Pharmacology Biochemistry and Behavior, Vol. 43, pp. 513–521, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Relationships Between Ingestion and Gustatory Perception of Caffeine

DAVID J. MELA,*[1] RICHARD D. MATTES,†
SHUYA TANIMURA‡ AND MARIA ROSA GARCÍA-MEDINA§

**Department of Consumer Sciences, AFRC Institute of Food Research, Earley Gate, Whiteknights Road, Reading RG6 2EF, UK*
†Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104
‡Kirin Brewery Co., Ltd., 6-26-1 Jingumae, Shibuya-ku, Tokyo 150, Japan
§Escuela De Salud Publica, Facultad de Medicina - UBA, Laboratorio de Investigaciones Sensoriales, M.T. de Alvear 2202, 1453 Buenos Aires, Argentina

Received 7 October 1991

MELA, D. J., R. D. MATTES, S. TANIMURA AND M. R. GARCÍA-MEDINA. *Relationships between ingestion and gustatory perception of caffeine.* PHARMACOL BIOCHEM BEHAV 43(2) 513–521, 1992.—We observed that taste detection thresholds for caffeine (CAF) are elevated in habitual CAF users relative to nonusers. A series of experiments were carried out to explore that relationship and assess the influences of salivary CAF and acute vs. chronic CAF ingestion. A significant correlation between CAF ingestion and taste threshold was noted in two studies of U.S. adults, although this was not observed in a parallel study involving an Argentinean population. Acute CAF ingestion (5.5 mg/kg) had no appreciable effect on taste thresholds. Threshold values greatly exceeded even peak salivary CAF levels, indicating that classical taste adaptation was an unlikely influence. Chronic CAF ingestion (450 mg/day for 3 weeks) also had no consistent effect on taste thresholds for CAF or other taste stimuli. Although a number of explanations are considered, we suggest that the sensory phenomenon may reflect preexisting differences between CAF users and nonusers or perhaps an effect of exposure to other bitter and/or CAF-containing foods and beverages.

Caffeine Gustation Saliva Humans Coffee Taste thresholds Taste sensitivity

IT has been proposed that functionally significant relationships exist between salivary constituents and gustatory perception of selected compounds (2,25,29). The preponderance of evidence indicates that there is a relationship between thresholds for NaCl and salivary sodium concentrations, and this data has been used to account for gustatory adaptation to salivary sodium. However, exceptions to this relationship have been noted, and it may only be apparent under certain test conditions and at extremes of salivary sodium (5,6). Other mechanisms have also been described through which salivary components of endogenous and exogenous origin may effect saliva-taste interactions (5).

Caffeine (CAF) is a commonly consumed bitter stimulus that reliably appears in saliva shortly after ingestion. Acute or chronic exposure of the gustatory epithelium via salivary CAF can therefore be readily manipulated for experimental purposes. Ingested CAF reaches a plasma peak in about 30–60 min and has a half-life of about 4–6 h (3,8,9). Salivary concentrations closely parallel plasma levels (8,40). Hence, individuals who frequently consume CAF are likely to maintain detectable levels of CAF in plasma and saliva at almost all times.

Pilot studies (see Experiment 1 below) indicated that taste detection thresholds for CAF differed between habitual CAF users and nonusers. It was therefore felt that CAF could present a useful model compound with which to evaluate novel interactions of diet and gustatory function.

GENERAL METHODS

Subjects were all healthy, young, adult males and females recruited by public advertisement and paid for their participation. Criteria for exclusion included use of any form of tobacco and current health disorders or use of medications reported to alter taste or smell. For experiments involving CAF ingestion, subjects were also excluded if they reported extreme reactions to CAF or any condition where CAF ingestion is contraindicated. Subjects were instructed to refrain from chewing gum, brushing their teeth, or consuming anything except water for at least 2 h preceding sensory testing. In some experiments, there were also specific restrictions against CAF consumption for stipulated time periods prior to testing. While many subjects had previous experience in other sensory

[1] To whom requests for reprints should be addressed.

evaluation experiments, all were naive as to the purpose of these studies. With the exception of Experiment 2B, all testing was conducted at the Monell Chemical Senses Center. The experiments were approved by the University of Pennsylvania Committee on Studies Involving Human Beings, and participants completed an informed consent document prior to any testing.

Whole-mouth-stimulated saliva was collected for 3 consecutive min while subjects chewed on an unflavored gum base at a metered rate of 80 chews/min. Collection commenced after subjects expectorated following a 1-min "practice" trial. Subjects sat quietly and expectorated into a preweighed funnel and test tube at the end of each minute. Resting saliva was collected in a similar manner except subjects sat completely still with their mouths slightly open and simply allowed saliva to drain continuously into the funnel. Flow rates were determined by weight. In experiments where salivary caffeine concentrations were determined, the extractions and analyses were achieved by the method of Hartley et al. (15) as described by Mela (27).

Taste testing in all sessions began by asking the subject to rinse several times with deionized water. All samples for threshold and suprathreshold testing consisted of approximately 10 ml solution at room temperature, which subjects were instructed to sip, evaluate, and expectorate. No stimuli were swallowed at any point in the experiments, and deionized water was used for rinsing between samples. All subjects were tested for thiourea taste sensitivity using the method described by Mela (28). No significant effect of this trait was observed in any study so the data are not presented.

Statistical analyses were carried out using the SPSS/PC+ software package (SPSS Inc., Chicago, IL). Descriptive data are reported as mean ± SD except as noted. Comparisons are based upon the studentized *t*-tests or full-factorial analysis of variance (ANOVA) with the criteria for statistical significance set at $p < 0.05$.

EXPERIMENT 1

An initial study was conducted to assess whether CAF taste thresholds differed between CAF users and nonusers.

METHOD

Subjects were 13 females and 11 males, aged 28.6 ± 7.7 years. They were questioned on habitual CAF ingestion and selected for the study based upon level of CAF use as calculated from published tables and manufacturers' data. "Users" were considered to be those who consumed 400 mg or more CAF per day, while nonuse was operationally defined as consumption of less than 70 mg (the approximate CAF content of one cup of tea) per day. Prospective participants were screened until there were 12 subjects in each group. Nonusers (eight females, four males) reported a mean CAF consumption of 4 mg/day. Ten of the nonusers reported a habitual intake of under 10 mg/day. Users (five females, seven males) reported a mean intake of 423 mg/day.

CAF taste detection thresholds and salivary flow were determined in each of two sessions conducted on separate days, with a 1-day hiatus. Threshold testing solutions consisted of CAF in deionized water at concentrations ranging from 7.9×10^{-3} to 3.155×10^{-5} M by 1/5 log dilution steps. The taste threshold evaluation procedure was a single ascending triangle test, starting with the lowest CAF concentration. Subjects were given three cups, one containing CAF and 2 containing water; their task was to identify the odd sample based upon taste. The sample concentration was increased following any single incorrect response. Once a correct identification was made, the same concentration was presented a second time. It was presented a third time if the preceding two identifications were correct. The threshold level was defined as the concentration correctly identified in three consecutive presentations. Subjects rinsed once between cups and twice between trials. The geometric mean of the two separate threshold determinations was used in data analyses.

RESULTS

CAF taste detection thresholds ranged from 0.16–1.99 mM, and the threshold measurements made on the two separate dates were positively correlated ($r = 0.45$, $p = 0.01$). There was a large, statistically significant difference in CAF taste detection threshold between the user (1.17 ± 0.50 mM) and nonuser (0.5188 ± 0.236 mM) groups as shown in Fig. 1, $t(23) = -3.90$, $p = 0.001$. There was also a low but statistically significant positive correlation between caffeine intake and thresholds ($r = 0.41$, $p = 0.025$). Salivary flow rates were found to be unrelated to CAF use or thresholds, and taste thresholds were not significantly related to sex or interactions of sex and caffeine intake.

EXPERIMENT 2A

Before exploring possible mechanisms for the findings of Experiment 1, a second experiment was conducted to replicate the findings with improved control over subject variables and reduce the possible influences of recent CAF ingestion. In addition, observations were extended to suprathreshold taste stimuli.

METHOD

Eleven males and 11 females, with a mean age of 24.2 ± 4.5 years, half CAF users and half nonusers (as defined in Experiment 1), were recruited after responding to an extensive questionnaire eliciting information on habitual use of caffeinated foods, beverages, and drugs. Because the actual CAF content of a "cup" of coffee and tea can vary widely with actual serving size, brand, and preparation methods (35), subjects specified these on the questionnaire. Nonusers (seven females, four males) reported a mean CAF consumption of 11 mg/day. Users (four females, seven males) reported a mean intake of 275 mg/day. The protocol was similar to Experiment 1 except: a) Subjects were to abstain from CAF use from a time at least 24 h before the first session through the end of the second session; b) both resting and stimulated saliva were collected and analyzed for CAF; and c) the sensory testing included a suprathreshold time-intensity rating procedure.

In addition to threshold testing, five CAF solutions ranging from 3.155×10^{-2} to 7.9×10^{-4} M by 1/5 log steps were used in a time-intensity procedure. To standardize mouth movement, subjects ingested a piece of unflavored gum base with each stimulus solution. After 3 s, the solution alone was expectorated and taste assessments were made as subjects chewed at a metered rate of 80 chews/min. Ratings were assigned by turning the knob on a strip chart recorder moving at 2.0 cm/min. The instrument was calibrated to allow the pen to deviate along a 200-mm path marked on the recorder at 0 ("no taste"), 100 ("moderate"), and 200 mm ("extremely strong"). Paper covered the output immediately beyond the pen so subjects could not see prior ratings. Each trial continued for 3 min or until a stable 0 reading was reached.

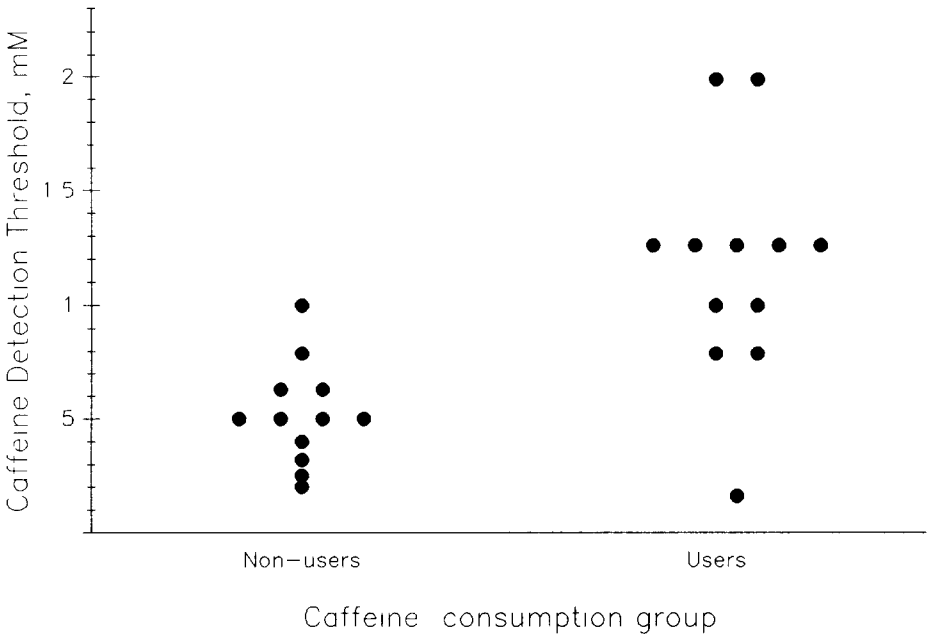

FIG. 1. CAF taste detection thresholds of habitual CAF users and nonusers in Experiment 1.

Measurements included maximum intensity, time to reach maximum intensity, time from maximum intensity to return to 0 (or minimum intensity), total time of perception, and total area under the curve (determined by cutting and weighing). Time-intensity data were collected from 17 subjects (9 users, 8 nonusers).

RESULTS

As in Experiment 1, mean individual CAF detection thresholds were positively correlated with habitual CAF intake in the total sample (Fig. 2; $r = 0.53$, $p = 0.006$). When only CAF users were considered in the computation, the correlation between thresholds and intake was even stronger (Fig. 2; $r = 0.79$, $p = 0.002$). However, in this experiment mean thresholds did not differ between groups. In addition, while habitual dietary CAF intake was positively correlated with thresholds determined at both test sessions thresholds at the two time points were not significantly correlated with each other. Despite the request to abstain from CAF ingestion, salivary CAF concentrations above 1.0 μM were detected in eight subjects in the first session and five subjects in the second session. While the presence and concentration of CAF in saliva were unrelated to thresholds, these may have contributed to the poor correlation between threshold measures taken at the two time points. Taste thresholds were not significantly related to sex or interactions of sex and caffeine intake.

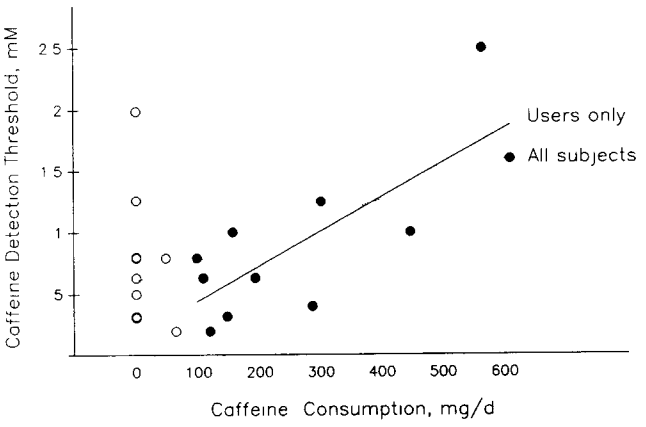

FIG. 2. Relationship between CAF taste detection thresholds and habitual CAF consumption of CAF users (●) and nonusers (○) in Experiment 2A, with first-order regression lines plotted for all subjects (----) and CAF users only (——).

Time-intensity variables were analyzed by a 2 × 5 (CAF intake group × concentration) repeated-measures ANOVA. There were no significant group or group × concentration effects on any measure, although there was a trend for CAF users to reach maximum intensity more slowly than nonusers, $F(1, 15) = 3.72$, $p = 0.073$. There were no consistent correlations of time-intensity measures with CAF intake, thresholds, or salivary flow or CAF concentrations.

EXPERIMENT 2B

Coincident with the conduct of Experiment 2A, a parallel experiment was carried out at the Laboratory of Sensory Investigations of The University of Buenos Aires, Argentina.

METHOD

Twenty subjects, mean age 23.2 years, with equal numbers having CAF consumption of greater than 400 mg/day (5 males, 5 females; mean CAF intake 615 mg/day) and below 100 mg/day (5 males, 5 females; mean CAF intake 74 mg/day), participated in two test sessions 1 week apart. Subjects were requested to refrain from CAF ingestion for at least 6 h prior to each sensory testing session.

CAF detection thresholds were determined using the meth-

ods described except the highest CAF solution was 3.155×10^{-3} M. Subjects also judged the perceived intensity of the five strongest CAF solutions, as well as quinine sulfate (QS, 1.31×10^{-4} to 8.17×10^{-6} M) and NaCl (1.37–0.085 M) solutions prepared by serial half dilution. Samples were presented in random order, and perceived intensity was assessed using the method of magnitude estimation (36). Using this method, subjects assign numerical values in proportion to the intensity of the sensation they perceive, with no fixed external scale or reference. To place subject ratings on a common scale, individual normalization factors for CAF and QS were computed as 10 divided by the geometric mean of each subject's intensity estimates for NaCl (37). The individual intensity data were then multiplied by this factor and log-transformed prior to statistical analyses by repeated-measures ANOVA.

RESULTS

Taste thresholds measured in the two sessions were significantly correlated ($r = 0.53, p = 0.009$). However, in contrast to the results of Experiments 1 and 2A there was no relationship between CAF intake and taste thresholds in this population. There were also no significant main or interactive effects of CAF user group on suprathreshold intensity ratings except the subjective range (ratio of perceived intensity of highest to lowest concentration solutions) for QS was greater for CAF nonusers (Mann–Whitney U-test, $p = 0.033$).

Experiments 1 and 2A indicate that chronic CAF consumption is associated with increased CAF taste detection thresholds. It is not clear why this was not seen in Experiment 2B. While the population was of a different ethnic origin, they were otherwise demographically similar. It is possible that these subjects may differ in their exposure to bitter compounds or methylxanthines other than CAF. Variations in dietary exposure to bitter food constituents have been associated with different sensitivities to and preferences for bitter compounds across populations (16,30). A genetic basis for sensitivity to certain bitter compounds, including CAF, has been suggested in some reports (11,14,34).

EXPERIMENT 3

The preceding experiments did not specifically test the possible effects of recent CAF consumption. A dosing experiment was therefore designed to determine whether thresholds would be specifically modified in response to acute CAF ingestion.

METHOD

Ten males and 2 females (mean age of 28.8 years), with a mean CAF intake of 190 mg/day, were newly recruited to participate in this experiment. Subjects were asked to refrain from CAF use for at least 24 h prior to testing, and compliance with this request was confirmed by salivary CAF analysis.

The test session began with a resting saliva collection, followed by determination of baseline taste detection thresholds for CAF and NaCl. The methods and CAF concentrations used for threshold determination were identical to Experiments 1 and 2A except presentations of CAF and NaCl samples were alternated such that both thresholds were determined simultaneously. NaCl concentrations ranged from 2.5×10^{-2} to 3.91×10^{-4} M by serial half-dilution. Data on thiourea taster status was not collected in this experiment.

After baseline testing, subjects ingested a gelatin capsule containing 5.5 mg CAF/kg body weight (approximately equivalent to three to four cups of brewed coffee for a 70-kg man). Body weight was determined on a clinical balance-beam scale at time of testing. Although there was no true placebo session, the study was double blind in the sense that both subjects and the technician administering the tests were led to believe that the capsules could contain either caffeine or an inactive placebo. In a previous study (27), we found that subjects given this dose did not reliably identify whether they had received CAF or a placebo.

Resting saliva collections began at 25 (time 1), 55 (time 2), 85 (time 3), 115 (time 4), 145 (time 5), 175 (time 6), and 355 (time 7) min after ingestion of the capsule, and detection threshold testing began 5 min after initiating each saliva collection. CAF detection thresholds were determined at all time points but, because of time limitations, NaCl thresholds were determined only at baseline and times 3, 6, and 7. During periods between taste testing, subjects sat quietly in the test room, reading or working on word puzzles.

RESULTS

Only two subjects had detectable levels of CAF in saliva at time 0, both below 2 μM. There was no statistically significant

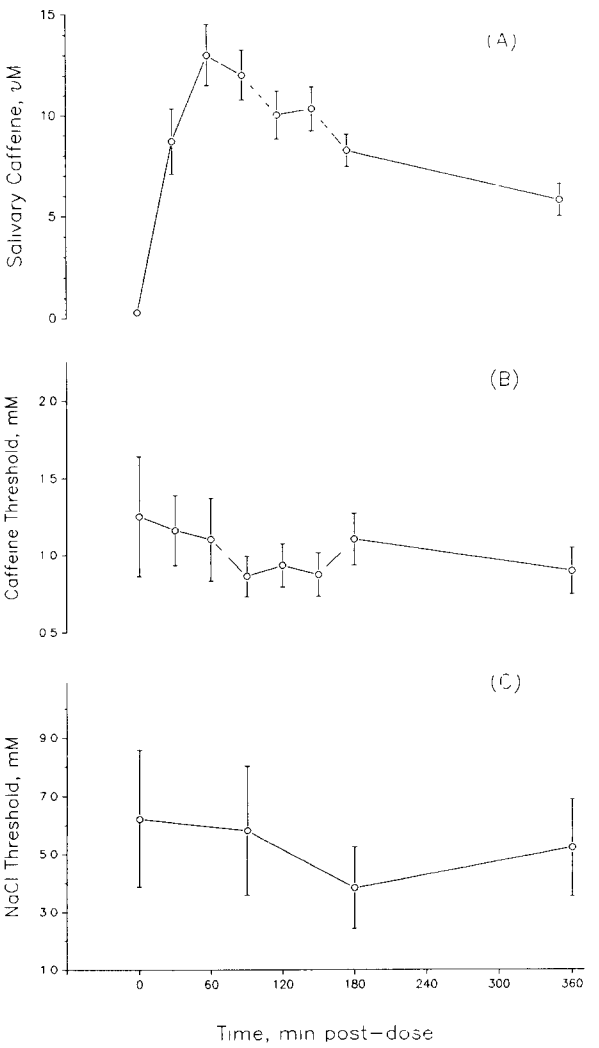

FIG. 3. Effect of 5.5-mg/kg CAF dose on (A) salivary CAF concentrations, (B) CAF taste detection thresholds, and (C) NaCl taste detection thresholds of subjects in Experiment 3. Means ± SEM.

association between habitual CAF usage and baseline CAF detection thresholds. This may be, in part, attributable to the small number of subjects in the experiment. Repeated-measures ANOVA identified no significant effects of acute CAF ingestion on CAF or NaCl thresholds or salivary flow over the time following dosing (Figs. 3A-3C).

Peak salivary CAF concentration was observed at time 2 for six subjects, with peaks for other subjects observed at times 1 (n = 1), 3 (n = 3), and 4 (n = 2). The mean peak salivary CAF concentration was 15.2 ± 4.42 μM (range 9.22-20.88). At the point of peak salivary CAF levels, individual CAF taste detection thresholds averaged 1.31 ± 0.91 mM, exceeding salivary CAF by about two orders of magnitude. At no time point for any subject did the two values come within a 10-fold difference.

EXPERIMENT 4

Salivary CAF levels and acute CAF exposure did not appear to account for the observed associations between habitual CAF use and taste sensitivity. These results prompted an additional study to examine the specific role of chronic CAF exposure. The study was designed to assess whether gustatory function of nonusers would systematically change over an extended period of moderate CAF consumption. The protocol included assessment of physiological measures reportedly altered by CAF use as additional indices of the effects of the experimental manipulation.

METHOD

Eighteen subjects were selected based upon a reported customary CAF consumption of less than 100 mg/day (n = 15) or abstention from CAF use for a 3-week period prior to the study (n = 3).

The 9-week experiment was conducted using a double-blind, placebo-controlled within-subject design, with half the subjects given CAF in the first experimental period and the other half given the placebo first (Table 1). During the CAF experimental periods, subjects were given gelatin capsules containing 150 mg CAF (with 100 mg corn starch as filler, for a total weight of 250 mg). The placebo contained 250 mg cornstarch alone. Vials containing the different capsules were coded by an investigator not in contact with the test subjects. Subjects were given a vial containing 1 week's capsules and instructed to take one capsule three times each day—upon rising, in midafternoon, and in the evening—for a total CAF intake of 450 mg/day. They were also asked to arrange on test dates to take one of their three capsules between 2 and 4 h prior to testing and avoid all caffeinated foods and medications throughout the duration of the study.

Three test sessions were conducted during the first week to establish firm baseline measures. Additional test sessions were conducted once per week thereafter. All sessions began by measurement of resting pulse and blood pressure and collection of a stimulated saliva sample. Subjects then completed two physical performance tasks described below (20).

Hand steadiness was measured using a hand-held metal stylus (1.5 mm diameter) and vertical metal plate with circular holes cut through it (Model 32011, Lafayette Instrument Co., Lafayette, IN). Subjects sat with their arm extended and attempted to hold the stylus in holes of 7.9, 5.15, and 4.0 mm without touching the sides. The stylus was connected to an impulse counter (Lafayette Model 58022), and the average number of contacts over 2 30-s periods at each hole size was determined.

In a rotary tracking task, subjects attempted to maintain contact between a flexible metal-tipped stylus and a 1.8-cm diameter disk located 5 cm from the edge of a 24.4-cm turntable moving at 45 rpm. Additional testing at 60 rpm was added midway through the experiment (n = 13 subjects). The stylus was connected to a stopclock (Lafayette Model 54030) and the average contact time was determined in 2 20-s trials at each speed.

Taste detection thresholds were determined for CAF, denatonium benzoate (DB), and NaCl. DB is a potent bitter stimulus; the molar detection threshold for this man-made compound is about five to six orders of magnitude lower than for CAF. This was selected as a sensitive medium with which to test for possible generalized effects on bitter taste perception. NaCl was used as a nonbitter stimulus to assess generalized effects of CAF on taste sensitivity. The following concentration series were prepared as serial half-dilutions: CAF, 5.0×10^{-2} to 6.1×10^{-6} M; DB, 1.0×10^{-7} to 1.22×10^{-11} M; and NaCl, 8.0×10^{-1} to 2.44×10^{-5} M.

Detection thresholds were determined by a two-cup forced-choice staircase method, judged likely to offer enhanced sensitivity and improved reliability over the time of the experiment, compared to ascending triangle tests used previously. In this procedure, a series of single taste solutions and water blanks were presented as randomly ordered pairs. Subjects sipped each cup, held it in their mouth 3 s, and then expectorated. Their task was to select the sample with the stronger taste. A single selection of the blank resulted in subsequent presentation of the next highest concentration of that tastant, while selection of the tastant sample prompted presentation of the same concentration again. Samples of CAF, DB, and NaCl were alternated such that all thresholds were determined simultaneously. Upon selecting the tastant sample in two consecutive presentations following an error, the sequence for that stimulus descended until another error was made and then ascended again. After seven such reversals in sequence, that tastant was removed from the procedure. The first reversal point was disregarded and thresholds were calculated as the geometric mean of the last six reversal point concentrations.

The perceived intensity of the top five concentrations of CAF, DB, and NaCl were determined using a modulus-free magnitude estimation procedure (36) as described in Experiment 2B. Subjects sipped and expectorated each solution and then immediately provided a value for its taste intensity. In this experiment,audition, rather than another taste modality, was used to place individual ratings on a common numerical scale (37). Subjects rated the intensity of 1,000-Hz tones at 30, 45, 60, 75, and 90 dB sound pressure presented to the right ear. These were randomly interspersed among the taste stimuli. The order of stimuli and concentrations within each stimulus were randomly ordered except all five concentrations

TABLE 1

PROTOCOL FOR EXPERIMENT 4

Week	Number of Test Sessions	Treatment	
		Group 1	Group 2
1 (Baseline)	3	Placebo	Placebo
2-4 (Experimental)	3	CAF	Placebo
5-6 (Washout)	2	Placebo	Placebo
7-9 (Experimental)	3	Placebo	CAF

of each taste stimulus were presented as a block. All stimuli were presented twice, and the mean response was used in further computations. These raw magnitude estimates were normalized as follows: Individual normalization factors were computed as 10 divided by the geometric mean of each subject's loudness estimates in a test session (37). Individual intensity data from the same session were multiplied by this factor prior to statistical analyses. Log-transformation of these data improved the relationship between concentration and response and served to normalize variance across concentrations for repeated-measures ANOVA procedures.

Up to six extra capsules were dispensed each week and the count of returned capsules was used as a check on compliance and malingering. Salivary CAF levels were used as an additional index of compliance.

Differences in mean responses between the 3-week experimental CAF and placebo periods were compared by paired *t*-tests, and full-factorial repeated-measures ANOVA were conducted to evaluate the main and interactive effects of group (G) (CAF vs. placebo first), condition (C) (CAF vs. placebo), and time (T).

RESULTS

The capsule count and other response measures indicated high compliance with the caffeine dosing schedule. CAF was not detected in any saliva sample from subjects at any point in the placebo period, whereas it was detected in all saliva samples during the CAF period. The data also show an effect of CAF on both hand steadiness and rotary tracking performance measures. Figures 4 and 5 present the change in performance over time on each treatment. In the hand steadiness test, overall mean performance on both the 5.15- and 4.0-mm holes were significantly worse during the CAF vs. placebo period [5.15 mm: 15.2 ± 8.8 (CAF) vs. 12.7 ± 6.9 (placebo) contacts/30 s, $t(17) = -2.13$, $p = 0.048$; 4.0 mm: 51.5 ± 17.0 (CAF) vs. 46.0 ± 14.9 (placebo) contacts/30 s, $t(17) = -2.77$, $p = 0.013$]. Differences were also noted on the rotary tracking test at both 45 and 60 rpm [45 rpm: 15.3 ± 2.4 (CAF) vs. 17.2 ± 2.1 (placebo) s contact time, $t(16) = 3.03$, $p = 0.008$; 60 rpm: 10.7 ± 3.4 (CAF) vs. 12.5 ± 3.9 (placebo) s contact time, $t(12) = 2.24$, $p = 0.045$, respectively].

ANOVA identified significant main effects of condition only for the CAF-induced decrement in hand steadiness at 5.15 mm, $F(1, 16) = 8.16$, $p < 0.01$, and 4.0 mm, $F(1, 16) = 7.44$, $p < 0.015$. There were significant interactive effects on a number of measures. Pulse tended to increase over time on the CAF but not placebo conditions [Fig. 6; C × T, $F(1, 16) = 5.08$, $p < 0.012$], and a similar effect was identified for hand steadiness at 5.15 mm [C × T, $F(1, 16) = 7.97$, $p < 0.002$]. The group getting placebo first showed more pronounced effects of CAF vs. placebo on hand steadiness at 5.15 mm [G × C, $F(1, 16) = 5.26$, $p = 0.036$] and 4.0 mm [G × C, $F(1, 16) = 7.44$, $p = 0.015$]. However, subjects getting CAF first exhibited comparatively greater effects of CAF on rotary tracking at 45 rpm [G × C, $F(1, 15) = 5.43$, $p = 0.034$] and 60 rpm [G × C, $F(1, 11) = 24.04$, $p < 0.001$].

Taste detection thresholds for CAF, DB, and NaCl all showed some fluctuations over the course of the experiment, as shown in Fig. 7, but these were unrelated to the specific treatments. Despite the inherent variation in thresholds over time, calculations of statistical power indicate that this experiment had a 90% probability of detecting a 0.2-M change in CAF detection thresholds. A change of this magnitude among nonusers increasing their CAF intake by 450 mg/day would have been consistent with the results of Experiments 1 and 2A. Analyses also indicated no significant effects related to CAF vs. placebo ingestion on any of the perceived intensity measures (data not shown).

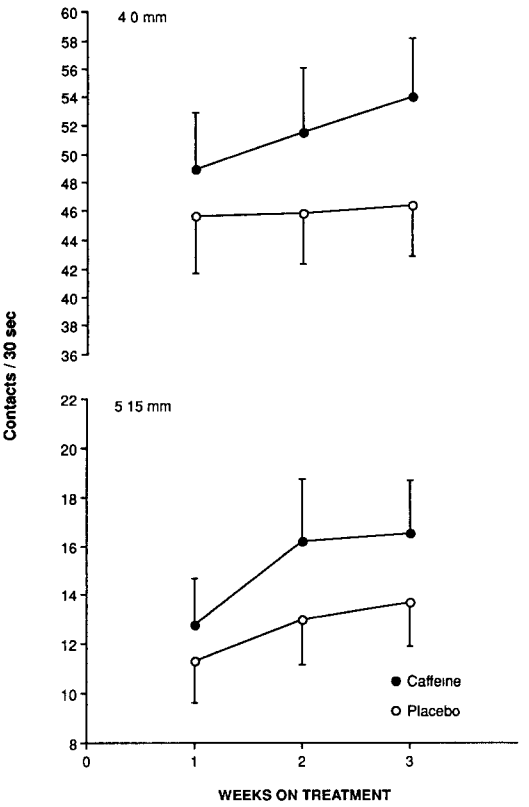

FIG. 4. Performance on a hand steadiness task at two levels of difficulty during chronic ingestion of CAF (450 mg/day) or placebo. Means ± SEM.

GENERAL DISCUSSION

These experiments document a relationship between habitual CAF intake and CAF taste detection thresholds. Observations consistent with this have also been made in a separate study on taste reactivity to CAF (Mela, unpublished). The relationship, however, does not appear to be attributable to acute or chronic CAF ingestion. Several possible explanations for these results may be considered.

Acute CAF ingestion seems particularly unlikely to affect taste perception through simple adaptation. It is clear that, even after a substantial CAF dose, taste detection thresholds consistently exceed peak salivary CAF levels by at least a factor of 10 in both CAF users and nonusers. Although use of higher doses might narrow that difference, the quantity of CAF ingested by subjects in Experiment 3 was considerably greater than would normally be encountered in a single sitting in a typical diet.

Pharmacological properties of CAF would seem a more likely mediator of the observed relationships between CAF intake and taste function. Plasma CAF levels in the 1- to 5-μM range, values well below the salivary levels observed here, elicit discriminable effects on other sensory systems (e.g., audition, vision) and mood (13,21). Antagonism of adeno-

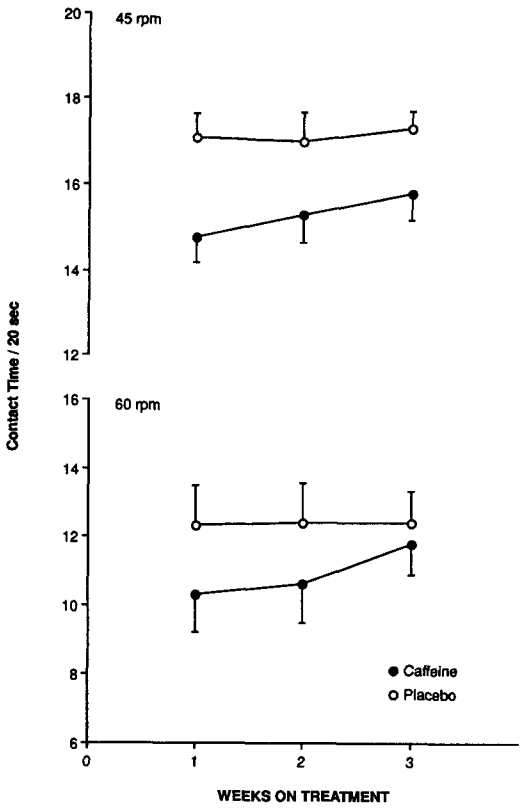

FIG. 5. Performance on a rotary tracking task at two speeds during chronic ingestion of CAF (450 mg/day) or placebo. Means ± SEM.

sine receptors is generally accepted as the primary mechanism underlying the behavioral and physiological effects of CAF (4,38).

With regard to taste, Schiffman et al. (32,33) reported that brief (4 min) exposure of the lingual surface to micromolar concentrations of CAF and other methylxanthines markedly

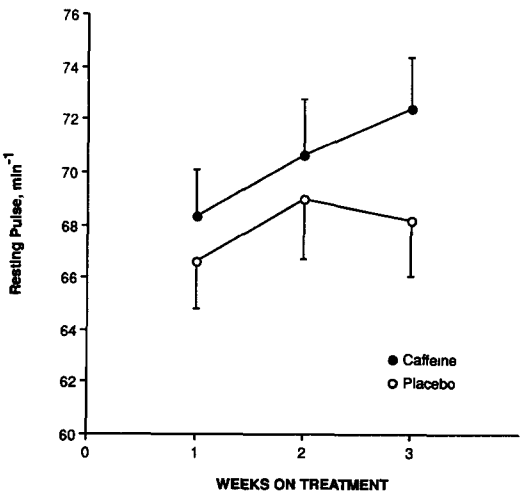

FIG. 6. Resting pulse during chronic ingestion of CAF (450 mg/day) or placebo. Means ± SEM.

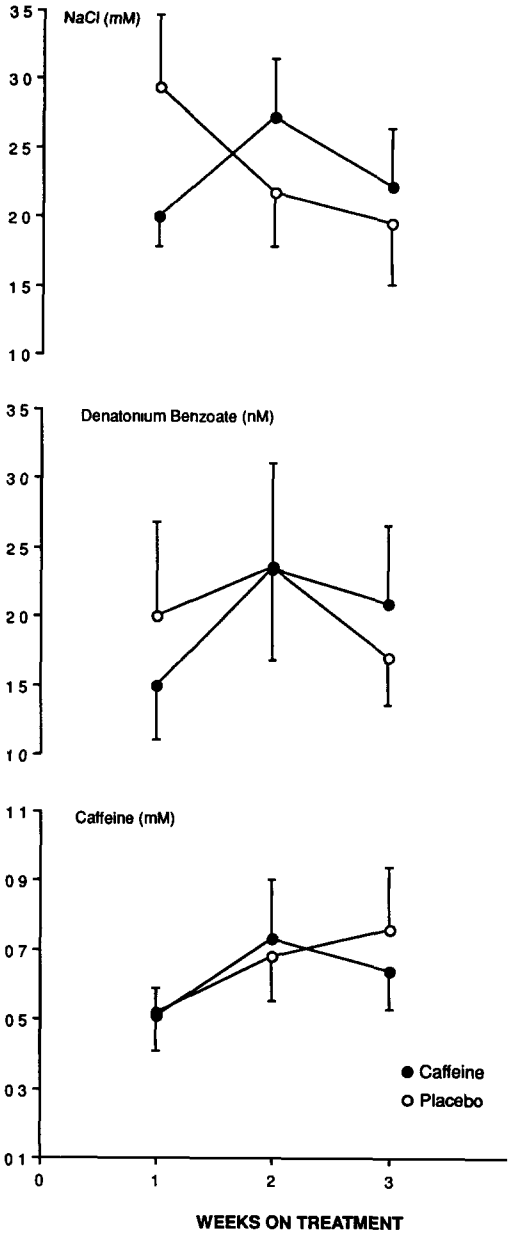

FIG. 7. Taste detection thresholds for NaCl, DB, and CAF during chronic ingestion of CAF (450 mg/day) or placebo. Means ± SEM.

enhances the perceived taste intensities of a number of sapid compounds, including quinine HCl. They suggested that lingual CAF exposure may enhance taste intensity by blocking adenosine binding and activation of putative inhibitor A_1 receptors in taste receptor cells. However, the presence or absence of such receptors on these cells has not been confirmed (J. G. Brand, personal communication), and the primary psychophysical findings have not been successfully replicated. Indeed, in a previous study (27), as well as the present work (Experiments 2A, 2B, and 4), we have been unable to confirm any relationships between lingual CAF exposure (via salivary CAF) and perceived intensity of taste stimuli. Moreover, the present studies suggest that, if anything, the presence of CAF would be associated with reduced taste sensitivity.

It is conceivable that long-term bathing of the taste receptor cells in a CAF-containing medium may modify their reception or transduction characteristics. The performance data from Experiment 4 provide evidence that at least some physiological influence was effected by CAF ingestion, yet we observed no changes in gustatory function during this period and no differences in perceived intensity in any experiment. It is possible that the period required for gustatory changes may be substantially longer than 3 weeks; however, this time period was selected because it represents about two times the typical half-life of gustatory epithelial cells. Thus, most of the cell population resident at the end of CAF exposure could be expected to have developed and matured in the presence (or absence) of CAF in the appropriate test periods. The present study involved both peripheral and central exposure to caffeine and it is possible, though unlikely, that some counterbalancing effect may exist.

It is perhaps more likely that the observed relationships between CAF intake and taste function are mediated by other components of dietary CAF sources. Coffee is by far the most significant dietary source of CAF, yet the CAF concentration of a typical cup of coffee in the United States is only 2–3 mM, ranging from about 1.5 up to 6mM (1,35), not far above the range of CAF taste detection thresholds. At these concentrations, CAF is not particularly bitter and, contrary to popular assumptions, CAF is not the major contributor to the bitter taste of coffee (7,26). There are a large number of other bitter compounds in coffee; the specific contribution of each of these to overall bitterness has not been established and may vary substantially with the source of beans and preparation of the beverage (26). Frequent exposure to these other potent bitter stimuli may mediate the relationships seen in Experiments 1 and 2A. This would not have been tested in Experiments 3 and 4, which used pure CAF rather than coffee. Similarly, differences in the composition of coffee and other CAF-containing or bitter foodstuffs might explain the dissimilar results seen in the Argentinean subject population in Experiment 2B. As previously noted, dietary and genetic influences on bitter taste sensitivity have been documented (11, 16,30,34).

Additional possibilities relate to preexisting differences and self-selection among the CAF user and nonuser populations. Hence, differences in taste function might precede and contribute to the initiation and continued use of CAF, or both intake and taste sensitivity may be manifestations of other characteristics of CAF users and nonusers, for example, level of arousal and extroversion (31). While the former view has not been tested, its importance is challenged by other evidence indicating that the relationships between taste thresholds, preferences, and intake of bitter foods are weak (17,23,24). With regard to the second possibility, a number of studies have identified links between personality measures and gustatory function, particularly bitter taste thresholds (10,12, 18,19,22,39). If this is correct, then acute or chronic administration of CAF might be expected to have little effect on taste thresholds, as observed in Experiments 3 and 4. The possibility also exists that the gustatory systems of these populations differ in sensitivity to CAF-induced changes. Although gustatory function was unaffected by chronic CAF use among the nonusers selected in Experiment 4, controlled withdrawal and reexposure of CAF users to CAF might have identified a differential response.

There were a range of different methods of gustatory function used in these experiments and a number of minor variations within the various methods. This makes absolute comparisons between experiments more difficult; however, with the exception of taste threshold data in Experiment 2B, the results were relatively consistent. Nevertheless, further clarification and verification of any gustatory effects related to ingestion of CAF (and other dietary components) is warranted.

In summary, these studies document an association between CAF intake and taste sensitivity. Attempts to demonstrate a direct influence of dietary exposure to CAF on taste were unsuccessful. We therefore suggest that the observed differences in gustatory function may not be causally related to CAF use but perhaps reflective of some other individual or environmental characteristic(s) associated with CAF ingestion. Explanations holding that CAF taste sensitivity is influenced by unidentified dietary components or that gustatory function and intake are both reflections of other individual traits remain plausible but unexplored. A better understanding of the bases for these relationships could provide novel insights on the interrelationships of sensory function, nutrition, and behavior.

ACKNOWLEDGEMENTS

The authors appreciate the technical assistance of Joan Algeo and Tracy Herbst. This work was supported in part by U.S. National Science Foundation Grant BNS 8418953 and carried out while D.J.M. was an Assistant Member and S.T. a visiting worker at the Monell Chemical Senses Center.

REFERENCES

1. Barone, J. J.; Roberts, H. Human consumption of caffeine. In: Dews, P. B., ed. Caffeine. Heidelberg: Springer-Verlag; 1984:59–73.
2. Bartoshuk, L. M. The psychophysics of taste. Am. J. Clin. Nutr. 31:1068–1077; 1978.
3. Benowitz, N. Clinical pharmacology of caffeine. Annu. Rev. Med. 41:277–288; 1990.
4. Choi, O. H.; Shamim, M. T.; Padgett, W. L.; Daly, J. W. Caffeine and theophylline analogues: Correlation of behavioral effects with activity as adenosine receptor antagonists and as phosphodiesterase inhibitors. Life Sci. 43:387–398; 1988.
5. Christensen, C. M. Importance of saliva in diet–taste relationships. In: Kare, M. R.; Brand, J. G., eds. Interaction of the chemical senses with nutrition. New York: Academic Press; 1986:3–24.
6. Christensen, C. M.; Bertino, M.; Beauchamp, G. K.; Navazesh, M. Salivary sodium levels and salt perception. Presentation at the 4th Annual Meeting of the Association for Chemoreception Sciences, Sarasota, FL, 1982.
7. Cines, B. M.; Rozin, P. Some aspects of the liking for hot coffee and coffee flavor. Appetite 3:23–34; 1982.
8. Cook, C. E.; Tallent, C. R.; Amerson, E. W.; Myers, M. W.; Kepler, J. A.; Taylor, G. F.; Christensen, H. D. Caffeine in plasma and saliva by a radioimmunoassay procedure. J. Pharmacol. Exp. Ther. 199:679–686; 1976.
9. Denaro, C. P.; Brown, C. R.; Wilson, M.; Jacob, P.; Benowitz, N. L. Dose-dependency of caffeine metabolism with repeated dosing. Clin. Pharmacol. Ther. 48:277–285; 1990.
10. Dess, N. K.; Chapman, C. D. Individual differences in taste, body weight, and depression in the "helplessness" rat model and in humans. Brain Res. Bull. 24:669–676; 1990.
11. Drewnowski, A. Genetics of taste and smell. World Rev. Nutr. Dietet. 63:194–208; 1990.

12. Fischer, R.; Ristine, L. P.; Wisecup, P. Increase in gustatory acuity and hyperarousal in schizophrenia. Biol. Psychiatry 1:209–218; 1969.
13. Griffiths, R. R.; Evans, S. M.; Heishman, S. J.; Preston, K. L.; Sannerud, C. A.; Wolfe, B.; Woodson, P. P. Low-dose caffeine discrimination in humans. J. Pharmacol. Exp. Ther. 252:970–978; 1990.
14. Hall, M. J.; Bartoshuk, L. M.; Cain, W. S.; Stevens, J. C. PTC taste blindness and the taste of caffeine. Nature 253:442–443; 1975.
15. Hartley, E.; Smith, I. J.; Cookman, J. R. Improved high-performance liquid chromatographic method for the simultaneous determination of caffeine and its *N*-demethylated metabolites in plasma using solid-phase extraction. J. Chromatogr. 342:105–117; 1985.
16. Hladik, C. M.; Robbe, B.; Pagezy, H. Differential taste thresholds among Pygmy rain forest populations, Sudanese, and Eskimo, with reference to the biochemical environment. C. R. Acad. Sci. Paris 303:453–458; 1986.
17. Jerzsa-Latta, M.; Krondl, M.; Coleman, P. Use and perceived attributes of cruciferous vegetables in terms of genetically-mediated taste sensitivity. Appetite 15:127–134; 1990.
18. Kimmel, H. L.; Lester, D. Personalities of those who can taste phenylthiocarbamide. Psychol. Rep. 61:586; 1987.
19. Knopp, W.; Fischer, R.; Beck, J.; Teitelbaum, A. Clinical implications of the relation between taste sensitivity and the appearance of extrapyramidal side effects. Dis. Nerv. Syst. 27:729–735; 1966.
20. Kuznicki, J. T.; Turner, L. S. The effects of caffeine on caffeine users and non-users. Physiol. Behav. 37:397–408; 1986.
21. Leiberman, H. R.; Wurtman, R. J.; Emde, G. G.; Roberts, C.; Coviella, I. L. G. The effects of low doses of caffeine on human performance and mood. Psychopharmacology (Berl.) 92:308–312; 1987.
22. Mascie-Taylor, C. G. N.; McManus, I. C.; MacLarnon, A. M.; Lanigan, P. M. The association between phenylthiocarbamide (PTC) tasting ability and psychometric variables. Behav. Genet. 13:191–196; 1983.
23. Mattes, R.; Labov, J. Bitter taste responses to phenylthiocarbamide are not related to dietary goitrogen intake in human beings. J. Am. Diet. Assoc. 89:692–694; 1989.
24. Mattes, R. D. Gustation as a determinant of ingestion: Methodological issues. Am. J. Clin. Nutr. 41:672–683; 1985.
25. McBurney, D. H.; Pfaffmann, C. Gustatory adaptation to saliva and sodium chloride. J. Exp. Psychol. 65:523–529; 1963.
26. McCamey, D. A.; Thorpe, T. M.; McCarthy, J. P. Coffee bitterness. In: Rouseff, R. F., ed. Bitterness in foods and beverages. Amsterdam: Elsevier Science Publishers B.V.; 1990: 169–182.
27. Mela, D. J. Caffeine ingested under natural conditions does not alter taste intensity. Pharmacol. Biochem. Behav. 34:483–485; 1989.
28. Mela, D. J. Gustatory perception of isohumulones: Influence of sex and thiourea taster status. Chem. Senses 15:485–490; 1990.
29. Morino, T.; Langford, H. G. Salivary sodium correlates with salt recognition threshold. Physiol. Behav. 21:45–48; 1978.
30. Moskowitz, H. R.; Kumaraiah, V.; Sharma, K. N.; Jacobs, H. L.; Sharma, S. D. Cross-cultural differences in simple taste preferences. Science 190:1217–1218; 1975.
31. Rapoport, J. L.; Berg, C. J.; Ismond, D. R.; Zahn, T. P.; Neims, A. Behavioral effects of caffeine in children. Arch. Gen. Psychiatry 41:1073–1079; 1984.
32. Schiffman, S. S.; Diaz, C.; Beeker, T. G. Caffeine intensifies taste of certain sweeteners: Role of adenosine receptors. Pharmacol. Biochem. Behav. 24:429–432; 1986.
33. Schiffman, S. S.; Gill, J. M.; Diaz, C. Methyl xanthines enhance taste: Evidence for modulation of taste by adenosine receptor. Pharmacol. Biochem. Behav. 22:195–203; 1985.
34. Smith, S. E.; Davies, P. D. O. Quinine taste thresholds: A family study and a twin study. Ann. Hum. Genet. 37:227–232; 1973.
35. Stavric, B.; Klassen, R.; Watkinson, B.; Karpinski, K.; Stapley, R.; Fried, P. Variability in caffeine consumption from coffee and tea: Possible significance for epidemiological studies. Food Chem. Toxicol. 26:111–118; 1988.
36. Stevens, J. C.; Marks, L. E. Cross-modality matching functions generated by magnitude estimation. Percept. Psychophys. 27: 379–389; 1980.
37. Stevens, J. C.; Plantinga, A.; Cain, W. S. Reduction of odor and nasal pungency associated with aging. Neurobiol. Aging 3:125–132; 1982.
38. von Borstel, W. R.; Wurtman, R. J. Caffeine and the cardiovascular effects of physiological levels of adenosine. In: Dews, P. B., ed. Caffeine. Heidelberg: Springer-Verlag; 1984:142–150.
39. Whittemore, P. B. Phenylthiocarbamide (PTC) tasting and reported depression. J. Clin. Psychol. 42:260–263; 1986.
40. Zylber-Katz, E.; Granit, L.; Levy, M. Relationship between caffeine concentrations in plasma and saliva. Clin. Pharmacol. Ther. 36:133–137; 1984.