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Judith S. DeVoe

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THE EFFECTS OF CAFFEINE ALONE, AND IN COMBINATION WITH NICOTINE, ON SEVERAL BEHAVIORS IN RATS

by

Judith S. DeVoe

A Thesis
Submitted to the
Faculty of The Graduate College
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Western Michigan University
Kalamazoo, Michigan
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THE EFFECTS OF CAFFEINE ALONE, AND IN COMBINATION WITH NICOTINE, ON SEVERAL BEHAVIORS IN RATS

Judith S. DeVoe

Western Michigan University

Several doses of caffeine-sodium benzoate (0.0, 2.5, 5.0, 10.0 & 20.0 mg/kg, stated as salt) were administered daily by intraperitoneal injections (IP) for an initial twelve consecutive days which constituted Phase I. A probe-dose of nicotine (2.0 mg/kg) was administered in combination with each caffeine dose for the following seven days comprising Phase II. Removal of nicotine subsequent to the last day of Phase II initiated a second caffeine-only (nicotine withdrawal) condition, Phase III. Tests of locomotion and aggression ensued at various points of the study and water intakes and body weights were recorded daily, prior to injections. Overall, locomotion increased as a function of caffeine dose with the exception on Day 1 of Phase II. Muricidal aggression was enhanced at higher doses of caffeine, but not with lower doses. Even though previous research indicated nicotine's ability to suppress the muricide, nicotine did not suppress the caffeine-induced attack analyzed in the present study. Caffeine markedly increased water consumption across all phases as a function of dose, and the 10.0 mg/kg dose was statistically different from all other doses. The 2.0 mg/kg nicotine dose in Phase II produced additional increases in water intake, in comparison to Phase I, regardless of the concurrently administered dose of caffeine. Caffeine dose was also found to subtly influence body weight.
ACKNOWLEDGEMENTS

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CHAPTER I

INTRODUCTION

Caffeine, one of the most widely self-administered central nervous system (CNS) stimulants in North America (Winsted, 1979), is found in coffee, tea, soda pop, chocolate and numerous over-the-counter preparations. When the drug is consumed in large quantities, a psychiatric disorder termed "caffeinism" can be induced (American Psychiatric Association, 1980) causing symptoms such as "tension", "urge to cry", insomnia, irritability, restlessness, and tremor (e.g., Neil, Hemmelhock, Malinger, Malinger, & Hannie, 1978; Bezchibnyk & Jeffries, 1981; Greden, 1974; Greden, Fontaine, Lubetsky & Chamberlin, 1978) which have sometimes confounded other psychiatric diagnoses. While caffeinism represents a severe symptomology, most often associated with a long self-administration history, some of caffeine's behavioral effects appear under acute, lower doses (e.g., Goldstein, Kaizer & Whitby, 1969). Increased irritability in humans (e.g., DeFreitas & Schwartz, 1979), altered water intake and a purported "tolerance liability" in rats (e.g., Wayner & Kleinrock, 1978; Wayner Jolicoeur, Rondeau & Barone, 1976) are among caffeine's noted behavioral actions.

Various laboratory, non-human assays have been employed to further analyze those behavioral effects of caffeine originally observed in human recipients. For instance, evidence of caffeine-induced irritability has been sought using certain models of aggression employing laboratory animals. Caffeine has increased shock-induced attack in
both the rat (Eichelman, Orenberg, Hackley & Barchas, 1978) and squirrel monkey (Emley & Hutchinson, 1983). Caffeine has not been found to enhance muricidal aggression (Eichelman et al., 1978), but has been seen to reverse the anti-muricidal effects of chlordiazepoxide (Quenzer & Feldman, 1974). In addition, stressed animals (chronically isolated beginning immediately at weaning) treated with caffeine have evidenced greater pathophysiology and higher mortality rates than do socialized subjects (Henry & Stephens, 1980). Frequent fighting in the stressed group possibly contributed to the higher percentage of deaths.

A second behavioral action of caffeine, altered water balance, has been examined, albeit sparsely, also using laboratory animals (e.g., Markel, Wayner, Jolicoeur & Mintz, 1981). Acute caffeine treatment yields diuresis, and chronic treatment can produce tolerance to diuretic effects (e.g., Rall, 1980). High doses, but not low, of acute caffeine have suppressed schedule-induced polydipsia in rats of reduced body weights and chronic administration has produced tolerance to this effect (Wayner et al, 1976). When rats returned to free-feeding weights, low caffeine doses enhanced polydipsic drinking. In another study, Merkel, Wayner, Jolicoeur & Mintz (1981) treated rats with several caffeine doses and showed dose-related effects on home-cage food and water intakes, dependent upon conditions. A high dose (100.0 mg/kg) attenuated both food and water consumptions, for all conditions. A low dose (3.125 mg/kg) increased 1-hour water consumption in the 23-hour water deprived condition, as did the 12.5 mg/kg dose in the free-feeding and drinking condition.
Caffeine, a psychomotor stimulant, increases locomotion in animals (e.g., Estler, 1973; Thethapandha, Maling, and Gilette, 1972). Mongolian gerbils significantly increase wheel running activity when treated with low to moderate caffeine doses, however, gerbils treated with high doses of caffeine display decreased wheel running as compared to those treated with saline (Pettijohn, 1979). Caffeine markedly increases locomotion, and simultaneously decreases social interactions in rats (File and Hyde, 1979). When illumination and environmental familiarity are manipulated during tests of locomotion and social interaction, anxiety indices have been inferred (e.g., File and Hyde, 1979). Assays have revealed that test illumination can modify the locomotor effects of certain CNS stimulants (e.g., d-amphetamine and methylphenidate) but not those of caffeine (Kallman & Issac, 1975).

In summary, caffeine is a widely self-administered, socially approved substance associated with numerous untoward behavioral side-effects. These influences have been observed in human caffeine recipients, consist notably of irritability, altered water balance and hyperactivity, and have been analyzed to certain degrees in various animal preparations. Few investigations have analyzed these three side-effects in the same subjects. Therefore, the major purpose of the present study was to examine effects of several caffeine doses on muricide, water intake, wheel running and body weight in the same rodent subjects. It was hoped that a unique constellation of effects would emerge indicating relative selectivity of caffeine's actions. To the extent that a unique, readily reproduced constellation of effects could be demonstrated, it was believed...
that future investigations of adverse drug-drug interactions involving caffeine might be expedited.

A drug's effects on behavior, when examined independently of another compound, are not necessarily the same as when studied in drug-drug combinations (e.g., Hansten, 1971; Griffin and D'Arcy, 1979; Lasagna, 1978; Melmon & Gilman, 1980). Drug-drug interactions may change the absorption, metabolism, distribution and/or elimination of a substance (i.e., its pharmacokinetics) (e.g., Hollister, 1978; Melmon & Gilman, 1980). For example, one chemical may effect the amount or rate of absorption of another drug so that effective serum levels are never reached, or, so that the rate of an effect's onset many be speeded or delayed. Other examples of pharmacokinetic actions would include when certain drugs increase or decrease drug metabolism and therefore speed or slow elimination of other chemical agents. In addition to pharmacokinetic interactions, drugs may act directly to facilitate or suppress the effects of other chemical agents and therefore produce pharmacodynamic interactions (e.g., Hollister, 1978; Melmon & Gilman, 1980). Accordingly, two substances may compete for, or alter, common receptor sites (e.g., Melmon & Gilman, 1980.)

Given the above, it is plausible that caffeine's behavioral influences can be substantially altered by drug-drug interactions (either of the pharmacokinetic and/or pharmacodynamic sorts), and various evidence has accrued which supports this notion. For instance, in humans certain oral doses of caffeine in combination with pentobarbitone actually produce effects similar to placebo, suggesting that the individual effects of the two drugs when given alone were cancelled.
by the two agents being given in combination (Forrest, Beliveau & Brown, 1972). In infrahuman subjects various caffeine doses as well as higher doses of phenobarbitone or pentobarbitone each individually increase locomotor activity, yet when caffeine-barbiturate combinations are given, activity effects have appeared additive (Waldeck, 1975). In mice, combined doses of ethanol and caffeine act to increase motility above those levels achieved under either drug administered alone (Waldeck, 1973). Further, caffeine with or without ethanol has facilitated the reversal of motor activity induced by ET495 + Clonidine (Waldeck, 1973).

Since caffeine and nicotine often are concurrently self-administered, the possibility exists that nicotine frequently modifies caffeine's behavioral influence. However, surprisingly little data has been collected regarding this plausibility. That nicotine exerts powerful behavioral actions is well-known and has been analyzed in numerous laboratory animal investigations. For instance, nicotine has been shown to selectively suppress various measures of aggression (e.g., Driscoll & Baettig, 1981; Waldbillig, 1980; Emley & Hutchinson, 1983), alter water balance mechanisms (e.g., Gritz and Jarvik, 1978; Sanger, 1978), influence body weight (e.g., Sanger, 1978; McNair, and Bryson, 1983), and to markedly change various indices of motor activity (e.g., Stolerman, Fink, and Jarvik, 1974; Clarke and Kumar, 1983; Bryson, Biner, McNair, Bergeranc, and Abrams, 1981). Given that caffeine and nicotine are frequently self-administered in combination and that nicotine itself is behaviorally active, a second objective of the present study was to examine how repeated nicotine administrations might modify reactions of rats already
receiving sub-acute caffeine treatments. A standard nicotine challenge (2.0 mg/kg) was selected for simultaneous administration to rats also already receiving any of five caffeine doses (0.0, 2.5, 5.0, 10.0, and 20.0 mg/kg). Finally, a third objective of the present study was to tentatively assess how an extended caffeine history might alter reaction to withdrawal from repeated nicotine treatments.
CHAPTER II

Method

Subjects

Thirty female Sprague-Dawley rats, weight $\bar{X} = 181 \pm S.E. 7.9$ gm served as subjects. Animals were bred and raised in this laboratory, and kept under conditions of constant temperature (23 C, ca) and lighting. Subjects were randomly selected and assigned to one of five groups, comprised of six rats each. For the duration of the experiment animals were individually housed and provided water and rat chow (Rat Chow 5012, Ralston-Purina Co., St. Louis, Mo.), ad libitum.

Apparatus

Water intake was monitored via 100 ml fluid reservoirs including No. 6 stoppers and attached drink spouts (Corning 100 ml polystyrene graduated cylinders, Corning Glass Works, Corning, N.Y.). The reservoirs were filled daily with tap water, inverted and attached by a wire spring to individual stainless steel cages (32 cm x 24 x 20 cm; Unifab Corp., Kalamazoo, Michigan).

Separate body weights, in grams, were recorded daily from a top-loading scale (Pelouze, Model 1000).

Motor activity was measured for individual performances on one of three standard Wahmann Running Wheels (Wahmann Co., Baltimore, MD). Each wheel (35 cm dia. x 11 cm wide) was contained in a separate, sound-attenuating chamber (61 cm x 61 cm x 61 cm) equipped with masking white
noise (80 db), forced air ventilation, and illumination (7.5 volt, G.E.). A microswitch and digital monitor served to measure revolutions in both directions.

Thirty female CF-1 mice, bred and raised in this laboratory, served as targets for aggressive responses. One mouse was placed in each rat's cage for a 24-hour period. Mouse mortality rate was recorded.

Procedure

Phase I: Once animals were assigned to respective groups to control for homogeneity of weight-between-groups, an eight day acclimation period followed in which daily water intake was recorded. Subsequently, control animals were administered daily intraperitoneal injections of physiological saline solution and four experimental groups were administered daily intraperitoneal injections of caffeine at one of four dose levels (2.5, 5.0, 10.0 & 20.0 mg/kg). Each group continued to receive that dose initially assigned. Prior to each of 12 daily injections, water intake and total body weights were measured and recorded for all groups (N = 6). On the twelfth day of drug, one-half hour after injection, each animal was placed in a running wheel for a thirty-minute test session. Once all animals were returned to home cages a single female CF-1 mouse was placed in each cage over a 24-hour period. Incurred death rates were recorded and used as indices of rat aggression (muricide).

Phase II: On the thirteenth day of study all animals received previously assigned doses of caffeine, followed by a standard (2.0 mg/kg) intraperitoneal (IP) injection of nicotine. One-half hour after injections,
animals were placed in one of three running wheels and number of revolutions were then recorded over single thirty-minute intervals. Subsequently one CF-1 mouse was placed in each animal's home cage for 24 hours in a test of muricide. Both total body weights and 24-hour water intake were recorded daily for seven days. Collection of locomotion and muricide data was again made, on the seventh day of Phase II.

Phase III: For the remaining six days of study there occurred a second caffeine-only condition; also termed the "nicotine withdrawal phase." Animals were administered their previous caffeine doses as in Phases I and II and both water intakes and body weights were recorded for the first five of these days. On the sixth day tests of locomotion and muricide were again obtained.

Drug Preparation and Administration

Caffeine-sodium benzoate (50% caffeine, 50% benzoic acid, sodium salt), Sigma Chemical Company, St. Louis, Missouri) was dissolved in physiological saline to concentrations of 2.5, 5.0, 10.0 and 20.0 mg/ml. All statements of caffeine concentration and dosage (2.5, 5.0, 10.0 and 20.0 mg/kg) are given in terms of the sodium benzoate salt. When nicotine treatments were given, these consisted of nicotine tartrate (ICN Pharmaceuticals, Inc., Life Sciences Group, Plainview, New York) dissolved into aqueous saline solution at a concentration of 2.0 mg/ml. All injections were administered via the intraperitoneal route (IP) at volumes of 1.0 ml/kg. All injections were administered subsequent to 24-hour water intake and body weight measures and 30 minutes prior to tests of locomotion.
**Statistical Analysis**

Effects of caffeine on water intake, body weight, and locomotion were analyzed by multivariate analysis. A general linear models procedure was utilized to contrast test days. Determinations for linear or quadratic effects indicate the shape of the function. Effects of caffeine on muricidal aggression were analyzed by a Generalized Cochran-Mantel-Haenszel test statistic for Average Partial Association in Three-way contingency tables (Landis, Heymon & Koch, 1978; Landis, Cooper, Kennedy & Koch, 1977).
CHAPTER III

Results

Effects on Water Intake

In the overall assessment of water intake there was observed main effects of caffeine dose (p < 0.01). For this measure, neither the linear dose-response evaluation nor the quadratic dose-response evaluation reached statistical significance for either the intercept or slope. Figure 1 presents the average water intake (milliliters) for each caffeine dose group, across the three phases. The dashed vertical lines serve to separate phases and the dashed horizontal lines represent average water consumption for saline controls during Phase I only and is reproduced upon each other dose group's data for comparison purposes. As can be seen in Phase I, water intake was increased for both the 5.0 mg/kg and 10.0 mg/kg dose groups compared to saline performance, and was significant for the 10.0 mg/kg group (p < 0.04). In addition the 2.0 mg/kg nicotine treatments appeared to clearly enhance water intake irrespective of the caffeine dose employed. The overall assessment revealed a main effect of phase (p < 0.01), and this was related to the intake enhancement under nicotine (Phase II). Overall assessment revealed no interaction between caffeine dose and phase of treatment (p < 0.31). Figure 2 displays the average water intake per phase (milliliters) for each group. In this figure, the intake-enhancing influence of nicotine is emphasized.
Figure 1. The effect of caffeine dose alone, and in combination with 2.0 mg/kg nicotine, upon average number of milliliters of water consumed (± standard error) plotted as a function of day of treatment. Superimposed upon the saline-control (0.0 mg/kg) graph is drawn a horizontal line which represents that average number of milliliters of water consumed in Phase I, and is reproduced on each of the other doses' graphs for comparison purposes.
Figure 2. The effect of caffeine dose upon average milliliters of water consumed (N=6/group) across Phases I, II and III.
Effects on Body Weight

In the overall assessment of body weight data there emerged a main effect of dose ($p < 0.01$). Multivariate analysis revealed statistical significance for caffeine dose on body weight for both the intercept ($p < 0.02$) and slope ($p < 0.03$) of the linear dose-response assessment, and for the intercept ($p < 0.01$), but not the slope ($p < 0.47$) of the quadratic dose-response evaluation. Figure 3 displays total body weights (grams) for all caffeine doses at various points in the study. From left to right and top to bottom, the data appear as follows: first day caffeine-only (Phase I); first day caffeine-plus-nicotine (Phase II); seventh day caffeine-plus-nicotine (Phase II); and sixth day of nicotine withdrawal (Phase III). A small expected weight increase was noted as the study progressed (growth was seen from Day 1, Phase I to Day 1, Phase II). There was, however, no main effect of phase upon weight nor was there a dose-byphase interaction.

Effects on Locomotion

The effects of sub-acute caffeine, alone and in combination with nicotine, upon locomotion are displayed in Figure 4. Four running wheel assessments were performed, one on each of the following occasions: Phase I, Day 12 (caffeine-only); Phase II, Days 1 & 7 (caffeine-plus-nicotine); and Phase III, Day 6 (nicotine withdrawal). As can be seen in Figure 4, caffeine produced a general increase in locomotion, at the higher dose levels, in Phase I. Following this, the addition of nicotine (2.0 mg/kg) to the treatment regimen yielded
Figure 3. The effect of caffeine dose upon average body weight (+ standard errors) at each of four junctures in the study: Phase I, Day 1; Phase II, Day 1; Phase II, Day 7; Phase III, Day 6.
BODY WEIGHT AND CAFFEINE

1st DAY CAFFEINE

1st DAY NICOTINE

7th DAY NICOTINE

6th DAY POST NICOTINE

DOSE OF CAFFEINE (mg/kg)

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Figure 4. The effect of caffeine dose given alone (Phase I, Day 12), in combination with 2.0 mg/kg nicotine (Phase II, Days 1 and 7), and under withdrawal from nicotine (Phase III, Day 6) upon number of running-wheel revolutions. Bars indicate the average number of revolutions (N=6/group) in 30-minute test sessions (± 1.0 standard errors).
WHEEL RUNNING CAFFEINE AND NICOTINE

DAY 12 CAFFEINE & NICOTINE
DAY 1 CAFFEINE & NICOTINE
DAY 7 CAFFEINE & NICOTINE
DAY 6 POST-NICOTINE

REVOLUTIONS

DOSE OF CAFFEINE (mg/kg)

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marked locomotor suppression for all groups on Day 1 of Phase II (horizontally striped bar); an effect which was significant (p < 0.01). By contrast, on the 7th day of Phase II all groups showed increased activity (solid bar) in comparison to both the first day of combined treatment (p < 0.01) and to the assessment taken on the twelfth day of Phase I (caffeine-alone) (p < 0.01) but was not significantly different than locomotion which occurred at the end of Phase III (p < 0.063). Overall analysis of locomotor data using a linear models procedure revealed significant main effects for both caffeine dose (p < 0.01) and phase of treatment (p < 0.01), but not for an interaction of caffeine dose and phase (p < 0.06). A comparison of test days, across all phases, showed a significant linear dose effect (p < 0.01).

Locomotion data, collected during various phases of nicotine exposure (acute, sub-acute, and post-withdrawal), were further analyzed by plotting number of revolutions change from baseline values. The results of this analysis are presented in Figure 5. In the top graph, data collected under exposure to acute 2.0 mg/kg nicotine (Phase II, Day 1) and sub-acute 2.0 mg/kg nicotine (Phase II, Day 7) are plotted as a function of caffeine dose-in-combination upon wheel-running activity. Data are expressed as average number of revolutions change (+ standard errors) from wheel-running values collected under caffeine alone (Baseline = Phase I, Day 12). The data collected under acute nicotine exposure displays a dramatic locomotor suppression, as contrasted with behavior under Day 12 caffeine-alone. Then, after seven daily administrations of 2.0 mg/kg nicotine, retesting of locomotion revealed a
Figure 5. Top graph: The influence of acute 2.0 mg/kg nicotine (Phase II, Day 1) and sub-acute 2.0 mg/kg nicotine (Phase II, Day 7) plotted as a function of caffeine dose-in-combination upon wheel-running activity. Data are expressed as average number of revolutions change (+ standard error) from wheel running values collected under caffeine alone (Baseline = Phase I, Day 12). Bottom graph: the influence of caffeine dose upon locomotion (Phase III, Day 6) six days after sub-acute history with 2.0 mg/kg nicotine treatments. Data are expressed as average number of revolutions change (+ standard errors) from wheel running values collected on the 7th day of nicotine-plus-caffeine.
NUMBER OF REVOLUTIONS CHANGE FROM BASELINE

DAY 7
NICOTINE
(2.0mg/kg)
+ CAFFEINE

DAY I
NICOTINE
(2.0mg/kg)
+ CAFFEINE

POST-NICOTINE DAY 6
(CAFFEINE ALONE)

DOSE OF CAFFEINE
(mg/kg)
substantial elevation of motor activity, vis a vis data from both Day 12 caffeine-alone and Day 1 caffeine-plus-nicotine. This effect was considered a preliminary indication of behavioral tolerance. In the lower graph of Figure 5, data collected six days after the termination of subacute nicotine are presented. The data are expressed as average number of revolutions change (+ standard errors) from wheel-running values collected on the 7th day of caffeine-plus-nicotine. As can be seen, locomotor behavior declined between Phase II and Phase III, yet this decline was clearly dependent upon caffeine dose.

Effects on Muricidal Aggression

Figure 6 on muricide shows four trials (T1, last day of Phase I; T2, first day Phase II; T3, last day Phase II and T4, last day Phase III) of mouse mortality rate for each subject across all groups as a function of caffeine dose (0.0, 2.5, 5.0, 10.0 & 20.0 mg/kg). In group one there occurred one death on T3. In group four (10.0 mg/kg) three subjects killed on all four trials. In the 20.0 mg/kg group, subject two killed on T2, T3 and T4. These results suggest that muricidal aggression as a function of caffeine dose is suppressed at lower doses and enhanced at higher doses. Apparently the standard 2.0 mg/kg dose of nicotine failed to suppress aggressive responding with higher doses of caffeine. Statistical analysis using the Generalized Cochran-Mantel-Haensel test for average partial association in three-way contingency tables revealed a significant muricide by trial effect on T3 versus T1 (p < 0.057) and no effect on T2 versus T1 or T4 versus T1. Muricide as a function of dose shows a quadratic effect (p < 0.03.)
Figure 6. The effect of caffeine alone, and in combination with 2.0 mg/kg nicotine, upon number of muricidal responses (24-hour exposures to one mouse per rate, N = 6/group) within each caffeine dose level. The four trials occurred respectively at Phase I, Day 12; Phase II, Day 1; Phase II, Day 7; and Phase III, Day 6. Each bar represents one mouse-kill.
CHAPTER IV

DISCUSSION

Moderate doses of caffeine, given alone (Phase I) produced increased water intake and the 10.0 mg/kg dose induced elevated water intake significantly higher than that under saline (see Figures 1 and 2). For comparison purposes, studies of caffeine's effects on water intake are generally sparse (e.g., see Merkel, Wayner, Jolcoeur, and Mintz, 1981). However, the present study's results stand at variance with Cooper (1982) who reported that water-deprived rats evidenced attenuated water drinking in 2-hour test sessions, and thus suggests that caffeine-induced changes in water balance depends upon deprivational state and certain other experimental conditions. Wayner et al., (1986) examined the effects of acute caffeine (3.125, 6.25, 12.5, 25.0, 50.0 and 100.0 mg/kg) on schedule-induced polydipsia (SIP) in 1-hour test sessions and on related home-cage intakes with rats at both 80% and 100% body weight. When at 80% weights, rats consumed significantly more water in home cages under 50 and 100 mg/kg treatments but drank less at 100 mg/kg in SIP test sessions. When at 100% weights, rats consumed more home-cage water at 50 and 100 mg/kg dose levels and displayed enhanced SIP under 3.125 mg/kg treatment but suppressed SIP under the 100 mg/kg dose level. A search for tolerance to caffeine's SIP effects under 100 mg/kg doses given 8 times, one every other day, did demonstrate tolerance by the 5th treatment. Merkel et al., (1981) further analyzed water intake effects of
acute caffeine (3.125, 6.25, 12.5, 25.0, 50.0 and 100.0 mg/kg) with rats maintained under various food or water deprivational conditions. This later study clearly demonstrated that caffeine's influence on water balance depends upon previous deprivational state. The present study corroborates those findings of Wayner et al. (1976), Merkel et al., (1976) and Cooper (1982) which have all shown caffeine as active in altering rodent water balance. The present study, however, appears to be the first to examine the water intake-effects of repeated, daily sub-acute caffeine treatments (0.0, 2.5, 5.0, 10.0 and 20.0 mg/kg). In this regimen, no evidence of tolerance emerged throughout the Phase I (12 days of caffeine alone) period.

In Phase II standard probe doses of nicotine (2.0 mg/kg) were given daily for seven days, to all subjects. Evaluation of the influence of this nicotine dose alone was made by scrutinizing those water intakes of the 0.0 mg/kg caffeine subjects during Phase II. Here it was seen that nicotine considerably enhanced drinking. When 2.0 mg/kg nicotine treatments were combined with the various caffeine doses (2.5, 5.0, 10.0 and 20.0 mg/kg), intake enhancements were again seen (see Figures 1 and 2, Phase II). Statistical analysis revealed that while there were main effects of Phase and of caffeine dose, there occurred no Phase X caffeine-dose interaction. Few studies have explored the potential dipsogenic influences of nicotine, in isolation, let alone in combination with other chemical agents. One previous study placed nicotine into rats' home-cage drinking water to examine whether nicotine self-administration could be induced (Falkenborn et al., 1981). In that experiment water intake was suppressed under
nicotine treatment yet it remains unclear whether confounding variables such as nicotine's taste and relative reinforcing properties were actually what accounted for the observed effects. In another study, Domino and Lutz (1973) injected nicotine intraperitoneally and effect on SIP were then analyzed. Here too, nicotine was found to suppress water intake. Our findings with nicotine at 2.0 mg/kg, given alone, indicate that nicotine can function as a dipsogen, and thus runs contrary to those data collected by both Falkenborn et al., (1981) and Domino and Lutz (1973). The potential determinants of the described across-reports variance remain obscure.

Phase III constituted a return to the caffeine-only condition and as such may be construed as the "nicotine-withdrawal" phase. In general, institution of Phase III re-established lowered water intake levels; comparable to those found in Phase I. This clearly discernable fall in water consumption occurred regardless of the concommitant dose of caffeine being administered. This within-groups reversal of water intake effects, as seen across phases, adds to the evidence with which it can be asserted that nicotine can function as a dipsogen, even though the 0.0 mg/kg nicotine control condition was not employed.

In summary, the present experiment appears to have been the first to examine caffeine's dipsogenic effect in combination with nicotine's influence upon water balance. The present work reveals no preliminary evidence of caffeine-nicotine interaction in the determination of water intake. However, a much expanded nicotine dose range is necessary for definitive remarks on this issue.
Caffeine dose was found to exert a relatively subtle influence on body weight with slight elevations occurring at certain doses, yet when various caffeine doses were each compared to saline, the results were neither robust nor significant. This finding is in keeping with those results of Henry and Stephens (1980) who chronically exposed mice to caffeine via home-cage water supplies. Statistical analysis in the present study revealed no main effect of phase, and thus, a body weight effect of nicotine was ruled out. Our lack of nicotine effect on body weight is at variance with those results reported both by Baettig, Martin, and Classen (1980) and by Falkeborn, Larsson and Nordberg (1981). In both of these later two experiments nicotine was found to clearly suppress rat body weights relative to vehicle-control subjects. It may be that differences in route of administration account for differences in nicotine's body weight effects across research reports. In both the Baettig et al. (1980) and the Falkeborn et al. (1981) studies nicotine was administered orally, in home cage drinking water, and it may be that gustatory variables altered nicotine's influence that were not operative in the present study which employed intraperitoneal injection. McNair and Bryson (1983) have reported inhibition of weight-gain in 3 month old rats subcutaneously treated with nicotine for male subjects but not for female animals. As such those findings of McNair and Bryson (1983) are in agreement with those results here reported.

In the caffeine-only condition (Phase I) locomotion significantly increased as a function of dose. These results are consistent with the findings by Pettijohn (1979) using identical doses of caffeine as
in this experiment. The highest dose produced no significant increases in activity from controls. Chronically caffeine-treated rats (11 weeks) demonstrated tolerance to the stimulant effects on locomotor activity (Holtzman, 1983). The sub-acute caffeine treatments in Phase I did not appear to produce "tolerance" to the stimulant effects of locomotor activity.

Upon the first administration of a standard "probe" dose of nicotine (Day I, Phase II) there occurred a marked depression of wheel running across all groups relative to activity recorded in Phase I. This depression has previously been noted in experimentally naive rats receiving nicotine alone (Stolerman, Fink & Jarvik, 1973). The depressant action of nicotine on locomotor activity in non-tolerant rats is blocked by mecamyllamine, both a peripheral and central (CNS) blocker (Clarke & Krumar, 1983). On the other hand, hexamethonium, a peripheral (CNS) blocker had no effect on locomotor activity suggesting central actions of nicotine.

By contrast, following seven consecutive days of caffeine-plus-nicotine treatment, performance emerged well above that observed on the two previous test sessions, thus suggesting the possible development of "tolerance". To assert that tolerance occurred, an additional group receiving nicotine on only the first and last days of Phase II would be required. Nonetheless, tolerance to the depressive effects of nicotine on behavioral measures is well documented (Hubbard & Gohd, 1974 & Dougherty et al., 1981). Stolerman et al. (1973) have demonstrated that animals receiving three daily doses of nicotine for eight consecutive
days remain tolerant up to 90 days after drug withdrawal. On Day 6 of nicotine withdrawal (Phase III) activity levels remained elevated showing a slight but not significant decrease from Day 7 of combined drug treatments, with the exception of the 20.0 mg/kg dose group. The apparent "tolerance" to the depressant effects of nicotine on activity has been shown to continue in the absence of repeated injections (Morrison & Stephenson, 1972).

Both graphic and statistical analysis revealed that caffeine dose had a major impact on the tendency of rats to kill mice within 24-hour aggression test sessions. This general finding, that caffeine increases animal irritability, has also been demonstrated in numerous other studies (eg. Emley and Hutchinson, 1983; Eichelmann, et al., 1978). In addition, Quenzer and Feldman (1975) have reported that caffeine can reverse the anti-muricidal effects of chlordiazepoxide. In the present study, nicotine neither induced muricidal responses nor did it suppress muricide which had been induced under the higher doses of caffeine. This later result is somewhat surprising in that various other reports have documented nicotine's aggression suppressive effects, employing various species and attack induction and recording procedures (eg. Waldbillig, 1979; Berntson, Beattie, and Walker, 1976; Emley and Hutchinson, 1983; Driscoll and Baettig, 1981). The variables which account for a lack of nicotine's ability to suppress caffeine-induced muricide remain unknown. The present report does suggest however, that nicotine may not interact with caffeine to alter aggressive responses within the muricidal paradigm. Complete address of this issue will, however, require an extended nicotine dose range.
In summary, the most striking effects occurred with the 10.0 mg/kg dose of caffeine. Water intake was significantly enhanced across phases at 10 mg/K, in comparison to the other doses (see Figures 1 and 2). Also, consistent mouse-killing was observed on all four repeated trials of aggression, and, nicotine was not shown to suppress this behavior (see Figure 6). Overall, locomotion significantly increased as a function of caffeine dose in Phase I and by comparison was significantly depressed on Day 1 of Phase II (see Figures 4 and 5). This initial depression on activity induced by nicotine in non-tolerant animals is well documented (Clarke & Kumer, 1983; Stolerman et al. 1973: Stolerman et al, 1974). However, by Day 7 of Phase II, apparent tolerance to the depressant effects of nicotine had developed, and activity levels even exceeded those observed on the first caffeine-only condition for all groups. Although no drug-drug interaction effects were statistically detected, it has seemed reasonable to expect interaction effects given certain "opposing" effects of nicotine and of caffeine on aggression and water intake as described in the literature. The present study lacked an appropriate caffeine-only control group across all phases of treatment with which to fully assess interaction possibilities. Additionally, several doses of nicotine in combination with several doses of caffeine would provide a more useful assessment of interaction possibilities.
BIBLIOGRAPHY


