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Evidence for Withdrawal from Caffeine by Rats[1]

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VITIELLO, M. V. AND S. C. WOODS. *Evidence for withdrawal from caffeine by rats.* PHARMAC. BIOCHEM. BEHAV. 6(5) 553–555, 1977. – Injections of caffeine produced avoidance of a novel flavor. Rats which had previously received injections of caffeine on each of twelve days avoided a novel solution which had been associated with the absence of caffeine. This and other controls suggest that physiological withdrawal from caffeine is the mechanism for this avoidance.

Caffeine Physiological withdrawal Avoidance

CAFFEINE is a potent central nervous system stimulant consumed worldwide in at least moderate amounts on a daily basis [2, 18, 20]. Although a wealth of anecdotal evidence suggests that many humans have at least a psychological dependence on caffeine, there has been little experimetation on the issue. Further, many commercial preparations of caffeine, particularly those produced for its mild analeptic effect, specifically state that caffeine is not habit-forming. Contrary to these claims, one recent study [22] suggests that prolonged exposure to caffeine results in a preference for the drug by rats. This finding supports a variety of suggestive evidence [3, 4, 7, 8, 9, 10, 12, 14] for the development of dependence on caffeine.

The question of a dependence on caffeine becomes more important in light of the recent evidence linking the consumption of coffee with the incidence of myocardial infarction [1, 5, 11, 13, 17, 21]. One report has contested these findings [6] and none of the studies are conclusive. The present experiment demonstrates that physiological withdrawal from caffeine occurs following its removal. Rats were able to associate a novel flavor with the absence of caffeine and subsequently avoided that flavor. This technique has also been used to demonstrate withdrawal from morphine by addicted rats [15,16].

METHOD

The animals were 96 naive male Sprague-Dawley rats, approximately 120 days old and with an average initial weight of 326 g. They were housed in individual stainless steel cages and received ad lib food throughout the experiment. They were randomly divided into 16 groups of 6 animals each. Each group underwent the same basic procedure. All rats received only 30-min access to water a day for 12 days. During this period rats in 8 of the groups received injections of physiological saline at the end of their drinking bout, and the other 8 groups received injections of caffeine (caffeine and sodium benzoate, U.S.P., Parke and Davis) at one of the following doses: 0.5, 1.0, 2.0 or 4.0 mg/rat, following their drinking bout. All injections of caffeine were at a concentration of 4 mg/ml. Two saline-injected groups received each of the four possible volumes in their injections. On the 13th day, rats in all groups received a 0.1% aqueous solution of sodium saccharin in place of water during the usual 30-min drinking session. Immediately after consuming the saccharin, 4 of the groups which had previously received saline injections were again injected with saline (groups S-S). The other 4 groups which had previously received saline were injected with caffeine at one of the 4 doses previously used 0.5, 1.0, 2.0 or 4.0 mg/rat (Groups S-C). Likewise, 4 of the groups which had received caffeine on Days 1–12 were injected with saline on Day 13 (Groups C-S) and 4 were injected with caffeine at the same doses they normally received (Groups C-C).

On the following day, rats in all groups were given a 2-bottle preference test between 0.1% saccharin and water. The position of the drinking tube on the front of the cage of each rat had been randomly determined each day during the experiment so that each rat was accustomed to drinking from either of two positions (right or left) on the front of its cage. On Day 14, the 2 bottles were placed in these 2 positions. Further, the thirsty rat was forced to sample each tube for at least 10 sec on this day by having the other tube briefly removed.

RESULTS AND DISCUSSION

Since total fluid consumption on the test day within each level of caffeine concentration did not differ significantly among groups, consumption data were analyzed by

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TABLE 1

MEAN ABSOLUTE CONSUMPTION IN ML OF SACCHARIN, WATER AND TOTAL FLUID INTAKE AS A FUNCTION OF GROUP AND CAFFEINE WHERE ADMINISTERED

		0.5	1.0	2.0	4.0
S to S	Sacc.	20.8 ± 0.87	23.0 ± 1.57	25.0 ± 1.37	20.7 ± 2.42
	Water	1.8 ± 0.40	5.2 ± 1.14	5.0 ± 1.24	4.8 ± 1.28
	Total	22.7 ± 1.05	28.2 ± 1.74	30.0 ± 1.21	25.5 ± 1.67
C to C	Sacc.	18.0 ± 1.37	15.7 ± 2.54	21.5 ± 1.77	20.3 ± 0.84
	Water	2.5 ± 0.34	9.0 ± 2.27	6.3 ± 2.09	7.5 ± 0.88
	Total	20.5 ± 1.18	24.7 ± 0.99	27.8 ± 2.09	27.8 ± 0.48
S to C	Sacc.	16.0 ± 2.06	9.7 ± 2.96	5.5 ± 1.50	6.5 ± 2.04
	Water	5.0 ± 1.39	16.2 ± 2.94	22.5 ± 1.20	19.0 ± 1.91
	Total	21.0 ± 1.06	25.8 ± 1.70	28.0 ± 1.15	25.5 ± 1.67
C to S	Sacc.	17.3 ± 1.71	11.3 ± 3.08	9.7 ± 1.87	10.2 ± 1.28
	Water	2.7 ± 0.92	16.8 ± 2.66	18.8 ± 1.47	14.2 ± 1.68
	Total	20.0 ± 1.06	28.2 ± 1.40	28.5 ± 1.12	24.3 ± 1.02

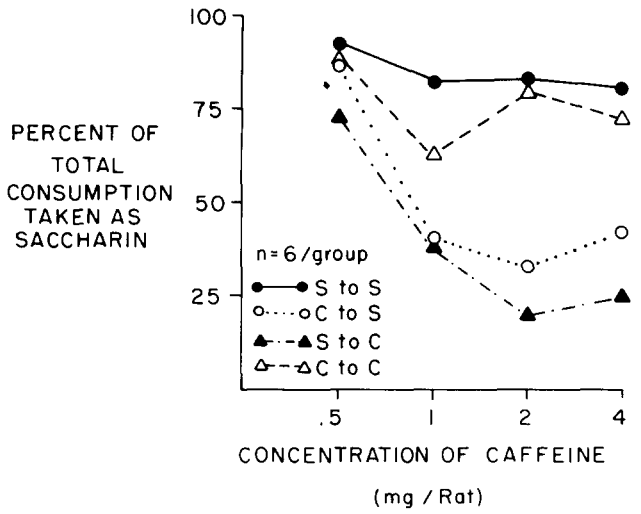

FIG. 1. Mean percent saccharin consumed on Day 14 as a function of group and caffeine concentration where administered. Percent saccharin was calculated as saccharin/(saccharin plus water). Rats in the S → S groups differed only in the volume of saline injected (0.13–1.0 ml/rat).

determination of the percent of the total liquid intake consumed as saccharin on the 2-bottle test (see Fig. 1 and Table 1). Data were analyzed via a 4 × 2 × 2 factor analysis of variance. The treatment of caffeine vs. saline on Day 13 and the dose-response effect were both significant ($p<0.01$). The interaction between rats receiving caffeine or saline on Days 1–12 versus rats receiving caffeine or saline on Day 13 was also significant ($p<0.01$). Paired comparisons between the S → S and S → C groups revealed significant differences at the 1.0, 2.0 and 4.0 mg/rat doses ($p<0.05$ for each comparison). Groups C → C and C → S differed significantly at the 2 higher concentrations ($p<0.05$ for each comparison).

For both the S-S and C-C groups, no aversion developed to saccharin as evinced by their relatively flat curves in Fig. 1. This was certainly expected of the S-S group, as this is the normal control used in experiments on taste aversions. The lack of an aversion by the C-C group presumably reflects the fact that there was no change of the drug they received throughout the experiment. They received caffeine injections following their drinking bouts regardless of whether the bottles contained water or saccharin, and the caffeine could not be considered to be a novel stimulus by the 13th injection. The S-C groups had a dose-dependent conditioned taste aversion; higher doses of caffeine administered in conjunction with saccharin led to significant aversions and subsequent avoidance of saccharin whereas the smallest dose had no reliable effect. This indicates that caffeine is an effective toxin for the formation of taste aversions when rats have never previously experienced the drug. This finding is consistent with previous reports of human behavior [4,9]. Rats in the C-S groups also developed significant aversions to saccharin when they had been accustomed to receiving the higher doses of caffeine. The implication is that the absence of caffeine in a situation where it had customarily occurred was aversive to the rats.

Previous research has shown that the association of a novel flavor with the withdrawal from morphine results in the subsequent avoidance of that flavor [15,16]. In the present experiment, rats given an injection of caffeine after consuming water in a daily drinking session received an injection that did not contain caffeine after consuming a novel flavor. They subsequently avoided the novel flavor, saccharin, if they had been accustomed to larger daily injections of caffeine. The implication is that the absence of caffeine was sufficiently aversive to the rats that they experienced withdrawal. While withdrawal signs are often opposite in nature from the effects of a given drug, this is the case when the parameter measured is a primary response to the drug (e.g., heart rate change following caffeine or analgesia following morphine). In the present study, a secondary response to the drug (consumption of a flavor) is the dependent variable and can be considered as one index of discomfort or unpleasantness. Therefore, although caffeine by itself causes avoidance of a novel flavor, absence of caffeine in a situation where it normally occurs would be expected to cause the same response only if it is associated with aversive or unpleasant sensations. This seems to be the case with the rats in this study. This is

in agreement with suggestive reports of human behavior [3, 4, 7, 9]. In light of these results, perhaps a more realistic attitude toward caffeine-containing compounds should be taken.

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