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Caffeine-Induced Taste Aversion and Mimetic Responses[1]

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WHITE, B. C. AND F. D. MASON. *Caffeine-induced taste aversion and mimetic responses*. PHARMACOL BIOCHEM BEHAV **23**(4) 515–518, 1985.—Novel tastes preceded a range of caffeine doses (10–80 mg/kg) in a taste aversion training trial. One week later rats which had doses of 30 mg or higher showed strong aversions as measured by a single bottle consumption test. The 10 and 20 mg dose produced the most hyperactivity and apparently enhanced intake of the taste paired with caffeine. During the training trial, rats receiving the 80 mg dose exhibited copious gapes and chin-rubs, mimetic responses to noxious tastes. Gapes also occurred in these subjects during the aversion test. Consumption was more sensitive than mimetic responding as a measure of the aversive effects of caffeine. Only the 80 mg dose produced neophobia. Tests with isotonic injections indicated that tonicity was not the source of the aversions.

Caffeine Locomotor activity Mimetic responses Neophobia Taste aversion

CAFFEINE, ubiquitous in the contemporary human diet, has now been included in many of the nonprescription weight control medications. The association of caffeine with dieting raises questions about the effects of this methylxanthine on food and water consumption. The use of caffeine in dieting is somewhat paradoxical in light of recent reports that in rats low to moderate doses may increase food consumption [3,10] or leave it unaffected [2]. At doses above 50 mg/kg there are consistent reports of decreased food intake and/or body weight gain [2, 10, 13].

The results of several of these studies suggest that flavor aversions may be involved in the decrease in food consumption and body weight gain. Following forced chronic consumption, rats developed a preference for caffeine but avoided the flavor of the drink in which it had been ingested [12]. A study from our lab [14] found that multiple injections of 60 mg/kg doses produced weight loss and copious displays of chin-rubbing and gaping, mimetic responses associated with noxious tastes [8] and conditioned flavor aversions [4].

In this context we have examined the range of caffeine doses that produce taste aversions, neophobia, mimetic behaviors and locomotor activity. An additional purpose was to assess the relative sensitivity of consumption and mimetic responding as measures of aversion.

EXPERIMENT 1

METHOD

Animals

Thirty female Long-Evans rats (191–293 g) were randomly assigned to five equal groups and housed individually in stainless-steel cages. During the 16 days prior to training, subjects were habituated to a restricted water intake schedule and maintained throughout the study at 85–90% of their ad lib body weight. Each group was assigned to either one of four caffeine doses (10, 20, 40, and 80 mg/kg, IP) or a sodium benzoate vehicle control injection (80 mg/kg, IP). The volume of injections was 1.0 ml/kg.

Procedure

For the last 9 days of the habituation period the animals were placed in the training-test cages for 15 min during which water was available. A graduated glass centrifuge tube (13.0 ml) with rubber stopper and stainless-steel spout was mounted at one end of the rectangular wiremesh cage ($20 \times 37 \times 17$ cm). On the day of training the rats were placed in the training-test cages for 30 min with saline (0.15 M) or saccharin (0.1%) solution available for drinking during the first 15 min. Half of each group received the saline drinking solution and the remainder the saccharin. Immediately following removal of the drinking tubes, the animals were injected with their designated dose of caffeine or sodium benzoate. For 15 min after injection an observer, blind to the treatments, recorded locomotor activity (crossings) and the mimetic responses of chin-rubbing and gaping [8]. A chin-rub was recorded when the animal lowered its chin to the substrate and moved forward, rubbing the chin on the floor of the cage. A gape was indicated when the mouth was opened wide which fully exposed the incisors. Behavioral observations were made from an adjacent room through a one-way mirror. The 15 min observation interval was selected because mimetic behaviors appeared within minutes of injections in an earlier study [14]. The half-time for absorption of a 25 mg/kg oral dose of caffeine has been reported to be on the order of 6 min [1]. This would suggest that behavioral effects could occur very rapidly, especially

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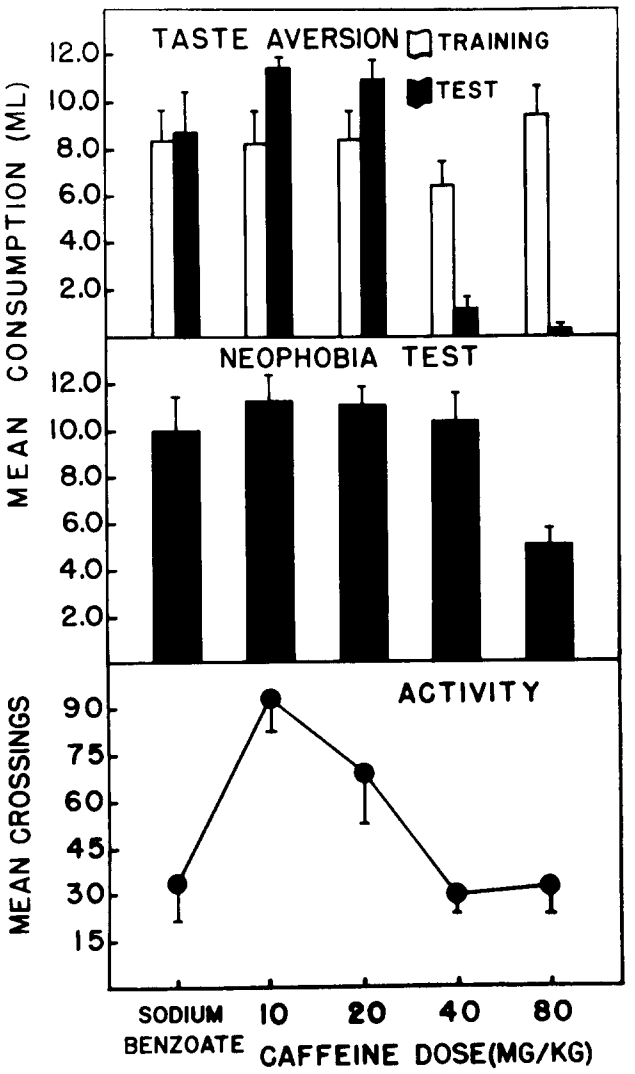

FIG. 1. Top panel. Mean and sem consumption of taste solution during training and aversion test. Middle panel. Consumption of novel taste solution during neophobia test. Bottom panel. Locomotor activity during 15 minute period following caffeine injection (training session).

if they are initiated by gastro-intestinal irritation as may be the case with mimetic responses.

Seven days after training the rats were again given access to the training solution for 15 min in a single bottle test for aversion. Gapes and chin-rubs were observed as above. Five days later each subject was given 15 min with a novel solution available as a test for neophobia. Saccharin was the novel solution for subjects trained with saline and *vice versa*. Only consumption was measured during the neophobia test.

RESULTS

The top panel of Fig. 1 illustrates the mean consumption of the taste solutions during training and the aversion test. Aversion training significantly affected consumption during the test session (between subjects: $F(4.25) = 35.11$, $p<0.01$), producing decreased drinking at the 40 and 80 mg/kg doses (p's<0.01, Newman-Keuls). The within subjects comparison of training with aversion test consumption was also statistically significant, $F(1,25) = 8.33$, $p<0.01$, as was the dose by training-test interaction, $F(4,25) = 15.28$, $p<0.01$. Individual comparisons of the 40 and 80 mg caffeine groups with their respective consumption during training revealed significant decreases during the aversion test (p's<0.01).

The lower caffeine doses appear to have enhanced consumption of the taste solution that preceded them. During the aversion test, the 10 and 20 mg groups consumed more of the taste solution than the sodium benzoate group ($p<0.05$). These groups also drank more during their aversion test than during their training exposure to the solutions ($p<0.05$). The sodium benzoate group's consumption did not differ significantly between training and testing. These results obscure the magnitude of the enhancement since 50% of the subjects in the 10 and 20 mg groups exhausted the capacity (13.5 ml) of the drinking tubes within the 15 min test session.

The middle panel of Fig. 1 shows the results of the neophobia test. Only the 80 mg dose of caffeine produced a significant attenuation of drinking, $F(4,25) = 5.33$, $p<0.01$). This group drank significantly less than each other group (p's<0.01), even though the previous day's water consumption was indistinguishable from that of the other groups.

Table 1 summarizes the observations of mimetic responses during training. The 80 mg caffeine group exhibited 91% of the observed gapes and 94% of the chin-rubs. Gaping was observed in each 80 mg subject and chin-rubbing was detected from all but one animal in this group. During the aversion test, each 80 mg subject was observed to gape upon tasting the solution. No chin-rubs were recorded during the aversion test. One sodium benzoate subject was judged to have gaped during the test.

EXPERIMENT 2

In Experiment 1 the injections were given at a constant volume (1.0 ml/kg), resulting in hypertonic solutions at the two highest doses. This procedure confounded the tonicity of the solutions with the doses that produced taste aversion. Accordingly, local irritations of the peritoneum may have produced the observed aversions. Consequently, a second experiment was conducted in order to determine whether aversions would occur at the highest dose when given in isotonic solution.

METHOD

Four groups of six female hooded rats (125–158 g) were habituated to water restriction and housed as in Experiment 1. All injections were made with isotonic solutions and administered intraperitoneally. The first group received an 80 mg/kg dose of caffeine dissolved in sodium benzoate solution. The second group was trained with a 30 mg/kg caffeine injection to provide more information about aversions from the middle of the dose range used in Experiment 1. Group three received isotonic sodium benzoate at a dose of 80 mg/kg and served as a vehicle control. A fourth group was a volume control and received isotonic saline at a volume similar to that of group one (4 ml/kg). Training and testing procedures were the same as in Experiment 1 with the exceptions that mimetic responses were observed only during training and there was no neophobia test.

TABLE 1
TOTAL MIMETIC RESPONSES DURING TRAINING

Mimetic Response	Sodium Benzoate	Saline	Caffeine (mg/kg) 10	20	30	40	80
Experiment 1							
Chin Rub	1	—	0	0	—	3	63
Gape	0	—	0	0	—	3	30
Experiment 2							
Chin Rub	1	0	—	—	0	—	115
Gape	0	1	—	—	1	—	44

RESULTS

The 80 mg caffeine dose remained a potent agent for inducing aversions when given as an isotonic solution (simple analysis of variance for experiment 2 aversion test: $F(3,20)=24.73$, $p<0.01$). Mean consumption (0.5 ml, sem = 0.2) of the averted solutions was virtually the same during the aversion test as that of the 80 mg caffeine group of Experiment 1. The profuse mimetic responding that occurred with the isotonic 80 mg caffeine dose (See Table 1.) is also consistent with the results of Experiment 1. Gapes and chin-rubs were very infrequent in the other isotonic groups.

The 30 mg caffeine dose appears to be close to the threshold for inducing aversions. Mean consumption (4.1 ml, sem = 1.7) was significantly ($p<0.01$, Newman-Kuels) below the saline and sodium benzoate groups (10.4 ml, sem = 0.8 and 10.7 ml, sem = 0.6, respectively) and significantly greater than the 80 mg caffeine group ($p<0.05$).

GENERAL DISCUSSION

The results of the aversion test indicated that caffeine is a potent agent for inducing taste aversions. The 30 mg/kg dose appears to be close to the threshold dose for conditioning aversions with a single injection when tested without alternative flavors available. This dose is well within the range of doses commonly used in behavioral studies of caffeine. The results of the present study suggest that food aversions may have been involved in the arrested body weight in rats given multiple caffeine treatments at 30 and 60 mg/kg [14]. Other investigators [2,10] have reported decreased food consumption following doses of 50 mg or higher. When food intake is measured within minutes of injection the attenuation of consumption is likely to be the result of the direct pharmacological effects of the drug. In the present study copious mimetic responding occurred during the 15 min following the 80 mg dose of caffeine, revealing the immediacy and severity of the malaise.

The single-bottle test of the present study may have contributed to the similarity in doses that produce aversion and doses that affect food consumption [2,10] and body weight [14]. In contrast, a two-bottle test has revealed aversion at doses as low as 3–6 mg/kg [12]. We believe that the single-bottle test is closer to the situation used in the feeding studies where alternatives are not offered. The relevance of either test to the human use of caffeine in weight control hinges on the availability of alternatives and the consistency of flavors in the diet.

In the case where feeding is measured over an extended period of time, depressed intake following caffeine treatment may involve conditioned flavor aversions. Merkel *et al.* [10] found food consumption attenuated for up to two days following caffeine doses of 50 and 100 mg/kg. Our results suggest that conditioned food aversion may have been involved; however, the authors discounted this explanation with the comment that aversion to familiar tastes "cannot" be conditioned. In fact, conditioned aversions to familiar tastes are well documented [4,9]. These aversions are not as strong and may require a more intense illness than those of novel tastes. Accordingly, our results show that the doses used by Merkel *et al.* [10] are well above those needed to produce aversions to novel tastes. In fact, one week after training our 80 mg dose produced extremely cautious sampling of the averted solution followed by gaping responses. Training was sufficiently aversive at the high dose to induce measurable neophobia twelve days later. Avoiding novel tastes following illness has obvious survival value for the animal [11]. There may also be survival value in being cautious with familiar foods following intense malaise. In the study by Merkel *et al.* [10], the absence of novel alternatives both before and after caffeine and the magnitude of the doses seem sufficient to have established conditioned aversion and temporary depression of food intake.

In the present study the high incidence of gaping and chin rubbing is indicative of the immediate noxious effects of the 80 mg caffeine dose. The fact that few of these mimetic responses were observed with the 30 and 40 mg doses during training reveals that gapes and chin rubs elicited by the aversive agent are not a very sensitive predictor of conditioned taste aversion. When elicited by the averted taste, mimetic responses indicated aversion at only the highest dose and then only gaping was exhibited. These results show that gapes and chin rubs are less sensitive than consumption as indices of aversion or malaise. However, in studies where consumption is not measured, mimetic responses may provide a useful index of toxic reactions to high doses of a drug.

Experiment 2 demonstrated that the 80 mg dose used in Experiment 1 retained its potency when injected as an isotonic solution. Intake was similar for the 80 mg caffeine groups of the two experiments. A substantial number of mimetic responses occurred in each 80 mg caffeine group with very few observed at other doses. Differences between

the two 80 mg groups were most likely the result of having different observers in the two experiments. The results from the two observers agree in that very few responses were recorded for the other groups.

In Experiment 1 the results of the 10 mg caffeine treatment suggest the possibility of a conditioned taste enhancement. One week after tasting the novel flavor followed by caffeine, the rats in this group consumed more of the taste solution than at their initial exposure and more than the sodium benzoate control group. Other researchers [3,10] have reported increases in feeding under the immediate influence of similar doses of caffeine. In this latter case feeding may be stimulated as part of the general arousal effects of the drug, since low doses of amphetamine have a similar effect on feeding [3]. In the present study the 10 and 20 mg doses produced the most locomotor activity. The conditioned enhancement of Experiment 1 may also be related to the activity effects of the lower doses. If the arousal effects of caffeine are accompanied by a positive affect as with humans [7], then conditioned enhancement may be the consequence of pairing this effect with a novel taste. This process may be similar to that reported by Green and Garcia [6] who found enhanced consumption of flavors associated with recuperation from illness.

If the effects of caffeine are similar in rats and humans, the utility of caffeine in dieting depends on the dose. High doses may be capable of reducing food consumption through either direct pharmacological effects or conditioned aversion. Lower caffeine doses may be included in diet medication for their arousing effects, but this may be counter productive given the potential for enhancement of consumption by stimulating doses.

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REFERENCES

1. Berg, A. W. Physiological disposition of caffeine. *Drug Metab Rev* **4:** 199-228, 1975.
2. Cox, R. H. and R. P. Maickel. Interactions of caffeine with various amphetamines on rat food consumption and avoidance responding. *Neuropharmacology* **15:** 767-771, 1976.
3. Dobrzanski, S. and N. S. Doggett. The effects of (+)-amphetamine and fenfluramine on feeding in starved and satiated mice. *Psychopharmacology (Berlin)* **48:** 283-286, 1976.
4. Garcia, J. and R. A. Koelling. A comparison of aversions induced by X-rays, toxins, and drugs in rats. *Radiat Res* **7:** 439-450, 1967.
5. Garcia, J., D. Forthman and B. White. Conditioned disgust and fear from mollusk to monkey. In: *Primary Neural Substrates of Learning and Behavioral Change*, edited by D. L. Alkon and J. Farley. New York: Cambridge University Press, 1983.
6. Green, K. F. and J. Garcia. Recuperation from illness: flavor enhancement for rats. *Science* **173:** 749-751, 1971.
7. Goldstein, A. and S. Kaizer. Psychotropic effects of caffeine in man. III. A questionnaire survey of coffee drinking and its effects in a group of housewives. *Clin Pharmacol Ther* **10:** 477-488, 1969.
8. Grill, H. J. and R. Norgren. The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain Res* **143:** 263-279, 1978.
9. McLaurin, W. A., J. A. Farley and B. B. Scarborough. Inhibitory effect of preirradiation saccharin habituation on conditioned avoidance behavior. *Radiat Res* **18:** 473-478, 1963.
10. Merkel, A. D., M. J. Wayner, F. B. Jolicoeur and R. Mintz. Effects of caffeine administration on food and water consumption under various experimental conditions. *Pharmacol Biochem Behav* **14:** 235-240, 1981.
11. Revusky, S. H. and E. W. Bedarf. Association of illness with prior ingestion of novel foods. *Science* **155:** 219-220, 1967.
12. Vitiello, M. V. and S. C. Woods. Caffeine: Preferential consumption by rats. *Pharmacol Biochem Behav* **3:** 147-149, 1975.
13. Vitiello, M. V. and S. C. Woods. Evidence for withdrawal from caffeine by rats. *Pharmacol Biochem Behav* **6:** 553-555, 1977.
14. White, B. C. and G. E. Keller, III. Caffeine pretreatment: Enhancement and attenuation of d-amphetamine-induced activity. *Pharmacol Biochem Behav* **20:** 383-386, 1984.