# THE EFFECTS OF CAFFEINE AND ASPARTAME ON FOOD INTAKE AND ANXIETY IN THE RAT

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# The Effects of Caffeine and Aspartame on Food Intake and Anxiety in the Rat

(c)Carolyn A. Fitzpatrick

A thesis submitted to the School of Graduate Studies in partial fulfilment of the requirements for the degree of Master of Science

Department of Psychology
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## Abstract

The present study investigated the effects of chronic low and high dose caffeine and aspartame on food intake and anxiety like behaviour in male Wistar rats. Anxiety-like behavior was assessed in the holeboard and elevated plus maze. Rats received caffeine ($3.125 \times 10^{-1}$ or $9.375 \times 10^{-1}$ mg/ml/day) and aspartame (2.5 or 7.5 mg/ml/day) in their drinking water for 7 days. Rats were then tested in the holeboard and elevated plus maze. High caffeine with and without aspartame decreased food intake relative to vehicle controls. However, rats treated with high caffeine alone developed a tolerance to the anorectic effects of caffeine while low or high doses of aspartame blocked this tolerance to high caffeine. A low dose of caffeine given with a low dose of aspartame synergistically reduced feeding. This suppression was transient. There were no anxiogenic effects in the holeboard and plus maze tests for any group, although baseline anxiety levels were high for all groups. Future research should examine the feeding suppression effects by high doses of caffeine over an extended period in addition to carefully controlling rats' basal levels of anxiety.

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# APPETITE AND ANXIETY EFFECTS BY ASPARTAME AND CAFFEINE IN RATS

## Introduction

Some 250-300 aspartame-sweetened products are available on the national market (Keegan, personal communication, 1992). Many of these products also contain caffeine (Keegan, personal communication, 1992). Caffeine is consumed in moderate amounts in the general population (Krahn, Hasse, Ray, Gosnell & Drewnowski, 1991). There are a number of reports that caffeine and aspartame independently produce anorectic effects in human and animal subjects (Stacher, 1986; Le Sauter, Goldberg & Geary, 1988; Wolkowitz, Gertz, Weingartner, Beccaria, Thompson & Liddle, 1990). In addition, caffeine has demonstrated anxiogenic properties (Baldwin & File, 1989). Since caffeine and aspartame are frequently ingested together, it is of interest to investigate if they interact.

In the first section, a brief introduction to the appetite suppressant effects of aspartame will be discussed. Following this, a brief introduction to the molecular and chemical properties of aspartame will be outlined. Finally, a review of the pharmacology of aspartame as it applies to appetite will be provided.

## Aspartame: Evidence for Feeding Reduction in Humans and Animals

There is direct evidence for an appetite suppressant effect by aspartame for humans and animals. This body of research has been conducted in response to the potential use for aspartame as a sucrose substitute in weight loss regimens. However, the findings have often been inconclusive due to the insufficient period allotted to intake measurement following an aspartame-containing regimen. The results of some of the studies designed to address this possibility will be outlined with humans and rats.

Porikos and Van Itallie (1982) examined the long term dietary response to aspartame ingestion in healthy volunteers. These researchers investigated the intake of 13 obese and 11 non-obese subjects as inpatients on a metabolic ward over periods of 15-30 days.

Three separate studies were conducted to assess any long term dietary adjustments by participants in response to a diet covertly reduced in calories with aspartame. In all experiments, subjects were offered an ad libitum meal and snack service. Consumption was weighed and caloric determinations of intake were computed.

Each study employed an ABA design where a baseline sucrose-containing diet was provided in the first and third periods of the study. In the second period, sucrose was covertly substituted with aspartame.

Across the three studies, caloric intake during the first three days of the aspartame diet was approximately 75% of baseline measures. From days 4-6 on aspartame, intake rose to 86% of baseline and stabilized. These intake patterns were similar for both obese and normal weight subjects.

Studies with animals using the same paradigm as for humans were conducted to assess the food intake suppressant properties of aspartame. Porikos and Van Itallie (1986) used 15 female Sprague-Dawley rats to assess intake using energy dilution with water. Rats were assigned to dietary obese or control conditions. Dietary obese rats were 37% overweight relative to controls. A baseline diet was formulated consisting of 58% carbohydrate in the form of sucrose. Rats were maintained on this diet for 30 days prior to caloric dilution of 20 or 40% by aspartame replacement. In the case where dilution was by 20%, both obese and control rats did not alter their intake. However, when dilution was by 40%, controls ate 76% and obese 74% of their baseline calories. Intake between control and obese groups did not differ significantly.

To sum, there is preliminary evidence for the direct influence of aspartame on consumption at the behavioral level. The findings of Porikos and Van Itallie (1982) demonstrate the need to examine intake over extended periods of time in a controlled environment. In addition, these studies show that it is necessary to investigate a subject's extended pattern of intake following aspartame administration, not just the amount of calories consumed at the next intake opportunity.

Aspartame: Methods to examine effects on consumption

Three methodological approaches have been used to investigate the effects of aspartame upon hunger, food intake, and body weight in humans. Each method differs in the degree to which accurate behavioral predictions can be formulated. Consequently, the results of such studies are often ambiguous and/or lack generalizability.

In the first method, the investigation takes place in the laboratory. The typical paradigm involves a comparison of short-delay consumption between two groups of subjects. One group receives an aspartame-sweetened preload and the other group receives the equivalent sucrose-sweetened preload. Intake is measured in a free selection situation after the preload. This method does not regard compensatory consumption beyond the experimental session. Nor does it control for a subject's satiety level prior to the experiment. Information

provided by this method is restricted to the immediate effects of aspartame upon subsequent ingestion.

For example, Brala and Hagen (1983) compared the effects of adding aspartame or sucrose to milk shakes on intake of protein and snack foods by male and female subjects in a session immediately following milk shake consumption. Subjects were then asked to rate their hunger. Intake was not influenced by the caloric differences between milk shakes. It is possible that subjective hunger differences could not influence subsequent intake using such a short delay. The researchers should have examined caloric regulation at post experiment intervals.

A second method to examine aspartame's effects entails an examination of natural eating and drinking behaviour. The advantage of this method is that the effects of aspartame can be studied in a naturalistic dietary environment (i.e., the effects of aspartame are examined as part of one's overall macronutrient intake). However, reliance upon self report measures of intake may provide inaccurate information concerning the effects of aspartame on eating behaviour. In addition, this method can only provide correlational evidence for the effects of aspartame. The hypothesis is that the addition of aspartame as a calorically-dilute sucrose substitute would facilitate compliance to a weight-loss diet

(Kanders, Lavin, Kowalchuk, Greenberg & Blackburn, 1988). Fifty-nine obese free-living men and women were randomly assigned to an isocaloric weight loss regimen with or without aspartame-containing substances. For Weeks 1-3 all subjects were instructed to avoid the use of aspartame-containing products. From Weeks 4-12, subjects were placed on their aspartame or no aspartame diets. At the end of a 12 week period, a weight loss of 11% for males and 7% for females was observed. These results were clinically significant (Kanders et al., 1988). Aspartame consuming groups lost slightly more weight than the nonaspartame group. While it was concluded that aspartame was not directly responsible for weight loss in the aspartame-consuming group, it was noted to enhance compliance to the experimental diet by adding variety and increasing the palatability of foods consumed.

The results of Kanders et al. (1988) indicate the need to validate the role of aspartame in facilitating weight loss in outpatient clinical trials. Self report may have also influenced the results of the study. Future research should attend to a possible motivational role of aspartame for the facilitation of dietary compliance.

A third approach examines the patterns of use of aspartame and its effects on weight change over a prolonged time interval. This type of prospective study can serve to

define patterns of sweetener use and to predict its health consequences (Stellman & Garfinkel, 1988). Again, this type of research is correlational and, therefore, cannot directly implicate aspartame as a mechanism for altering eating behaviour.

Stellman and Garfinkel (1988) examined patterns of artificial sweetener use and weight change in 78,694 women ages 50-69 years. The percentage of users increased with body mass index and decreased with age. 21.6% of these subjects used aspartame. In the year prior to the study, (1981-82) the percentage of women who gained weight was greater in the user versus non-user group at every level of body mass index. For the subjects who lost weight during that year, the amount lost differed between users and non-users only among obese participants (Stellman & Garfinkel, 1988). Food consumption patterns differed between aspartame consumers and nonconsumers. Aspartame users ate more calorically dilute foods while nonusers ate more calorically-dense food items. However, these authors did not report serving size.

Stellman and Garfinkel (1988) acknowledged the need to undertake a controlled clinical study in order to examine the putative effects of aspartame on eating behaviour. It would be necessary to ensure an accurate measure of intake (overall

calories) between aspartame users and nonusers in future experiments.

In summary, it is difficult to assess the direct effects of aspartame on intake behaviour without being able to assess accurately subjects' caloric intake. However, the artificiality of confining individuals to experimental settings to study intake creates a problem for the external validity of a study. In order to interpret aspartame's behavioral effects, it is also necessary to examine the chemical properties of aspartame and to examine the mechanisms of its actions.

Aspartame: An Introduction

Aspartame (L-aspartyl- -L-phenylalanine methyl ester) is a synthetic sweetening compound (Stegink & Filer, 1984). It's sweetness has been found to be inversely related to sucrose concentration (Stegink & Filer, 1984). Aspartame is 100-200 times a more potent sweetener than sucrose (Stegink & Filer, 1984). Thus, a small concentration of aspartame can add sweetness without providing the energy of sucrose. This characteristic of aspartame is advantageous because it reduces an individual's overall daily energy intake by caloric depletion (Anderson & Leiter, 1988). Body weight reduction is a potential consequence of lowered intake. In addition,

individuals who consume products with aspartame reduce their risk of dental caries (Stegink & Filer, 1984).

The former advantage will now be elaborated upon in consideration of both pre- and post-absorptive satiety signals. Following this, the theoretical mechanisms by which aspartame could suppress appetite will be discussed.

## Pharmacology of Aspartame: Pre- and Post-Absorptive Signals

Anderson and Leiter (1988) suggest that the amino acid composition of aspartame contributes to a decrease in feeding following ingestion of the compound. Aspartame is broken down in the lumen to L-phenylalanine, L-aspartic acid and methanol (Stegink & Filer, 1984).

Phenylalanine may act to provide pre-absorptive signals to the brain in two ways. First, it can stimulate amino acid receptors on vagal nerve fibres in the duodenum by release of CCK (Jeanningros, 1982), a peptide present in both the peripheral and central nervous systems (Weller, Smith & Gibbs, 1990). It has both gastrointestinal and behavioral effects.

## Aspartame: Mechanisms of Appetite Suppression

The food inhibition effect which accompanies aspartame ingestion has been explained by a cholecystokinin mediation

hypothesis (Rogers, Pleming & Blundell, 1990). There is evidence that CCK does reduce food ingestion when administered via chronic gastric cannulas.

Le Sauter, Goldberg & Geary (1988) tested real and sham feeding in gastric vagotomized rats. For rats food deprived for 18 hours, 0.3-4 g/kg of CCK administered by cannula inhibited food intake. Le Sauter et al. (1988) then examined food intake after doses of 0.5-4 g/kg in rats that received a total vagotomy, gastric vagotomy or a control operation. The feeding inhibition effect was present with equal potency for gastric vagotomized and control animals but was not detected after total vagotomy. However, total vagotomy did not block the inhibitory effect of a dose of 6 g/kg CCK. Le Sauter et al.'s (1988) findings suggest that abdominal vagal fibres mediate the satiety effect of CCK released by phenylalanine. Cholecystokinin is a peptide that is present in both the peripheral and central nervous systems (Weller, Smith & Gibbs, 1990). It has both gastrointestinal and behaviourial effects. Novin, Sanderson & Gonzalez (1979) demonstrated that the depression of food intake which occurs after amino acid infusion does not take place in vagotomized rats. This finding supports the conclusion that abdominal fibres mediate the satiety effect of CCK.

Aspartame and specific appetites

Wurtman (1983) has speculated that aspartame could increase appetite when consumed in conjunction with a high carbohydrate source for the following reasons. Carbohydrate ingestion produces a release in insulin. Insulin release could be responsible for aspartame increasing the uptake of amino acid into tissues (Wurtman, 1983). This increase is less for tryptophan than for other amino acids (Wurtman, 1983). Therefore, plasma tryptophan increases, and competition for the transport mechanisms across the blood-brain barrier for large neutral amino acids, including tryptophan, also increases. Consequently, more tryptophan is carried across the blood-brain barrier. Since tryptophan is a precursor to serotonin, brain serotonin levels increase and carbohydrate ingestion is suppressed (Wurtman, 1983). Aspartame ingestion increases blood phenylalanine (PHE) and tyrosine levels (Stegink & Filer, 1984). PHE and tyrosine are large neutral amino acids which compete with tryptophan for brain transport (Fernstrom, 1988). When aspartame is consumed with carbohydrates, tryptophan uptake and serotonin synthesis may be attenuated. Consequently, the decrease in serotonin synthesis and release would minimize the suppression of carbohydrate appetite (Fernstrom, 1988).

Other investigators contend that carbohydrate ingestion is not modulated by a negative feedback loop involving serotonin (e.g., Holder & Huether, 1990; Holder & DiBattista, 1994). Rather it is the combined taste and sensory properties of macronutrients that predict their subsequent ingestion. For example, Holder and Huether (1990) altered plasma amino acid levels and brain serotonin levels by injecting insulin (2.4U/kg) (ip) or tryptophan (40mg/kg) (ip) in rats. Insulin increased plasma tryptophan and the ratio of tryptophan to other large neutral amino acids , therefore decreasing brain serotonin while tryptophan injections increased serotonin.

According to this theory of carbohydrate ingestion, it was hypothesized that tryptophan injections should selectively decrease carbohydrate consumption relative to protein intake (Holder & Huether, 1990). This hypothesis was not supported by behavioral data. Although tryptophan injections reliably increased brain serotonin levels, they did not decrease carbohydrate consumption relative to pellets high in protein. These investigators suggested that food selection may not be strongly controlled by brain serotonin levels. Further, they suggest that the type of macronutrient and its sensory qualities may better serve to determine what quantity will be consumed.

Holder and DiBattista (1994) examined the effects of time-restricted access to protein and of oral-sensory cues on protein selection. Access to protein was manipulated (1 hour with access only to a carbohydrate-fat diet or continuous access), and the type of protein provided was controlled (soy-based or casein-based). Rats whose protein access was restricted consumed a greater amount of protein when available than did rats whose access was not restricted. Rats that had unlimited access to protein consumed 60% more protein during a one hour test period when the type of protein was different at the time of testing (Holder & DiBattista, 1994).

Together, these results led Holder & DiBattista (1994) to conclude that oral-sensory qualities of the food, rather than brain serotonin levels, are more important for regulation of food intake. If this is true, then Wurtman's speculation that aspartame would increase food intake are in error, and one would not expect aspartame to alter food consumption.

Anderson and Leiter (1988) argue that future research should attend to the effects of artificial sweeteners on food intake rather than examining their influence upon constituent amino acids. Anderson and Leiter (1988) suggest that the effects of aspartame upon appetite cannot be assessed by measuring simultaneous consumption of other substances or consumption immediately after ingestion. Therefore, it is

important to examine consumption over an extended period of time. In addition, the consistent failure to find support for complete caloric compensation in immediate or short delay consumption suggests further that the time period for consumption measurement after aspartame ingestion is too short. Alternatively, compensation mechanisms may be limited.

Caffeine: An Introduction

Caffeine is a member of the methylxanthine class of stimulant drugs (Holtzman, 1986). It is found in coffee, tea, cola beverages, diet medications and sleep-prevention compounds (Murray, 1988). In humans, caffeine increases anxiety, nervousness and tension while it decreases performance on fine motor tasks (Baldwin & File, 1989). In addition, it can enhance performance in many learning and cognitive tasks.

Caffeine has appetite suppressant, diuretic and stimulant effects (Fahey & Treasure, 1991). Although caffeine has been deemed safe when used in moderate amounts (Murray, 1988) there is a potential for caffeine to produce harmful consequences.

The next section will provide a review of the investigational procedures employed to assess appetite suppression. In addition, the role of caffeine-induced anxiety in feeding reduction will be proposed.

## Caffeine and appetite suppression: Experimental evidence

There are a number of studies which indicate that the food intake of rats diminishes following caffeine administration. Dietze and Kulkosky (1991) investigated the effects of combined administration of caffeine and bombesin on ethanol and food intake in deprived rats. In single doses, 50 g/kg and 10 g/kg respectively, ethanol and food consumption were significantly decreased (Dietze & Kulkosky, 1991). The decrease in ethanol intake was less than the expected additive effect while the food intake depression represented a simple additive effect (Dietze & Kulkosky, 1991). These authors concluded that caffeine could affect endogenous satiety signals for alcohol intake (Dietze & Kulkosky, 1991).

## Caffeine: Central and Behavioral Effects

Caffeine affects the benzodiazapine and catecholamine systems (Nakamoto, Roy, Gottschalk, Yazdani & Rowsowska, 1991). At a dose equivalent to ten cups of coffee, 20% of benzodiazapine receptors may be blocked (Paul, Marangos, Goodwin & Slotnik, 1980). However, Snyder (1984) reports that this blockade is insufficient to affect behaviour. Caffeine influences catecholamine neurons in the peripheral and central

nervous systems. Caffeine inhibits calcium independent phosphodiesterase (Choi, Shamim, Padgett & Daly, 1988) and blocks adenosine receptors (Popoli, Caporali & de Carolis, 1989). Endogenous adenosine may exert a tonic inhibition on the dopaminergic system. The blocking of adenosine receptors by caffeine would potentiate dopamine functioning and could induce hyperactivity (Nakamoto et al., 1991).

In addition to hyperactivity, caffeine induces anxiety. Baldwin and File (1989) found that rats injected with caffeine (20 and 40 mg/kg/day) displayed less social interaction than control animals. The effects of chronic administration of caffeine in rat's drinking water were also examined (File, Baldwin, Johnston & Wilk, 1987). Rats were given 50 or 100 mg/kg/day of caffeine for a 21 day period. A subsequent acute dose of caffeine (20 or 40 mg/kg) was administered (ip) to the animals (File et al., 1987). Anxiogenic effects were assessed using the holeboard apparatus (File et al., 1987). A variety of behaviours may be monitored using this device including the number of holes explored, the number of head-dips made by the animal (both eyes disappearing into the hole) and activity (amount of time spent moving about the holeboard) (File & Wardill, 1975). Acute administration at the low dose of caffeine increased motor activity. The higher dose given acutely reduced head-dipping behaviour (File et al., 1987).

Baldwin and File (1987) suggest that caffeine does not produce an effect at either adenosine or benzodiazapine receptors. Though the researchers were able to block the anxiogenic effect of caffeine in the social interaction test using the anxiolytic chordiazepoxide, they concluded that chordiazepoxide reversed caffeine's effects in a non-competitive manner (File & Baldwin, 1988).

In addition, Baldwin and File (1988) attempted to reverse the anxiogenic action of caffeine in the social interaction test with 2-chloradenosine, an adenosine receptor antagonist. The antagonist was ineffective.

Meliska, Landrum and Landrum (1990) investigated the effects of chronic and subchronic caffeine administration on wheelrunning in rats. Meliska et al., (1990) compared the effects of repeated oral caffeine ingestion when administration was chronic (consecutive days) and subchronic (alternate days). Meliska et al. (190) hypothesized a tolerance effect for locomotor stimulation with chronic administration while they anticipated an increase in stimulation with subchronic administration.

There were three treatment conditions: chronic caffeine access (24 hour of 0.5 mg/ml hydrous caffeine alkaloid), subchronic caffeine access (24 hour 0.5mg/ml caffeine solution alternating with 24 hour tap water) or continuous access to ta

water. Baseline wheelrunning was taken for 2 days with rats with rats given tap water. Drinking bottles were weighed daily. Consumption of 1.0 ml was deemed equivalent to a reduction of 1.0 gram of bottle weight (Meliska et al., 1990). Percentage of baseline wheelrunning was recorded for 16 days (Meliska et al., 1990). Rats receiving subchronic caffeine administration were significantly more active than those receiving continuous caffeine in the last half of the experimental testing period (sessions 9, 11, 13 and 15). In addition, the subchronic caffeine rats demonstrated significant increases in wheelrunning behaviour when compared with the tap water only group. Continuous caffeine access rats did not differ significantly from rats given tap water. Meliska et al. (1990) explained the observed differences in terms of tolerance and sensitivity effects. Meliska et al. (1990) reported that sensitization occurs with a 24 hour-on/off alternating regimen of oral caffeine access when rats are provided with continuous opportunity for wheelrunning, while complete tolerance to the locomotor stimulating effects of caffeine occurs when caffeine is administered chronically. Meliska et al. (1990) proposed that daily exposure to caffeine leads to metabolic, drug-dispositional or pharmacodynamic alterations that do not develop when administration is given on alternate days. This finding is important when considering

the potential for the interaction of caffeine and aspartame. It is possible that not only dose magnitude but also the temporal spacing of doses influence whether or not behavioral effects are observed when caffeine and aspartame are ingested together. Therefore, it may be important to evaluate the chronicity of the dosage levels of caffeine and aspartame to investigate feeding suppression and anxiety effects by these compounds. There could be a relationship between feeding and anxiety that is only exhibited when there is a sustained metabolic or pharmacodynamic response to caffeine and aspartame.

Feeding and Anxiety: A potential relationship

Several studies have examined the relationship between feeding and anxiety. Herman and Polivy (1975) examined a two way relationship between eating behaviour and anxiety in humans. They dichotomized obese and normal subjects by eating restraint levels. Low and high anxiety was induced by the threat of a mild or painful electric shock, respectively. In a posttest, they assessed the efficacy of eating as an anxiety-reducing mechanism. Herman and Polivy's (1975) findings indicated that subjects who were classified as unrestrained eaters ate significantly less when anxious while restrained eaters ate slightly more. The latter difference was not significant (Herman & Polivy, 1975). For all

subjects, eating did not reduce anxiety level significantly. Therefore, anxiety can decrease feeding when cognitive restraint level does not influence intake. On the other hand, there is little empirical support that eating decreases anxiety.

In another study, Herman, Polivy, Lank & Heatherington (1987) examined the effects of anxiety and food deprivation on food consumption of dieters and nondieters. They determined that anxiety effectively suppressed hunger in nondieters only when these subjects were initially hungry. However, for dieters, the effect was reversed: anxiety increased hunger when subjects were initially hungry.

The results of Herman and Polivy (1975) and Herman et al. (1987) demonstrate the need to examine the relationship between feeding and anxiety both in low and high satiety and anxiety states. Therefore, it is important to determine both the independent effects of caffeine and aspartame and their interactive effects. It is possible that different levels of these compounds differentially affect anxiety. If this is the case, then the relationship between feeding and anxiety may not be a linear one.

Interactive Effects of Caffeine and Aspartame: Behavioral Evidence

The evidence reviewed suggests that ingestion of aspartame and caffeine may suppress feeding and potentiate anxiety. Since these compounds are frequently consumed together (Keegan, personal communication, 1992) it is of interest to investigate whether the effects of caffeine and aspartame interact. It is anticipated that high doses of aspartame or caffeine alone will suppress appetite in rats, but that low doses will not. It is possible that combining lower doses of caffeine and aspartame will produce synergistic effects on behaviour, however. These possibilities will be tested in the proposed research.

Although the anxiogenic properties of caffeine are known, the literature does not cite evidence that aspartame may have anxiety-producing potential. It is important to determine if this is the case. In fact, there are reasons to believe that aspartame might be anxiogenic. First, CCK release is a product of aspartame catabolism and CCK has been shown to be anxiogenic (Woodruff & Hughes, 1991). Second, CCK antagonists are known to possess anxiolytic action in caffeine induced anxiogenesis (Baldwin & File, 1989). If aspartame is an anxiogenic compound, then CCK might mediate this effect.

Moreover, if both the anxiogenic effects of caffeine and aspartame involve CCK, then these two compounds might be expected to act synergistically.

Therefore, another goal of the proposed work is to determine the anxiogenic potential of aspartame alone or in combination with caffeine.

It is also of interest to know how the anorectic effects of caffeine and aspartame are produced. Appetite suppression may occur by the direct influence of CCK on feeding. On the other hand, feeding could be reduced indirectly by an increase in anxiety. A further goal of this thesis is to investigate these possibilities.

## Method

### Subjects

A total of seventy two juvenile albino Wistar rats (Charles River, Quebec) were used for the study. Rats were 30-45 days old at the start of the experiment. Subjects were housed individually in wire mesh hanging cages (22 x 24 x 22 cm). Rats were maintained on a twelve hour light:dark cycle with lights on at 0300. The room temperature was held constant at 20 C $\pm$1 C. Water was continuously available in glass water bottles attached to the side of each cage. Animals were given unlimited access to pelleted rat chow (Purina Laboratories). The food dispenser provided access to 2 gram rat pellets. Consumption was calculated to the nearest 0.1 gram after subtracting spillage.

### Drugs

Caffeine-sodium benzoate (50:50 (w/w): Sigma Chemical) was dissolved in tap water. Doses of $3.125 \times 10^{-1}$ mg/ml and $9.375 \times 10^{-1}$ mg/ml were prepared for oral consumption. Aspartame (N-L- -aspartyl-L-phenylalanine methyl ester: The NutraSweet Company) doses of 2.5 mg/ml and 7.5 mg/ml were used for oral administration. Aspartame was dissolved in tap water. All doses were prepared using 40mL of water solvent, according to baseline drinking levels.

Apparatae

A wooden holeboard was used to examine activity and exploratory behaviour. The holeboard was a square wooden box 60 cm on a side with four sides rising 35 cm above the floor of the box. The floor of the box was elevated 12 cm above the ground. The holes were 3.8 cm in diameter. They were placed at the corners of a square drawn inside the box, with sides 14 cm from the walls of the holeboard. The entire apparatus was painted with flat grey enamel paint. A video camera was positioned above the holeboard to permit videotaping of behaviour.

A wooden elevated plus maze was also used to investigate exploration, activity and anxiety-like behaviour. The apparatus consisted of four equal-length arms arranged in the shape of a plus sign. Each arm was 10 cm wide, 50 cm long, and elevated 50 cm above the ground. The four arms were joined at the centre by a 10 cm square platform. Two of the arms opposite each other had no sides. The other two arms were closed with walls 40 cm high, but open on top. The walls did not extend into the centre of the maze. The maze was painted with flat grey enamel paint. A second video camera was positioned above the plus maze so that behaviour could be videotaped.

Procedures

Photoperiod Adjustment

The 72 rats were divided into 9 equal groups. A seventeen day photoperiod adjustment time was allowed before testing began to adapt the animals to the five hour shift in the 12 hour light:dark cycle. The purpose of the shift was to enable the researcher to conveniently record consumption measures during the dark phase of the cycle. Food and water intake were recorded daily after the first hour of the dark phase of the cycle for two seven day sessions. Nine days separated the two sessions.

Food Intake Measurement

Baseline consumption levels were measured in Session 1. Six 24 hour consumption measures were obtained for each animal. Each rat received one drug combination throughout Session 2. Rats were assigned to one of nine treatment conditions on the basis of food and water consumption in Session 1. Assignment was such that all groups showed equivalent baseline water and food intake. Three groups were given vehicle (tap water) or two doses of caffeine ($3.125 \times 10^{-1}$ mg/ml [low dose] and $9.375 \times 10^{-1}$ mg/ml [high dose]). Three groups were given vehicle or two doses of aspartame (2.5 mg/ml [low dose] or 7.5 mg/ml [high dose]). The last four groups were given combined doses of caffeine and aspartame as

caffeine with low dose aspartame, high dose caffeine with high dose aspartame, low dose caffeine with high dose aspartame and, high dose caffeine with low dose aspartame.

Six twenty four hour consumption measures were collected in Session 2 for each rat. All animals were then weighed on the last day of the experimental period.

Behaviour Testing

For anxiety testing, all groups were halved. One half of the animals were tested while still consuming caffeine and/or aspartame. In these rats, anxiety was tested 24 hours after the last intake measurements. The other half of the rats were tested 48 hours after the last intake measurements and 24 hours after having been taken off caffeine and/or aspartame.

All animals were then placed individually in the centre of a holeboard for five minutes. Immediately after the holeboard test, behaviour in the plus maze was tested. Each rat was placed in the centre of the maze facing an open arm. Behaviour was videotaped for 5 more minutes. Then the rats were returned to their home cages. Testing in the holeboard and maze took place between the hours of 3pm and 12am.

Behavioural measures taken in the holeboard included the number of head-dips, rearings, and the total activity. A head-dip was counted when the rat dipped its head into one of the holes with both eyes disappearing into it. Rearing was

measured when a rat stood on its hind paws with both front paws elevated. The total activity was considered to be the sum of the times that a rat was non-still. The holeboard was employed because it permits the separation of directed exploration (head-dipping) from locomotor activity and rearing (File and Wardill, 1975). A comparison of the holeboard and plus maze tests allows the determination of what degree behaviour on the plus maze reflects changes in the exploration or locomotor activity of the animals (Pellow, Chopin, File & Briley, 1985).

Two measures of anxiety-like behaviour were taken. The first was ratio of the number of entries into the arms of the maze divided by total entries to any arm of the maze (Ratio Entry). The second was time spent in the open arms of the maze divided by time spent in any arm of the maze (Ratio Time). A measure of activity/exploration was total arm entries.

**Statistical Analysis**

Solution and Food Consumption

Consumption measures were each divided into two sessions. Session 1 covered baseline intake over Days 1-6. Session 2 included consumption measures over Days 1-6 after the start of solution administration.

Changes in solution and food consumption across days was assessed separately for Sessions 1 and 2. In both instances a two way analysis of variance was used assessing group and day effects. There were no effects for Session 1 for either measure. Therefore, a single baseline value averaged over the six days of Session 1 was computed for each group's solution and food consumption.

In the Session 2 analysis, there were 7 levels of the days variable. The first "day" was the Session 1 baseline average. The remaining days were the six 24 hour measures taken in Session 2. Group differences were assessed post hoc with Duncan's multiple comparisons test.

**Anxiety Tests**

For the analysis of hole board and plus maze data, rats from each group were assigned to one of two levels of a Time variable. Animals that still had access to caffeine and/or aspartame were assigned to Time A. These rats were tested 24 hours after their last bottle of water containing caffeine and/or aspartame was given to them. Time B included animals whose caffeine and/or aspartame solutions had been exchanged for tap water 24 hours prior to testing. Two way ANOVA procedures were employed to investigate time and group

differences in the measures taken in the plus maze and hole board tests.

### Intake and Anxiety Correlations

The relationship between feeding and anxiety was investigated with Pearson Product moment correlation. Correlations were computed between food intake and Ratio Time and Ratio Entry scores from the plus maze. Six sets of correlations were calculated for each of the six days of Session 2.

## Results

### Dose Consumption for Session 2

Solution consumption for each of the six days of Session 2 was converted to dose (mg/kg). Dosing of caffeine and aspartame were analyzed separately. Two way ANOVA were used to assess differences between independent groups and repeated measures over days. Groups included in the aspartame consumption analysis were those receiving aspartame alone or in combination with caffeine. Groups included in the caffeine consumption analysis were those receiving caffeine alone or in combination with aspartame.

There were significant differences in dosing between groups for caffeine [$F(5,42)=28.10$, $p<.001$], and for aspartame $F[(5,42)=99.36$, $p<.0001]$. However, dosing was constant over days for all caffeine and aspartame conditions. Therefore, dose measurements for each condition were collapsed over Session 2 and then averaged. Group means were compared using Duncan's Multiple Range test ($p<.05$) (Figure 1).

For aspartame consumption, rats given a high dose of aspartame alone (high aspartame) or in combination with a low dose of caffeine (low caffeine/high aspartame) consumed equivalent doses of aspartame. Together these groups consumed more aspartame than all other groups. Rats given a low dose of aspartame alone (low aspartame ) or in combination with a low dose of caffeine (low caffeine/high aspartame) consumed equivalent doses of aspartame. Together these groups consumed less aspartame than all other groups given aspartame alone or in combination with a low dose of caffeine.

Though low doses of caffeine were without effect on aspartame consumption, high doses of caffeine did affect aspartame intake. Rats given a low dose of aspartame in combination with a high dose of caffeine (high caffeine/low aspartame) consumed less aspartame than rats given a low dose of aspartame alone, and less than all other groups. A similar suppressive effect of caffeine on aspartame consumption was

observed for the rats given a high dose of aspartame with a high dose of caffeine (high caffeine/high aspartame). These animals drank less aspartame than the other high aspartame groups, but more than the low aspartame groups (Figure 1).

In contrast, aspartame was without effect on caffeine consumption, while the expected differences in caffeine intake in high and low caffeine groups were observed. Animals given a high dose of caffeine consumed more caffeine than low caffeine rats. Moreover, caffeine consumption of rats given a high dose of caffeine alone or in combination with low or high doses of aspartame did not differ. Similarly rats given low doses of caffeine alone or in combination with low or high doses of aspartame did not differ. Together these low dose caffeine groups consumed less caffeine than the high dose caffeine groups.

**Overall Nutrient Intakes**

**Baseline Intake**

As described in the analysis section of the methods, food intake over Days 1-6 of Session 1 was stable and equal in all groups (Figure 2).

**Intake over sessions 1 and 2**

There was a significant group x days interaction [$F(378,48)=1.82$, $p<.01$]. Multiple t-test (2 tailed) planned

comparisons were then computed to determine when Session 2 values differed from baseline and vehicle control (Figure 3). Duncan's Multiple Range Test ($p<.05$) was used to determine group differences in food intake between groups other than vehicle on each day of Session 2.

**Vehicle control group consumption.** Vehicle controls consumed at their own baseline and that of all other groups over all days of Session 2. Therefore means of groups given aspartame and caffeine were compared to the average intake of vehicle controls over all days of session 2 and vehicle baseline (mean $\pm$ SEM - 31.2 $\pm$ 1.3 g). Unless otherwise noted, t test p values are two tailed probabilities.

**Consumption of rats given aspartame and caffeine.**

There was a dose related suppression of feeding by caffeine over Session 2. The low dose caffeine group showed no suppression of feeding over Session 2. Moreover, except for two days of Session 2, consumption did not differ from vehicle control. There was a transient increase in feeding over vehicle control on Day 4 of Session 2 ($t[378]=1.906$, $p<.05$). There was a trend toward an increase in feeding on the preceeding Day 3, as well ($t[378]=1.670$, $p<.05$ 1 tailed).

In contrast, the high dose caffeine group ate less than vehicle controls over days 1-5 of Session 2 (all $t[378]\geq$

2.366, $p<.01$). The suppression of feeding waned by day 6, and food consumption returned to vehicle control levels.

Aspartame had little effect on feeding over Session 2. Rats given a high dose of aspartame consumed at control levels over all days of Session 2. Animals given a low dose of aspartame behaved similarly, eating at control levels over days 1-4 and day 6 of Session 2. However, there was a transient suppression of feeding in low aspartame rats on day 5 ($t[378]=2/163$, $p<.05$).

Though aspartame had little effect when given alone, it did affect the duration of the suppression of feeding by high doses of caffeine. Like rats given high caffeine alone, rats given high doses of caffeine with either a low dose of aspartame (high caffeine/low aspartame) or a high dose of aspartame (high caffeine/high aspartame) ate less than controls over nearly all days of Session 2. High caffeine/high aspartame rats ate less than controls on days 1,2 and 4-6 (all $t[378]\geq 2.994$, $p<.01$), though feeding of this group tended to be less than controls on day 3, as well ($t[378]=1.621$, $p<.06$ 1 tailed). High caffeine/low aspartame rats ate less than controls over days 2-6 of Session 2 (all $t[378]\geq 2.395$, $p<.01$). Importantly, rats given a high dose of caffeine with either low or high doses of aspartame did not

return to control levels on day 6, unlike rats given only a high dose of caffeine.

As described above, a low dose of caffeine increased feeding over control on days 3 and 4. In contrast, a low dose of caffeine with a low dose of aspartame suppressed feeding on days 1 and 5 of Session 2 (all $t[378] \geq 2.220$, $p<.05$). The suppression of feeding on day 5 could be a replication of the suppression of feeding on day 5 produced by a low dose aspartame alone. It is likely, however, that caffeine and aspartame interacted. Low caffeine/low aspartame rats ate less on day 5 than the low dose aspartame alone group (Duncan's test, $p<.05$).

Suppression of feeding on day 1 is clearly an interaction between low doses of aspartame and caffeine, since neither given alone affected feeding on day 1 of Session 2. Moreover, the low caffeine/low aspartame rats ate less than low dose caffeine alone animals on day 1 (Duncan's test, $p<.05$).

Finally, groups given low aspartame alone or with low caffeine did not differ from each other (Duncan's test) or vehicle control on days 2,3,4 and 6 of Session 2. The feeding suppression tended to reverse by day 6, when the low caffeine/low aspartame group tended to eat more than controls ($t[378]=1.716$, $p<.05$ 1 tailed). Similarly, rats given a low dose of caffeine with a high dose of aspartame tended to eat

more than controls on day 6 $t[378]=1.722$, $p<.05$ 1 tailed). Unlike low caffeine/low aspartame rats, low caffeine/high aspartame animals consumed at control levels for days 1-5 of Session 2. So any synergistic effects of aspartame on feeding suppresion of low dose caffeine appears only with a low dose of aspartame.

**Food intake differences between groups given caffeine**

It was of interest to compare rats given different doses of caffeine alone or in combination with aspartame over days of Session 2. Duncan's tests ($p<.05$) were used.

The dose related suppression of feeding by caffeine was also evident in these comparisons. Rats given a high dose of caffeine ate less than rats given a low dose of caffeine over days 1-5 of Session 2.

In addition, aspartame transiently attenuated the anorectic affects of high doses of caffeine. Over days 1-3 of Session 2, high dose caffeine animals ate less than high caffeine/high aspartame and high caffeine/low aspartame groups which did not differ. On days 4 and 5, rats given a high dose of caffeine alone or with a low or high dose of aspartame did not differ.

On day 6, rats given a high dose of caffeine alone did not differ from low caffeine alone animals. These two groups

ate equal amounts and more than high caffeine/low aspartame or high caffeine/high aspartame rats, which did not differ. Together these findings reflect the waning of feeding suppression in high caffeine rats, and the persistence of feeding suppression in high caffeine plus aspartame rats.

A low dose of aspartame may initially interfere with the anorectic effects of high doses of caffeine. Recall that on day 1 rats given a high dose of caffeine alone or with a high dose of aspartame ate less than low caffeine rats or vehicle controls. In contrast, on day 1, rats given a high dose of caffeine with a low dose of aspartame did not differ from low caffeine alone animals or from vehicle controls. This pattern changed after day 1. Over days 2-6 of Session 2, rats given high doses of caffeine with a low dose of aspartame ate less than rats given low doses of caffeine alone.

On days 1 and 5 only, rats given a low dose of caffeine with a low dose of aspartame ate less than rats given a low dose of caffeine alone. These findings are consistent with those reported above. Recall that a low dose of caffeine had no anorectic effects. But when given in combination with a low dose of aspartame, feeding was suppressed relative to vehicle on days 1 and 5.

**Food intake differences between groups given aspartame**

It was of interest to compare rats given different doses of aspartame alone over days of Session 2. Duncan's tests ($p<.05$) were used.

Rats given a low dose of aspartame did not differ from rats given a high dose of aspartame over days 1-6.

**Body Weight**

There were no group differences in body weight at the end of the experiment.

**Anxiety Tests**

**Holeboard Data**

There were no time, group or group by time effects for any measures in the holeboard (see Table 1 for means).

**Plus Maze Data**

**Time Effects.** There were no significant differences between Time A and B rats. Rats given access to caffeine and/or aspartame prior to behaviour testing were no different in anxiety-like behaviour than rats whose access to caffeine and/or aspartame had been removed 24 hours prior to testing.

**Group Effects.** There were no group effects on measures taken in the plus maze. It should be noted that the overall mean values of the anxiety measures were low (.047 $\pm$ .014 and

.186 ± .024 mean ± SEM for Ratio Time and Ratio Entry respectively). While these means differ from zero (all $t[72] \geq 3.24$, $p < .01$ testing difference from zero), they are low, suggesting a relatively high anxiety baseline.

### Intake and anxiety correlations

Intake measures for each day of Session 2 were correlated with Ratio Entry and Ratio Time measures over all rats. Day 1 of Session 2 intake was negatively correlated with Ratio Time, $r = -.425$, $p = .0001$. Therefore, as consumption increased, the time spent in the open versus the total time spent in the arms of the maze decreased. Put another way, the more "anxious" the rat (smaller Ratio Time), the more food consumed. All other intake measures were not significantly correlated with either Ratio time or entry.

## Discussion

### Effects of dosing on Caffeine and Aspartame Consumption

Low and high aspartame and caffeine groups dosed themselves differently. This result was anticipated and suggests the dosage manipulation was effective.

### Effects of Aspartame on Caffeine Consumption

Aspartame did not affect caffeine consumption. Rats given low or high doses of aspartame in combination with

caffeine consumed as much caffeine as rats given equivalent doses of caffeine alone.

Effects of Caffeine on Aspartame Consumption

Caffeine produced a dose-related reduction in aspartame consumption. Low doses of caffeine did not change the amount of aspartame consumed relative to the low aspartame alone group. However, high doses of caffeine decreased the consumption of aspartame in all groups.

Effects of Caffeine Alone and with Aspartame on Food Consumption

High doses of caffeine administered alone and in combination with both low and high doses of aspartame consistently reduced food intake below control over days of Session 2. None of the other dosage combinations so consistently reduced food intake below control. When high doses of caffeine were administered alone, food intake decreased relative to baseline on Day 1 of Session 2 and remained less than baseline until the final day of Session 2. On Day 6 of Session 2, consumption did not differ from baseline. Since animals dosed equally over days, this result suggests that these animals developed a tolerance to the effect of a high dose of caffeine. Tolerance to caffeine does develop in that dependence and withdrawal symptoms have been reported in humans (Nehlig, Daval & Debry, 1992). Moreover,

tolerance to the locomotor stimulant effects of caffeine has been seen in rodents (Chou, Khan, Forde and Hirsh, 1985).

Food intake levels were also decreased on Day 1 of Session 2 when a high dose of caffeine was administered in combination with a high dose of aspartame. Consumption was less than control on all but Day 3 of Session 2. On Day 3 there was a trend toward suppession of feeding, however. Unlike the feeding pattern of high caffeine rats, consumption of high caffeine/high aspartame rats did not return to control levels on the final day of Session 2.

Unlike the other high caffeine groups, food intake of the high caffeine/low aspartame animals did not decrease until Day 2 of Session 2. Like the other high caffeine groups, high caffeine/low aspartame rats consumption was reduced below control on Days 2-5. Unlike high caffeine but like high caffeine/high aspartame rats, feeding remained suppressed on Day 6.

The failure of the high caffeine plus aspartame groups to return to control levels suggests that a tolerance to the anorectic effects of caffeine had not developed. The finding that low and high doses of aspartame prolong the anorectic effect of caffeine even though they do not have anorectic properties of their own, suggests that aspartame prevented tolerance to caffeine from developing.

There are a few possible explanations for why a high dose of caffeine reduced food intake in the present study. However, it is uncertain whether any one of them alone may account adequately for the results in the present study.

Caffeine ingestion has been shown to be a prandial behaviour in both humans and rats (Newland and Brown, 1992). These researchers demonstrated that high doses of caffeine (4 mg/ml) (comparable to the higher dose in the present study), were only consumed by rats in their drinking water in the presence of food. It could be that the taste of the solutions prepared with a high dose of caffeine altered the flavour of the chow, making it unpleasant to consume. Alternatively, it may be that the taste of the solution itself was unpleasant as rats given a high dose of caffeine with or without aspartame consumed less than the targeted doses of their respective solutions. If true, then it is possible to conclude that prandial drinking facilitates mastication and swallowing. Therefore, if fluid intake is decreased, so too is food intake (see Figures 1 and 3). However, because fluid intake was constant over days (Figure 1) while food intake returned to baseline levels at least for rats given a high dose of caffeine alone, other explanations deserve consideration.

There is evidence to suggest that caffeine affects the activity of certain catecholaminergic neurotransmitters that

are known to be involved in the regulation of feeding. For example, caffeine increases intracerebral dopamine concentration (Nehlig et al., 1992). In addition, caffeine selectively depresses the firing rate of dopamine neurons of the ventral tegmental area that project to the frontal cortex and limbic structures therefore not inhibiting the firing rate of neurons projecting to the striatum (Nehlig et al., 1992). In addition, caffeine dose-dependently decreases the local release of dopamine in the caudate nucleus. Together, these actions suggest that caffeine could stimulate motor activity. It is possible that caffeine-induced locomotion may interrupt feeding as movement and feeding are incompatible with another. Given that tolerance to caffeine had developed in rats given a high dose of caffeine alone, it is possible that tolerance had developed to the locomotor stimulatory effects of caffeine. Therefore, when activity levels returned to normal, feeding resumed.

Serotonin is also affected by caffeine ingestion. Generally, caffeine increases in vitro serotonin concentration in the brainstem (Nehlig et al., 1992). The intensity of this effect depends on the emotional level of the animal. However, data that suggest caffeine involvement in serotonin regulation point to a facilitation of feeding by caffeine-induced serotonin level changes. For example, caffeine increases

concentrations and rates of cerebral use of noradrenaline, dopamine and serotonin. Concentration and rate increases in these neurotransmitters serve to increase, rather than decrease feeding (Morley, pp.1268-9, 1987). Serotonin also affects sleep and arousal patterns (Nehlig et al., 1992). Therefore, it is possible that caffeine's effects on serotonin are selective for motor pathways, not thalamic ones concerned with feeding. If true, then caffeine might decrease feeding indirectly through serotonin-modulated arousal/inactivity cycles.

Together, the above possibilities could suggest that caffeine reduces food intake directly by its sensory qualities and indirectly, by modulating activity cycles and facilitating behaviours that may be incompatible with feeding.

Effects of Aspartame on Feeding Changes induced by Caffeine Consumption

Low and high doses of aspartame alone had no effect on food intake. However, when administered with either dose of caffeine, there were some complicated and time dependent interactions between aspartame and caffeine.

In general, high caffeine reduces food intake below control levels. On the other hand, low doses of caffeine do not affect food consumption except on Days 3 and 4 of Session

2. On these days low doses of caffeine increased food consumption above control.

The interactions of aspartame with caffeine are dose dependent only when a low dose of caffeine is given. In this case it is a low, not a high dose of aspartame which acts synergistically with caffeine to reduce food consumption. The interaction of caffeine and aspartame appears only on Days 1 and 5 of Session 2. On these days low caffeine plus low aspartame reduces feeding below control. Low caffeine alone does not affect feeding on these days. Low aspartame does reduce feeding on Day 5 but not on Day 1. In any event, food consumption of low caffiene/low aspartame rats is below that of low caffeine alone and low aspartame alone groups on both days. In contrast, rats given a high dose of aspartame in combination with a low dose of caffeine do not differ from rats given only a low dose of caffeine.

These effects are not due to differences in dosing of caffeine. The low caffeine alone group consumed as much caffeine as the low caffeine/low aspartame and the low caffeine/high aspartame groups. Moreover, the low caffeine/low aspartame group consumed less aspartame than the low caffeine/high aspartame groups. So it is the lower dose of aspartame which interacts with the low dose of caffeine.

These results suggest the possibility of an inverted U dose-effect curve. The high dose of aspartame given here might fall in the part of the curve which is back to baseline. The low dose of aspartame given may fall within the rising part of the curve. If true, giving more doses of aspartame below and just above the low dose given here should reveal a more normal dose response effect.

In contrast, both low and high doses of aspartame interact equally with high doses of caffeine to alter the food intake expected from a high dose of caffeine alone. Rather than facilitate anorectic effects, however, aspartame attenuates the anorectic effects of a high dose of caffeine. This attenuation appears only in the first 3 days of Session 2. Thereafter all high caffeine groups show the same degree of suppression of feeding.

A reason for the transient suppression of the anorectic effects of caffeine by aspartame is because of the tolerance to the anorectic effects of caffeine shown by high caffeine alone rats. High caffeine plus aspartame groups did not change their food consumption over Days 2-5 (Duncan Test, no differences). In contrast, the high caffeine alone group increased its food consumption to the same level of the high caffeine and aspartame groups by Days 4 and 5, and then exceeded that level on Day 6 (Figure 3). The only other

change over days was seen in the high caffeine/low aspartame rats. These animals ate more on Day 1 than on Day 2. Day 1 consumption equalled intake on Days 3-6, however. Taken together, these data show a pattern that suggests a continuous action of aspartame in interaction with caffeine. Though there is an attenuation of the the anorectic effects of high caffeine by aspartame, aspartame also prevents the tolerance to high caffeine seen in the high caffeine alone group.

The difference between the interaction of aspartame with low and high doses of caffeine are not due to aspartame dosing differences between the groups, though high doses of caffeine do reduce aspartame consumption. In the case of the high caffeine/low aspartame groups, consumption of aspartame was the lowest of all groups given aspartame (312.9 mg/kg). On the other hand, rats given high caffeine/high aspartame consumed a dose of aspartame (920.9 mg/kg) between the low caffeine/low aspartame group (460.7 mg/kg) and the low caffeine/high aspartame group (1390.9 mg.kg). So the attenuation of the anorectic effects of a high dose of caffeine by aspartame appears over a dose range of 312.9 to 920.9 mg/kg. This range includes the dose of aspartame (460.7 mg/kg) which facilitated anorectic effects of a low dose of caffeine.

Implications of the present findings

It is likely that different mechanisms mediate these two types of interactions of aspartame with caffeine. First the timing of their appearance is different. The attenuation of the anorectic effects of high caffeine by low and high doses of aspartame appears on Day 1 . It likely persists over Days 1-6, since consumption of these groups is unchanging over days of Session 2. Given that the metabolic and excretion processes for aspartame occur over a 24 hour period (Stegink, 1984, p. 513) it is plausible that aspartame has prolonged effects. In contrast, the synergistic effect of low dose aspartame on the anorectic effects of low dose caffeine appears on Days 1 and 5. Why these two particular days is unclear.

It is possible that there is a cyclic pattern to this interaction with a period of 3 days. If true, then extending the observations to at least 9 days of drug administration would have shown a third suppression of feeding. In fact, a study by Porikos and Van Itallie (1982) in humans reported that changes in food intake by aspartame occur between days 3 and 4 of a six day aspartame consumption period.

It is also worth noting that no study has reported changes in consumption over days. Rather, analyses have focussed on average consumption over a defined period of days.

Therefore, it is possible that any patterns emerging over days were not noticed in previous research.

Effects of Low and High Doses of Aspartame Alone on Food Intake

Aspartame alone did not decrease food intake relative to controls in the present study. This result is contrary to the findings of Porikos and Van Itallie (1982) in humans. These researchers administered aspartame to normal and obese humans over a 6 day period. Food consumption was averaged over 3 day blocks. They found that aspartame decreased food intake in the first 3 day block. In the second 3 days block, consumption rose, but not back to baseline. Given that they averaged over days, their analysis may have missed a complete return to baseline of consumption.

Rogers et al. (1990) speculated that the suppression of feeding by aspartame is determined by at least two interacting influences: an inhibitory post-ingestive effect and a stimulatory effect of its sweet taste. The potency of these influences could be modified by the nutrient content of the aspartame-containing product (Rogers et al., 1990). As rats do not taste aspartame as sweet (Scalafani & Abrams, 1986), the inhibitory postingestive effect of aspartame is likely the primary factor influencing food intake.

Aspartame may not have decreased food intake in the present study for several reasons. First, the postingestive inhibitory effects of aspartame may not have occurred at the time rats ate. Since rats were provided with 24 hour access to food and aspartame solutions, it is possible that eating and drinking behaviours did not occur together. The duration of any postingestive inhibitory effect by aspartame may have not been sufficient to decrease food intake at a later time. Smith (1991) reported that aspartame did serve to decrease meal size in rats but that this effect was brief and rapid. This finding suggests that aspartame is a more potent food intake depressant when food is provided simultaneously rather than subsequently with aspartame. Nevertheless, there was some persistent action on food consumption of aspartame in combination with caffeine. If the above speculation is correct, then some second set of processes triggered by aspartame combined with caffeine mediates the effects on food consumption.

Body Weight and Feeding Suppression

Though there was feeding suppression in three of the groups given high doses of caffeine, this did not reduce their weight relative to controls or other groups. The relatively mild feeding suppression of the high caffeine plus aspartame groups apparently was not severe enough to reduce body weight.

The gradual tolerance and rising food intake over days of the high caffeine alone group most likely compensated for the more severe reduction in consumption.

Rats given a solution containing 0.2% saccharin with either 0.055, 0.11 or 0.22% aspartame did not differ in body weight from controls after an 8 week period (Porikos & Koopmans, 1988). Aspartame did not affect body weight in male rats given aspartame as an admix to chow, although there was a dose-dependent suppression of weight gain after about 60 weeks (Ishii, 1986). Ishii (1986) concluded that the observed suppression of body weight corresponded to a sustained decrease in energy intake. However, Kanarek & Orthen-Gambill (1982) report that rats offered powdered sweet or nonsweet carbohydrate do not consume more calories or gain more weight than animals fed only chow and water. In addition, sex differences might exist in rats' responses to non-nutritive sweeteners (Porikos and Koopmans, 1988). These researchers speculate that the facilitation of weight loss by non-nutritive sweeteners could be restricted to female rats.

Effects of Caffeine on Anxiety and Exploration

Although high doses of caffeine decreased food intake, neither low or high doses of caffeine, alone or in combination with aspartame had any effect on anxiety-like behavior in the

plus maze. Nor were there any effects on activity or exploration in the hole board.

The lack of effects on exploration and activity in the hole board is consistent with the literature (File et al., 1988). File et al. (1988) demonstrated that a 21 day schedule of chronic caffeine (50 or 100 mg/kg/day) followed by an injection (ip) of 20 or 40mg/kg caffeine did not affect motor activity, rearing activity or the number of headdips. Moreover, the lack of effects in this study cannot be attributed to too acute an exposure to caffeine, since File et al. (1988) exposed their rats to caffeine over a time period that was three times the duration used in the present study.

The lack of an anxiogenic effect of caffeine is consistent with the findings of File et al. (1988). File et al. reported an anxiogenic effect of caffeine in the social interaction test. Thirty minutes after a systemic injection of caffeine (40 mg/kg) there was a decrease in social interaction. This was not seen after an injection of 20 mg/kg. Exposure to chronic caffeine (50 or 100 mg/kg per day in the drinking water for 21 days) followed by an injection of 20 or 40 mg/kg caffeine did not change social interaction. In contrast, chronic exposure to caffeine per se was without effect on social interaction, though it did increase motor activity. These data suggest that chronic exposure to

caffeine by drinking does not affect anxiety in rats. On the other hand, it does produce a tolerance to the anxiogenic effects of systemic injection of caffeine. Since rat in this study were given doses of caffeine within the range of the chronic exposure groups in the File et al. (1988) study (50 and 150 mg/kg per day), the findings of the present study are consistent with those of File et al. (1988).

In contrast, the present findings are not entirely consistent with studies of the effects of caffeine on anxiety measured in the elevated plus maze. Golda (1990) reported that 10mg/kg caffeine decreased the number of entries into the centre and open arms of the elevated plus maze in female Wistar rats. This anxiogenic like response is unlike the present observations. Dose and sex differences may account for the discrepancy. Baldwin, Johnston & File (1989) found that caffeine 40 mg/kg (ip) (approximate equivalent to 100 mg/kg oral dose according to File et al., 1988) injected 30 minutes before testing in the elevated plus maze significantly reduced the percentage of time spent in the open arms compared with controls. This clear anxiogenic effect is unlike the present findings. However, though the dose is likely equivalent, the route and method of administration are different.

In the present study the route of administration was

oral, not injected. An injected bolus of caffeine could have produced blood plasma levels of caffeine at the time of testing in the plus maze which were considerably higher than those achieved by free access oral administration. Higher plasma levels of caffeine may have accounted for the anxiogenic effects.

Since anxiety testing was begun just after lights out in the present study, it is likely that not all rats had not eaten or drunk much caffeine prior to the test. Recall that caffeine ingestion is a prandial behaviour (Newland & Brown, 1992). It is possible then that the plasma levels at the time of testing were considerably lower in the present study than the plasma levels in the studies demonstrating anxiogenic effects following acute systemic injection.

Caffeine changes brain function in ways which might lead one to suspect an anxiogenic potential. Caffeine causes a release of intracellular calcium via an interaction wth the ryanodine receptor. This calcium release depresses the $GABA_A$ receptor response (Desaulles et al., 1991). Depression of $GABA_A$ response is associated with increased anxiety in rodents (Skolnick, 1982).

In addition ryanodine receptors are localized in the rat cortex and dorsal hippocampus. Benzodiazepine receptor binding in rat cortex has been shown to change with chronic

caffeine (Wu and Coffin, 1984; Wu and Phillis, 1986). Moreover, Shi, Nikodijevic, Jacobson and Daly (1993) showed that chronic caffeine administration (100mg/kg/day) over four consecutive days in male NIH-Swiss strain mice is sufficient to produce changes in the density of a variety of central receptors including benzodiazepine-binding sites associated with GABA receptors. Specifically, the density of cortical benzodiazepine-binding sites associated with GABA receptors increased by 65% while the affinity slightly decreased (Shi et al., 1993). These changes were likely due to chronic blockade of adenosine $A_1$ receptors, which were also upregulated. Similar upregulation of benzodiazepine receptors following chronic caffeine consumption have been reported in the rat (Wu and Coffin, 1984; Wu and Phillis, 1986). However, these researchers were unable to definitively conclude which receptor system mediates caffeine's effects (Wu & Coffin, 1984).

These effects of caffeine are similar to the effects of chronic blockade of the benzodiazepine receptor by an antagonist (Flumazenil) or an inverse agonist (FG-7142) (Kulkarni and Ticku, 1990; Miller, 1991; Pritchard, Galpern, Lumpkin & Miller 1991). Since repeated doses of FG-7142 may be anxiogenic (Jeevanjee, Little, Nicholass & Nutt, 1985), one might expect an anxiogenic action of chronic caffeine under

these circumstances. On the other hand, Shi et al (1993) report complicated effects of chronic caffeine on other neurotransmitter systems. They found a down regulation of $\beta_1$ and $\beta_2$ adrenergic receptors, but an upregulation of serotonin type 1 and type 2 receptors. While serotonin is involved in anxiety, different receptors play different roles. The data to date suggest that type 1 receptor activation is anxiolytic, while type 2 receptor blockage is also anxiolytic, though this may be an oversimplification (Handley and McBlane, 1993). If the up regulation of type 1 and type 2 receptors reflects decreased binding, then opposite effects on anxiety might be expected. Given the complexity of the neurotransmitter changes produced by chronic caffeine, it unclear what the expected outcome might be.

There may also be a simple behavioral explanation for the lack of anxiogenic effects of caffeine in the present study. Given the rather high levels of anxiety of the groups including the controls (i.e. Ratio Time and Entry measures were low), it is possible that a putative anxiogen like caffeine would not increase anxiety in rats already so anxious. Baseline anxiety does affect the ability to demonstrate anxiogenic effects of putative anxiogenic compounds in other tests of anxiety which use shock (Skolnick, 1982), or home cage behavior (Carey, 1992). In these paradigm

steps are usually taken to decrease basal anxiety like behavior when anxiogenic compounds are given. In fact Carey et al. (1992) found that detecting an anxiogenic effect of caffeine in marmosets depended on using a "low anxiety" inducing test. Higher anxiety inducing tests failed to demonstrate an anxiogenic effect of caffeine. So there is the possibility that an anxiogenic effect of caffeine was not demonstrated in this study, because the rats were already as anxious as caffeine might have made them.

Whatever the reasons, there is little evidence that chronic doses of caffeine consumed in this study are anxiogenic. Therefore an anxiety induced suppression of feeding does not account for the anorectic effects of high doses of caffeine. Even if baseline anxiety was too high in the present study to detect an effect of caffeine on anxiety, one can still conclude that a caffeine induced suppression of feeding in this study was not mediated by an increase in anxiety. Moreover, it is unlikely that anxiety suppressed feeding in this study given the correlation of anxiety and feeding. Consumption on day 1 of Session 2 directly correlated with degree of anxiety. The more anxious the rat, the more food consumed.

Effects of Aspartame on Behaviour in the Plus Maze and Holeboard Tests

There was no evidence that aspartame alone or in combination with caffeine had an effect on anxiety-like behavior in the plus maze. Nor did aspartame affect exploration or activity in the hole board. If aspartame were anxiolytic, the high basal levels of anxiety in the plus maze would not have prevented its detection. On the other hand, these high basal levels might have prevented a detection of a possible anxiogenic effect of aspartame. So one cannot conclude definitively that aspartame has no anxiogenic actions. More research in this area is required with careful control of basal levels of anxiety. In any event, changes in anxiety do not account for the interactions of asparatame and caffeine on feeding.

Directions for Future Research

Future studies should explore the influence of basal levels of anxiety on anxious response to caffeine and asparatame. In addition, it would be of interest to examine anxiety levels at each day of caffeine administration. Though the data in the present study do not support the hypothesis that caffeine reduces food consumption by increasing anxiety,

a more conclusive test should involve testing anxiety levels as anorectic effects first begin to develop.

With respect to food consumption, food and solution intake might be given over a longer period of time to test whether there are cycles in interaction of aspartame and caffeine on feeding. In addition, availability of caffeine and aspartame and food should be restricted to a defined time period to ensure co-consumption. In addition, it would be interesting to vary the temporal spacing of food and solution availability to resemble the often spontaneous pattern of intake demonstrated by human subjects. This manipulation could enhance the ecological validity of the study.

Another way to ensure that caffeine and aspartame are present in the body at desired times would be to monitor the plasma levels of caffeine and phenylalanine, respectively. As this would constitute an invasive measure, it would be desirable to control for animal stress by having a comparison group that received the solutions and food but no monitoring.

Another suggestion would be to examine in further detail the tolerance and withdrawal effects suggested by the results of caffeine and aspartame administration in this study. It would be necessary to investigate in further detail the pharmacologic properties of aspartame in the presence of caffeine.

A final suggestion would be to increase the number of doses of aspartame around the low dose level. Some of the dosage data suggested an inverted U dose response curve might account for the lack of effects of high doses of aspartame while there were effects of low doses. Moreover, those cases where the effects of low and high doses of aspartame in interaction with caffeine were the same suggest that the doses of aspartame may have been too high, above the ceiling for the effect. In this case more doses of aspartame below the low dose are needed to test this possibility.

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Tables

TABLE 1

The Effects of Caffeine and Aspartame on Headdipping, Rearing and Total Activity in the Holeboard Test.

| Group | Headdips[1.] | Rears[1.] | Total Activity[1.] |
|---|---|---|---|
| Control | 5.875 (1.311) | 2.125 (.9889) | 277.625 (6.738) |
| Low CAF | 8.000 (1.311) | 4.125 (.9889) | 294.750 (6.738) |
| High CAF | 7.125 (1.311) | 2.625 (.9889) | 283.125 (6.738) |
| Low APM | 7.625 (1.311) | 2.750 (.9889) | 284.500 (6.738) |
| High APM | 5.375 (1.311) | 3.750 (.9889) | 281.375 (6.738) |
| Low CAF/Low APM | 4.367 (1.311) | 4.667 (.9889) | 277.033 (6.738) |
| High CAF/High APM | 3.733 (1.311) | 2.000 (.9889) | 287.133 (6.738) |
| Low CAF/High APM | 7.233 (1.311) | 2.367 (.9889) | 282.267 (6.738) |
| High CAF/Low APM | 3.133 (1.311) | 4.233 (.9889) | 269.667 (6.738) |

1. Mean and SEM (in parentheses) of scores over the 5.0 minute test for all treatment groups. Headdips and rears are frequency, and time active is in seconds of activity. Group abbreviations are: CAF=caffeine (low=$3.125 \times 10^{-1}$ mg/ml/day, high=$9.375 \times 10^{-1}$ mg/ml/day); and APM=aspartame (low=2.5 mg/ml/day, high=7.5 mg/ml/day).

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entries into all arms of the maze. Ratio Measures are as defined in the text. Group abbreviations are: CAF=caffeine (low=$3.125 \times 10^{-1}$ mg/ml/day, high=$9.375 \times 10^{-1}$ mg/ml/day); and APM=aspartame (low=2.5 mg/ml/day high=7.5 mg/ml/day).

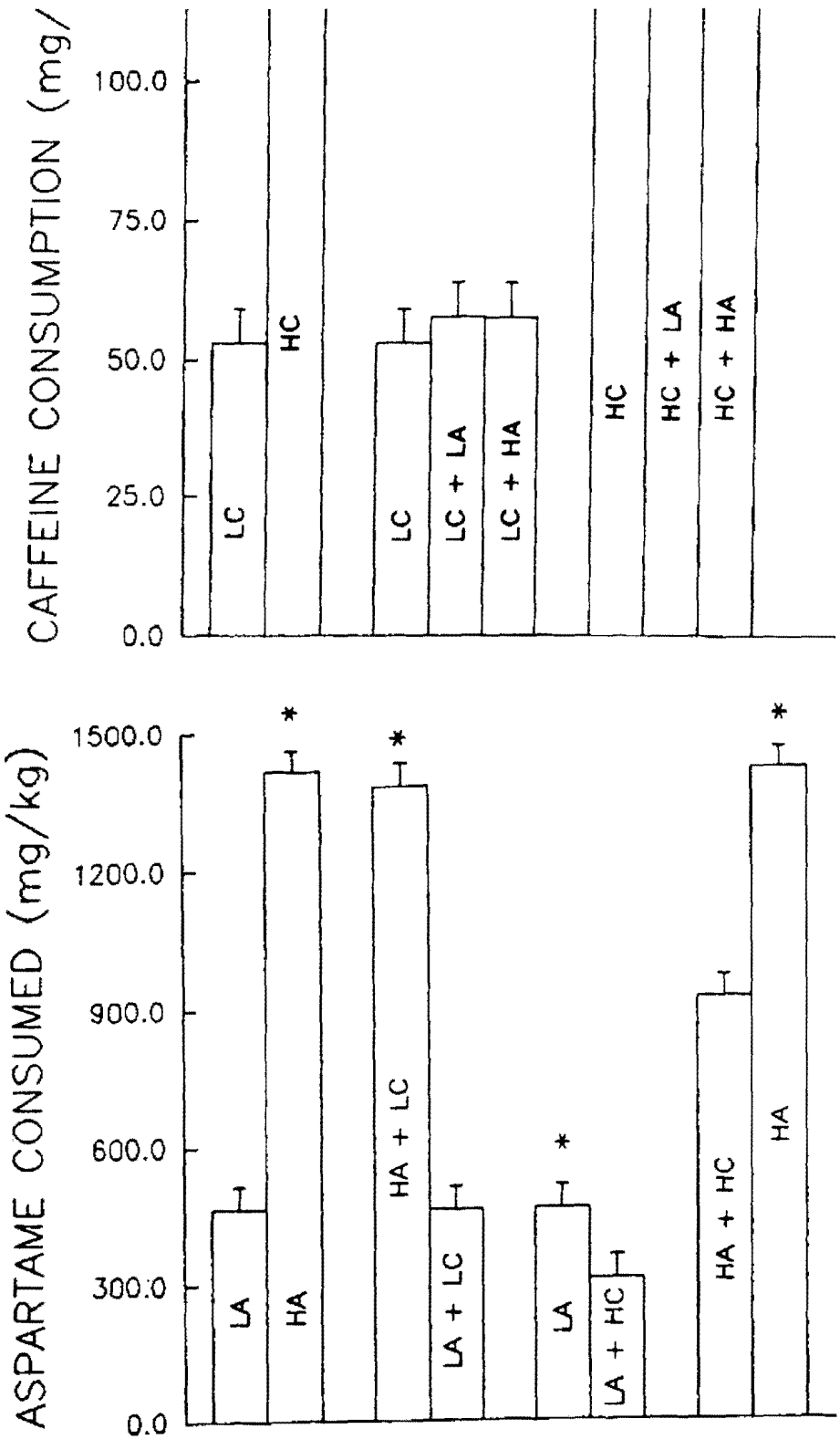
Figure Legends

Figure 1. Mean doses (mg/kg) of caffeine or aspartame (+SEM) consumed by rats given caffeine or aspartame, alone or in combination are plotted in the figure. The data are collapsed over the six days of consumption measurement for each group. Some means are repeated in the figure to facilitate comparison with related groups. In any given cluster of means, means marked with an asterisk differ from unmarked means, which do not differ from each other. The plot of caffeine consumption shows that the intended high and low dosing was achieved. Moreover, aspartame consumed with caffeine had no effect on the dose of caffeine consumed. Similarly, the plot of aspartame consumption illustrates that the intended high and low dosing of aspartame was also achieved. In contrast, high doses of caffeine reduced the aspartame dose consumed in the low and high aspartame dose groups (Duncan's tests, $p<.05$, following ANOVA)..

Figure 2. This figure shows control feeding patterns. Mean daily food intake during a 7 day continuous access to chow and water is plotted versus days. Feeding patterns for each of the nine groups are plotted separately. None of these animals differed in consumption between groups or across days of intake measurement (ANOVA).

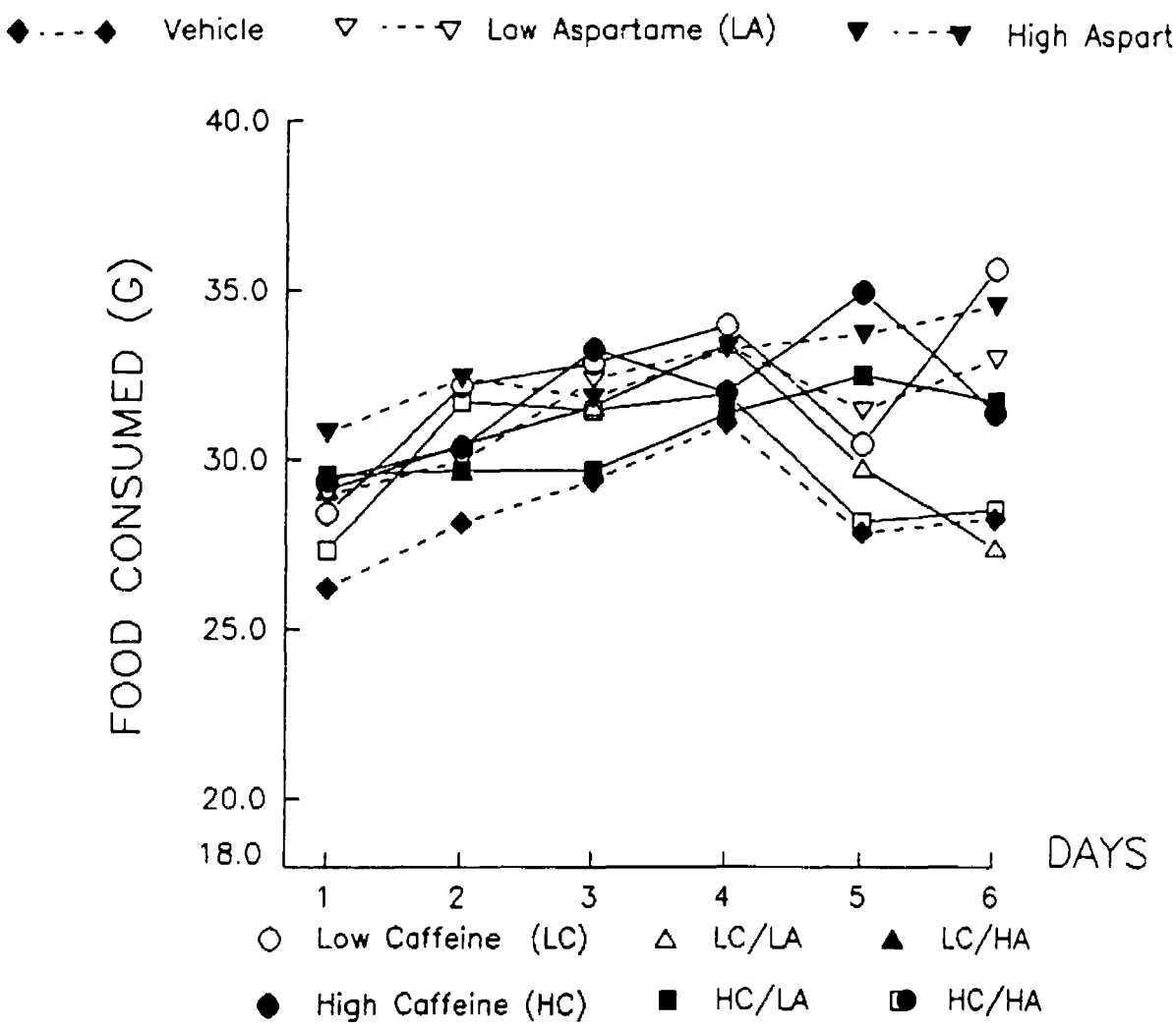
Figure 3. Mean daily food intake during a 7 day continuous access period to chow for groups receiving caffeine or aspartame alone or in combination in their drinking water at the time of measurement. Doses of caffeine were either .3125 or .9375 mg/ml/day. Doses of aspartame were either 2.5 or 7.5 mg/ml/day. Data from the nine groups are plotted separately. Vehicle is plotted with a dotted line, as are the low and high aspartame groups, which did not differ from vehicle. The solid horizontal line is baseline food consumption averaged over all groups and over the seven days prior to dosing (see Figure 2). Vehicle control consumption did not differ from this baseline. This figure demonstrates the suppression of feeding which occurs when high doses of caffeine are consumed (closed circles). It shows a tolerance produced by high caffeine when given alone, and a blocking of this effect when aspartame is given in combination with a high dose of caffeine (closed squares and open squares) (Duncan's tests, $p<.05$, following ANOVA). Other patterns illustrated in the figure are discussed in more detail in the text.

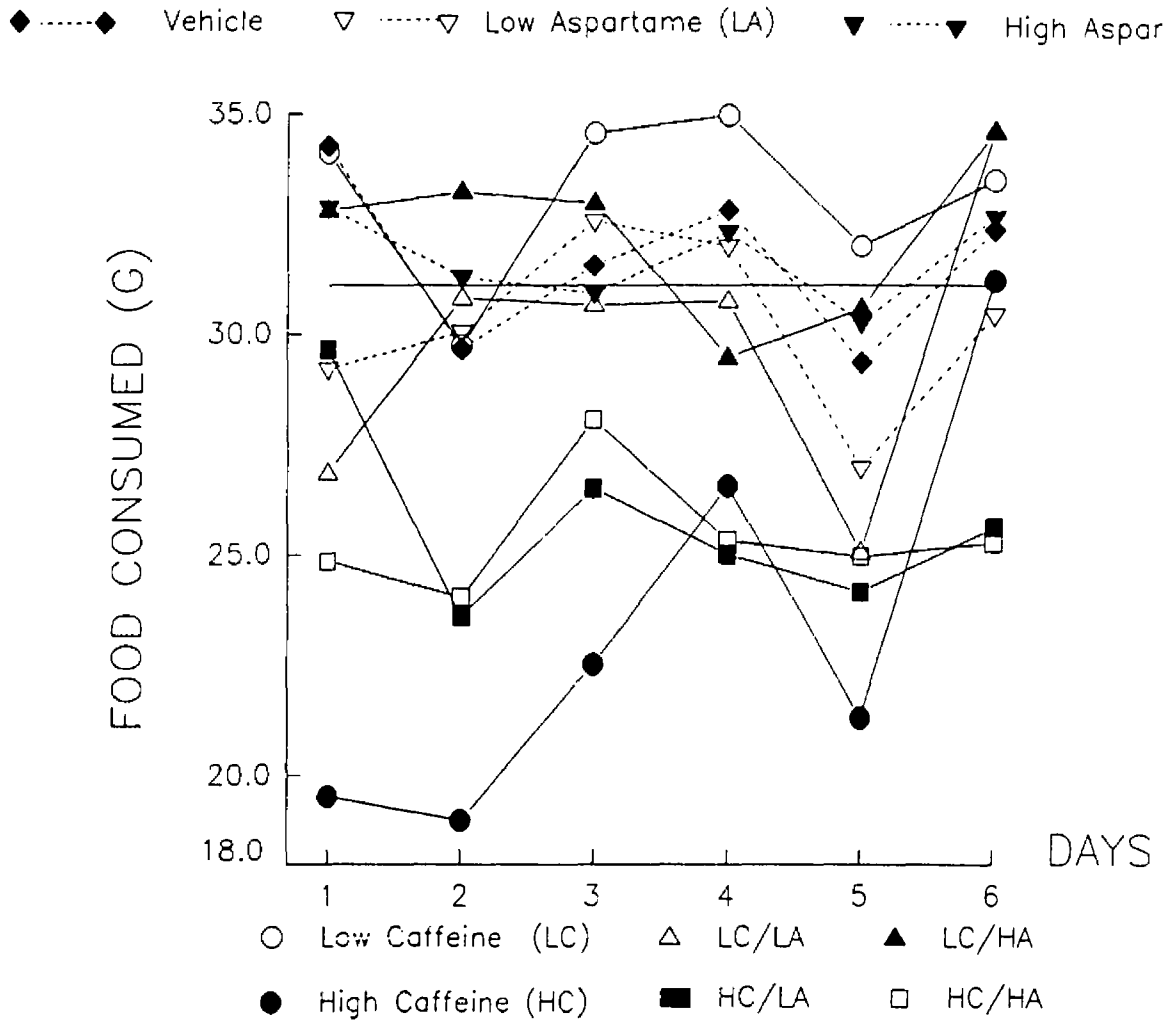
Figures

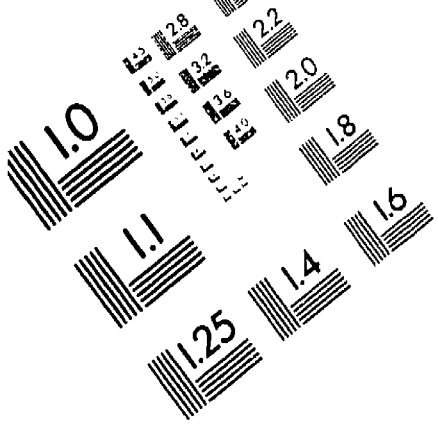


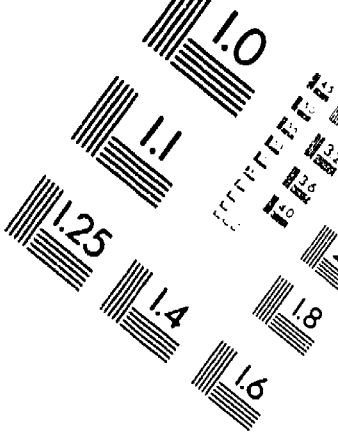

LA – LOW ASPARTAME
HA – HIGH ASPARTAME

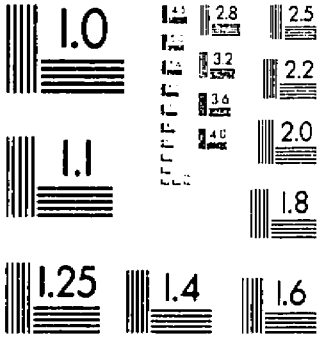
LC – LOW CAFFEINE
HC – HIGH CAFFEINE

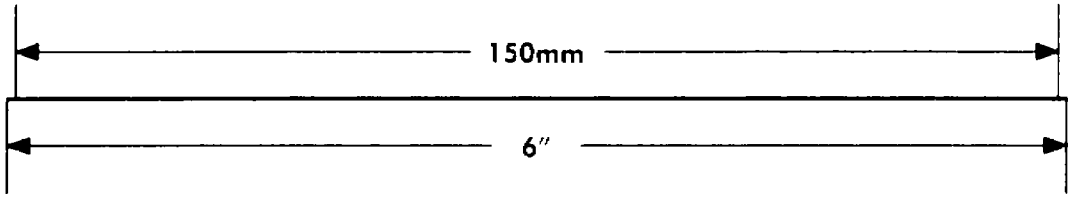

Vehicle
Low Aspartame (LA)
High Aspartame (
FOOD CONSUMED (G)
40.0
35.0
30.0
25.0
20.0
18.0
DAYS
1
2
3
4
5
6
Low Caffeine (LC)
LC/LA
LC/HA
High Caffeine (HC)
HC/LA
HC/HA

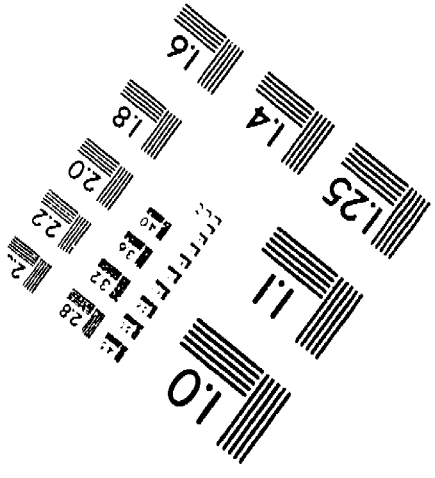

Vehicle
Low Aspartame (LA)
High Aspartame (HA
FOOD CONSUMED (G)
35.0
30.0
25.0
20.0
18.0
DAYS
1
2
3
4
5
6
Low Caffeine (LC)
LC/LA
LC/HA
High Caffeine (HC)
HC/LA
HC/HA

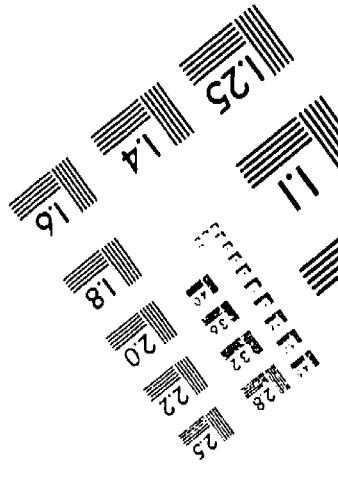
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