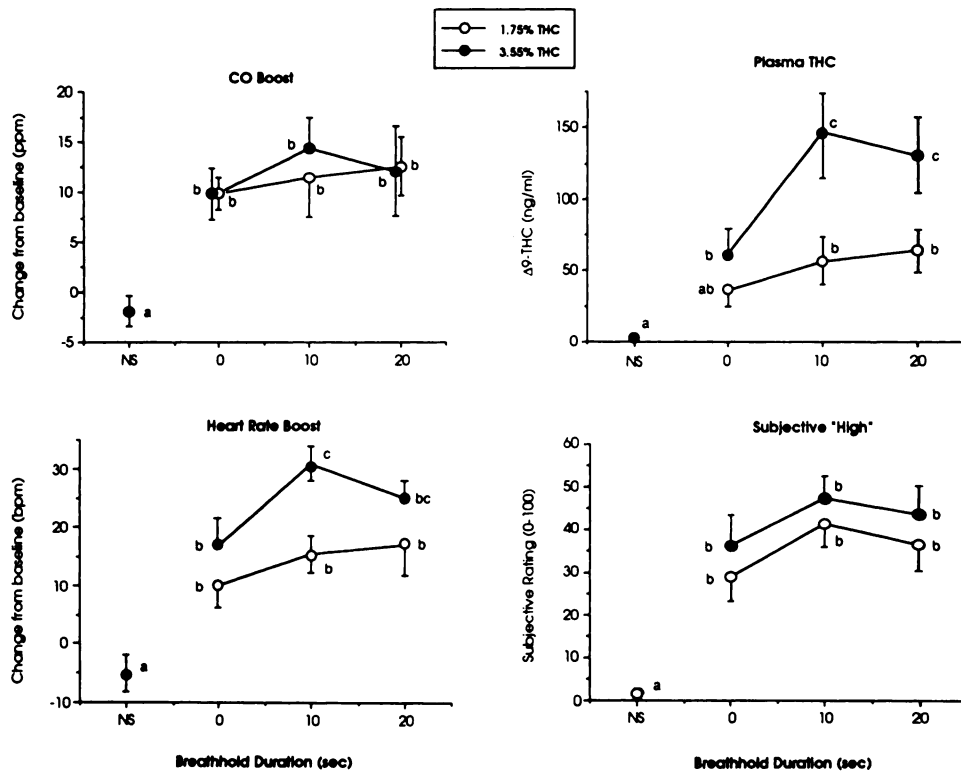


Fig. 2. Expired air CO increase from presmoking baseline to immediately after smoking (top left), plasma THC level immediately after smoking (top right), presmoking to postsmoking HR increase (bottom left) and postsmoking subjective "high" (bottom right) as a function of breathhold duration and cigarette THC content. NS on the X-axis indicates the no smoking control session. Each data point represents the mean ± S.E. of seven subjects. If the S.E. is not visible, it was less than the radius of the symbol. Lower case letters next to data points indicate Tukey *post hoc* test results from the overall (7 condition) analysis. Data points not sharing a common letter are significantly different ($P < .05$); those with common letters are not significantly different.

TABLE 4
Plasma THC: breathhold duration study*

Measurement Time Point	Cigarette Potency					
	1.75% THC			3.55% THC		
	Breathhold (sec)			Breathhold (sec)		
	0	10	20	0	10	20
Presmoking base line	1.8 (1.1)	1.5 (0.7)	2.1 (1.3)	2.0 (0.9)	1.8 (1.0)	2.1 (1.0)
Postsmoking (min)						
0	35.7 (10.3)	56.2 (13.4)	64.3 (14.1)	61.2 (18.7)	146.6 (29.7)	130.6 (28.2)
15	14.5 (3.7)	18.1 (4.8)	23.9 (5.2)	23.2 (7.4)	47.9 (12.1)	42.9 (12.1)
30	9.6 (2.7)	10.7 (2.5)	14.0 (4.9)	15.8 (4.9)	31.7 (7.5)	27.9 (8.1)
45	7.4 (2.1)	10.1 (2.5)	11.1 (4.0)	12.5 (3.5)	25.0 (5.3)	21.6 (5.9)

* Sample mean ($n = 7$, S.E. below in parentheses) plasma THC levels (ng/ml) for the six marijuana dosing conditions: 0, 10 and 20 sec breathhold times after inhalation of smoke from cigarettes containing 1.75% and 3.55% THC.

$F(1,6) = 7.49$. Mean HR boost was also significantly affected by breathhold time (10 sec > 0 sec; mean = 23.1 and 13.4 bpm, averaged across cigarette potency; 20 sec, mean = 20.9 bpm), $F(2,12) = 6.33$. Although breathhold effects were more prominent for high than for low potency cigarettes, the interaction term was not significant, $F(2,12) = 1.22$.

Plasma THC. Table 4 shows mean plasma THC levels obtained at each sample collection time for each marijuana dose condition, whereas figure 2 (upper right) depicts the first postsmoking values. In all conditions, THC levels were maximal immediately after smoking and decreased rapidly. At 45 min postsmoking, plasma levels declined to about 17 to 20% of peak values. Analysis of THC levels immediately after

smoking (fig. 2) indicated a significant condition effect, $F(6,36) = 11.15$. *Post hoc* tests indicated that 10-sec and 20-sec breathhold durations for high potency cigarettes produced plasma THC levels that were significantly higher than all other conditions. In the low potency cigarette conditions, plasma THC levels were not significantly different after 10 sec and 20 sec than after 0 sec of breathholding. However, as seen in table 4, there was a trend in that direction with plasma levels after 10 sec and 20 sec of breathholding (56 ng/ml and 64 ng/ml, respectively) being somewhat higher than levels seen after 0-sec breathholding (36 mg/ml). The supplemental ANOVA, excluding the no smoking session, yielded a significant breathhold time effect, $F(2,12) = 5.22$,

potency effect, $F(1,6) = 20.20$ and a marginal interaction, $F(2,12) = 3.29$, $P = .07$.

Subjective effects. Significant condition effects were obtained for 4 of 10 analog scale items—high, stoned, impaired and confused, $F_s(6,36) = 8.87, 8.27, 6.06$ and 2.71 , respectively, indicating mean differences between one or more marijuana smoking conditions and the no smoking control session. There were no condition \times time effects. Figure 2 (lower right) shows that, although ratings of subjective “high” after marijuana smoking were reliably elevated above no smoking levels, they were not significantly related to either breathhold duration, $F(2,12) = 2.04$, cigarette potency, $F(1,6) = 1.60$, or their interaction. Subjective ratings of “stoned,” “impaired” and “confused” showed very similar patterns. For ratings of cigarette “potency,” 3.55% THC cigarettes were rated as being significantly more potent than 1.75% THC cigarettes (mean = 1.7 ± 0.2 versus 1.2 ± 0.2 , respectively), $F(1,6) = 7.32$, although these ratings were not significantly related to breathhold duration.

Psychomotor performance. There were no significant effects of the experimental conditions on the psychomotor performance measures.

Study 2: Summary

Varying the duration of marijuana smoke breathholding from 0 to 20 sec did not produce consistent changes in biological exposure and behavioral effects. When high potency (3.55% THC) marijuana cigarettes were used, there was a clear effect of breathholding on plasma THC levels. Breathholding for both 10 and 20 sec, boosted plasma THC levels over no breathhold, but 20-sec breathholds did not produce an additional increase beyond that seen with 10-sec holds. Effects of breathholding on plasma THC levels were more equivocal when low potency (1.75% THC) cigarettes were used. Here, there were no significant effects across breathhold conditions, but a trend in the direction of higher THC levels with longer breathholds. These and other data (*cf.* Tashkin *et al.*, 1991a) are generally consistent with the conclusion that breathholding of marijuana smoke enhances absorption, but this study suggests that a ceiling effect may occur with breathholds longer than 10 sec. In this study, HR boost data reflected plasma THC levels. However, this was not the case for either CO boost or subjective effects. The latter were significantly greater in all smoking conditions relative to control, but were not differentially influenced by breathhold duration. Thus, even in the case of the high potency cigarettes, where marked differences in plasma THC levels were seen as a function of breathholding, plasma THC levels did not necessarily translate into increased subjective effects.

General Discussion

The two studies presented use a method described by Azorlosa *et al.* (1992) to measure the biological and behavioral impact of varying marijuana smoke exposure. Varying smoke dose via manipulation of puff volume (study 1) produced orderly changes in CO boost, plasma THC levels and subjective reports. Breathholding of inhaled smoke (study 2) boosted plasma THC levels in the high potency cigarette condition, but this was not clearly reflected in CO boost or subjective effects.

Dose delivery can be varied in several ways with smoked

drugs—in particular, both number and size of puffs are important. Study 1 extends previous observations on the effect of number of puffs (Azorlosa *et al.*, 1992) by showing an orderly relationship between puff volume and delivered dose, with parallel effects on subjective report measures. This demonstration extends to marijuana smoking a principle known to tobacco—*i.e.*, that amount of smoke exposure cannot be accurately assessed from gross measures of intake (*e.g.*, cigarettes/day). Few marijuana studies have measured puff volume. The present study varied volumes over a wider range than that used in a recent study by Tashkin *et al.* (1991a) and provided a more definitive demonstration that systematic variations in marijuana cumulative puff volume produce orderly biological exposure and subjective effects. The absence of differential HR boost effects in study 1, which are typically a reliable index of marijuana dose (Chait and Pierrri, 1992), appeared to be due to measurement problems. A potential confound in the puff volume study is that subjects may have smoked further down the rod in the 90-ml puff volume conditions with a consequently greater amount of THC extraction at the distal end of the cigarette (Tashkin *et al.*, 1991b). This would influence the absolute differences across dose conditions but not the principle that cumulative dose variation influences effects of smoked marijuana.

In this study, we varied cumulative puff volume, but another interesting and relevant clinical issue is whether size and/or spacing of puffs influence subjective effects at a given cumulative puff volume dose. In the study by Tashkin *et al.* (1991a), per-puff volume was varied although the total amount of smoke inhaled was equated across conditions by changing puff number. Variations in volume per puff did not influence THC levels, HR increase or “high.” However, the study did find some orderly effects of cumulative puff volume on these measures. Thus, the total smoke volume inhaled but not the volume per puff appears to determine smoked marijuana effects.

Effects of breathhold time are of interest because marijuana smokers engage in a stereotypical pattern of extended breathholding, presumably to maximize THC absorption. Study 2 showed that breathholding boosts plasma THC levels, although this effect was statistically significant only in the high and not the low potency cigarette condition. Plasma THC levels increased with 10-sec compared with 0-sec breathholding but did not increase further with a longer breathhold duration (fig 2). The same pattern of plasma THC levels seen across breathhold and cigarette potency conditions was reflected in the HR boost measure, which lends support to their validity. These findings are consistent with data from the one previous study (Tashkin *et al.*, 1991a) that examined breathhold effects on THC levels and found reliable increases in plasma THC levels when breathhold was increased from 4 to 14 sec. Thus, the plasma THC data suggest that the stereotypic behavior of marijuana smoking is useful for maximizing absorption; however, our study suggests there are diminishing returns with longer breathhold durations.

Although marijuana subjective effects (*e.g.*, “high”) have shown reliable dose-related increases in studies examining puff number (Azorlosa *et al.*, 1992) and cumulative puff volume (study 1), subjective effects were not significantly related to increasing breathholds in study 2. This was true despite the fact that the range of plasma level changes was

similar to those seen in study 1. These data are intriguing because they suggest the possibility that nonblind features of administration including the amount and potency (THC concentration) of smoke inhaled may in some cases be a more important determinant of subjective effects than are resulting plasma THC levels. Interpretation of the subjective data from study 2 should be made cautiously, however, because these subjects failed to make differential subjective responses when cigarette THC content was varied, which suggests that they were relatively insensitive to marijuana dose manipulation. Nevertheless, the lack of reliable breathholding effects on subjective report measures is consistent with observations in previous studies (Tashkin *et al.*, 1991a; Zacny and Chait, 1989, 1991). Taken together, the data from several studies suggest that, unlike other parameters of marijuana smoking, breathholding may produce greater biological exposure—and a resultant array of health risks (Beaconsfield *et al.*, 1972; Hoffman *et al.*, 1975; Tashkin *et al.*, 1991a; Wu *et al.*, 1988)—without producing an increase in subjective effects that are associated with the reinforcing value of the drug. By eliminating breathholding, marijuana smokers might be able to reduce somewhat the hazards of this activity without diminishing the desired subjective effects.

The magnitude of postsmoking HR increase seen in study 2 generally paralleled plasma THC levels, but HR effects were statistically less reliable (*e.g.*, differences between 0 and 20 sec conditions were not significant). Three previous studies have examined the effect of breathhold duration on HR increase. Zacny and Chait (1989) using low potency cigarettes (1.3% THC) failed to observe reliable alterations in HR boost after breathholds of 0, 10 or 20 sec. A replication study (Zacny and Chait, 1991) with better control procedures and more potent cigarettes (2.3% THC) again failed to find effects of breathhold (0 *versus* 20 sec) on HR boost. In the study by Tashkin *et al.* (1991a), where subjects smoked cigarettes containing 1.24% THC, longer breathholds resulted in significantly greater HR acceleration at a lower cumulative puff volume (270 ml) but breathhold effects were unreliable at higher cumulative volumes (420–450 ml). Thus, results across studies are generally consistent in failing to find reliable HR boosts associated with breathhold manipulation. The reasons for this are not clear, but it may simply be that HR changes produced by breathholding are relatively small and thus difficult to detect in small sample studies.

Two of three previous studies (Tashkin *et al.*, 1991a measuring plasma COHb; Zacny and Chait, 1991 measuring alveolar air) have found that magnitude of CO boost is related to breathhold duration. Further, Zacny *et al.* (1987) found that increasing breathhold duration from 0 to 16 sec approximately doubled the amount of CO absorbed from tobacco smoke. In contrast, our study and one previous study (Zacny and Chait, 1989), both measuring alveolar samples, have not found a relationship between breathholding and CO boost during marijuana smoking. In both these latter studies, relatively high CO boosts (8–10 parts per million) were observed under all conditions. It is possible that the measurement procedure obscured relationships between CO boost and breathhold in our study, because previous research has shown that transient changes in expired air CO levels occur within the first 5 min after smoking (Guyatt *et al.*, 1988; Woodman *et al.*, 1987). Thus, the predicted relationship might have been observed if readings had been delayed to a

postsmoking time (*e.g.* 5 min) when a more stable equilibration between lung and plasma CO has been established (Woodman *et al.*, 1987).

Cigarette potency was also varied in the present studies to assess the reliability of puff volume and breathhold time effects. The high potency cigarette containing 3.55% THC, which is comparable to street grade marijuana (USDHHS, 1991), produced reliable effects on plasma THC levels (both studies), subjective effects (study 1) and HR boost (study 2), but not CO boost. Inasmuch as expired CO reflects the amount of smoke inhaled and not the pharmacological content of the cigarette, the latter negative finding is expected. Examining topography manipulations with two different cigarette potencies enhanced generality of the findings for puff volume. In the case of breathhold duration, it is important to note that different conclusions may have been reached if only one cigarette potency had been used. This highlights the importance of assessing smoking effects at different dosing levels.

The marijuana doses delivered in this study produced reliable subjective effects (compared to no smoking) at average postsmoking plasma levels ranging from 33 to 164 ng/ml but these same doses failed to produce performance decrements on the laboratory tests used (digit span, digit symbol substitution and divided attention tracking). This is consistent with the data from Azorlosa *et al.* (1992) who found that performance decrements were observed only at marijuana doses producing plasma levels of more than 175 ng/ml. This observation may be relevant to the naturalistic use of marijuana, because users may engage in hazardous behavior (*e.g.*, driving a car) while feeling “high” but judging themselves able to perform complex tasks. Although different conclusions about marijuana-induced performance impairment may have been reached if more sensitive performance measures had been used (see Chait and Pierri, 1992, for a review), it is interesting that marijuana appears to be more potent in producing subjective than performance-impairing effects. It would be worthwhile in future research to determine how subjects’ marijuana exposure histories or experimentally induced tolerance levels influence the threshold at which performance decrements emerge relative to subjective effects.

In summary, the present two studies systematically varied marijuana puff volume and breathhold duration in conjunction with cigarette potency while holding constant other smoking parameters. This controlled method permits a more precise specification of smoked marijuana dose than is often the case in published reports. Presmoking to postsmoking changes in CO exposure, plasma THC and subjective reports (particularly acute intoxication) were significantly dose-related to puff volume, whereas only plasma THC level was influenced by breathhold duration. No psychomotor performance decrements were observed at average postsmoking plasma THC levels from 33 to 164 ng/ml. These findings, in conjunction with those reported by Azorlosa *et al.* (1992), confirm the usefulness of the controlled smoking technology, support the notion that puff volume reliably influences biological exposure and subjective effects and show that breathholding may increase the amount of THC absorbed. However, the findings cast doubt on the common belief that prolonged breathholding of marijuana smoke significantly enhances classical subjective effects that are associated with its reinforcing value in humans.

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