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Reluctance of Rats to Drink Hashish Suspensions: Free-Choice and Forced Consumption, and the Effects of Hypothalamic Stimulation

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Abstract. For over 30 days male Wistar rats drank a concentrated aqueous suspension of hashish in the absence of alternative fluids, but they rejected the drug when water was also made available. In another experiment rats given a choice between water and varying concentrations of hashish also rejected concentrated suspensions, but they appeared less reluctant to drink dilute concentrations. Neither a schedule of alternate-day presentation of hashish nor forced electrical stimulation of lateral hypothalamus induced rats to increase their home-cage intake of an aversive concentration of hashish, suggesting that the enhanced consumption of concentrated ethanol solutions obtained with these two procedures is not due to a nonspecific tendency to ingest any drug offered. Thus rats are generally reluctant to self-administer hashish via the oral route, and their reluctance is not affected by several procedures which can increase their intake of other drugs.

Key words: Cannabis — Self-Administration of Drugs — Ethanol — Lateral Hypothalamus — Electrical Stimulation of Brain.

Studies of the self-administration of various drugs by infrahuman organisms can provide important comparative evidence by allowing a contrast between the nonhuman's response to the drug and that of humans. Rats, monkeys, and other infrahuman species will under appropriate circumstances self-administer nearly every drug that humans seek (Schuster and Thompson, 1969). However, one drug which to date has not been examined in detail in the self-administration paradigm is *Cannabis sativa* (marijuana, hashish).

The present series of experiments was intended to serve two purposes: First, it is an attempt to induce the self-administration of cannabis by rats offered aqueous suspensions of hashish extract for oral consumption. A second purpose of these experiments was to examine the specificity of the increased intake of ethanol solutions induced by electrical stimulation of the hypothalamus (Amit, 1970) by determining whether such stimulation also induces enhanced consumption of aversive hashish suspensions.

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It is worth noting that cannabis is often consumed via the oral route by human beings, especially in India, where it is prepared as a decoction in milk or water or as an additive in sweetmeats (Farnsworth, 1968; Taylor, 1966).

General Procedure

The Department of National Health and Welfare of Canada supplied a purified extract of Moroccan hashish (hereafter referred to simply as hashish) with the following composition: delta 1-tetrahydrocannabinol [Δ^1-THC, the presumed major psychoactive ingredient of cannabis (Mechoulam, 1970)]: 55.14%; cannabichromene: 4.64%; cannabidiol: 17.65%; cannabigerol: 3.48%; cannabinol: 11.02%; unknown: 8.07%. A stock preparation of hashish was prepared by suspending a given amount of resin in a vehicle of 19 parts propylene glycol to 1 part 95% ethanol (v/v). In Experiment 1 an aqueous suspension of hashish was formed by mixing water with a stock preparation of 40 mg hashish/ml vehicle. In the subsequent experiments the concentration of the stock preparation used to make the aqueous suspension was 10 mg hashish/ml vehicle. As a precaution, the stock preparations, as well as unused hashish, were stored in an unlighted refrigerator until minutes before use, although it is known that the hashish resin remains chemically stable even in a lighted room at room temperature. When hashish is added to tap water a milky white suspension results, with the density of white proportional to the concentration of hashish. Even when stored at room temperature no significant change occurs in the ratio of the components of the hashish for a minimum of 48 h, as determined by gas-liquid chromatography (R. A. Graham, personal communication, 1971). The aqueous suspensions of hashish used in these experiments were therefore stored in polyethylene bottles at room temperature for 2 days, at which time the remainder was disposed of and fresh aqueous suspensions were prepared.

Experiment 1

Studies of the self-administration of drugs via the oral route generally are designed to induce a preference for the drug over water or some other alternative fluid. The rationale for this approach presumably is based on the fact that human drug users usually have alternatives to drug seeking available in their environment, and thus that animal studies should attempt to mirror this free choice aspect of human behavior. A common means of attempting to induce a drug preference is initially to force the subjects to consume the drug and then later to offer them a free choice, by which time they should have learned to associate any reinforcing drug effects with the act of drinking the drug preparation available.

Forcing rats to consume morphine solutions does result in the acquisition of a preference for the morphine over water (Stolerman and Kumar,

1970); but no such preference develops when rats are forced to drink barbiturates (Kleinman, Schmidt, and Stewart, 1966; Stolerman, Kumar, and Steinberg, 1971) or *d*-amphetamine (Stolerman *et al.*, 1971). The results with forced intake of ethanol solutions are mixed: some have reported increased intake of ethanol after a period of forced consumption (e.g. Cicero, Snider, Perez, and Swanson, 1971; Richter, 1956), while others have observed aversions to the ethanol solutions (e.g. Amit, Corcoran, Charness, and Shizgal, 1973; Essig, 1968). The first experiment of this paper is an attempt to induce a preference for hashish by forcing rats to drink a concentrated suspension of hashish for a prolonged period and then offering a free choice with water.

Method

Subjects. Ten male Wistar rats weighing 280—300 g at the beginning of the experiment were used. The rats were housed individually in metal cages with a wire-mesh floor and front panel. Purina chow was available *ad libitum*, and fluids were presented in calibrated glass Richter tubes (Kimax) mounted at the front of the cage.

Procedure. For 32 days the only fluid available to the rats was an aqueous suspension of hashish at a concentration of 0.2500 mg/ml of water, presented in a single Richter tube containing 50 ml of the suspension. The intake of hashish was measured each day, and the side of the cage on which the tube was mounted was alternated daily. A second Richter tube containing tap water was introduced on day 32, and the intake both of hashish and water was measured for the next 15 days. The positions of the water and hashish tubes were alternated daily to control for position habits.

Results

The rats' intake of fluid under both forced and free-choice conditions is shown in Fig. 1. During the 32 days of forced intake the mean daily

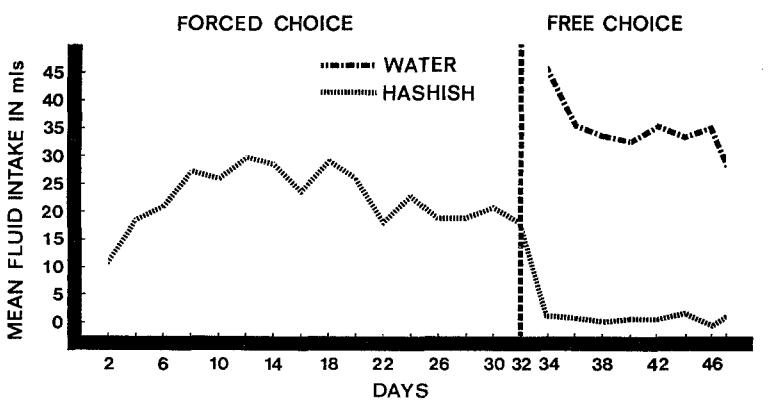

Fig. 1. Forced and free-choice intake of hashish suspension. Hashish was the only fluid available until day 32, when a water bottle was introduced as a second source of fluid

consumption of hashish suspension was 23.4 ml, or 5.8 mg of hashish resin. During the subsequent 15-day free-choice period the rats exhibited an aversion to hashish, with the mean daily intake dropping to 1.3 ml (or 0.3 mg). The mean daily intake of water during the free-choice period was 34.7 ml, comprising a mean intake of 97.5% of total fluid. Each rat's mean free-choice intake of water was significantly greater than its mean forced intake of hashish ($t = 8.1$, $df = 8$, $P < 0.005$). While the levels of water intake on days 33 and 34 were not significantly different, both were significantly greater than the levels of intake on the remaining days of the experiment (each $P < 0.01$).

Discussion

Adult male rats that had been forced to drink a concentrated aqueous suspension of hashish for a prolonged period failed to exhibit any substantial intake of the drug in the subsequent free-choice situation. Their intake of water on the first 2 days of the free-choice condition was significantly above the level of intake observed on subsequent days, similar to the effect Essig (1968) noted in rats whose fluid intake had been restricted to a 20% solution of ethanol for several months. Because ethanol is known to function as a diuretic, Essig speculated that the rats might have been maintained in a chronic state of fluid deficit for the period of forced ethanol consumption, and that they compensated for the deficit by enhanced water consumption when water became available. A similar explanation might be offered for the results of the present experiment, since Barry, Perhach, and Kubena (1970) have reported that administration of Δ^1-THC to rats causes an inhibition of antidiuretic hormone, as indicated by a doubled urine output during the 8-h interval following drug treatment. The consequences of such a progressively-increasing chronic dehydration as well as the noxious acute pharmacological effects of cannabis which motivate the learning of an avoidance response to novel tastes paired with cannabis injections (Corcoran, 1973; Elsmore and Fletcher, 1972) may have contributed to the reluctance of the rats in the present study to consume hashish once water was also available. However, aversive sensory factors are probably sufficient to account for this reluctance, since naive rats in a pilot experiment also rejected this concentration of hashish in a free-choice situation, presumably before they had ingested a quantity of drug sufficient to produce any unpleasant postingestional effects.

Experiment 2

The reluctance of rats to consume hashish in a free choice with water in the previous experiment may have been specific to the particular concentration of hashish offered. Alternatively, any potentially rewarding

properties of the hashish may have been counteracted by some aversive effects of prolonged forced consumption of the drug. In the second experiment rats were not forced to drink hashish but were offered a choice between water and a concentration of hashish determined individually for each animal. Experiment 2a examined the effects of exposing rats to either an ascending or a descending series of concentrations of the drug, while Experiment 2b was designed to determine if repeated experience with an initially nonpreferred concentration of hashish results in a gradual increase in the intake of the drug.

Method

Subjects. A total of 30 male Wistar rats weighing 250—300 g at the start of the experiment were used. The rats were housed as in the previous experiment with unrestricted access to chow and fluids available in glass Richter tubes. They were offered either a free choice between hashish and water (or the hashish vehicle in water, in the case of several rats), or water (vehicle) alone. Their source of fluid was never restricted to an aqueous suspension of hashish alone.

Procedure of Experiment 2a. The effects of exposure to a choice between water and a descending series of concentration of hashish were compared to choice between water and an ascending series. Ten rats each were in the ascending and descending groups, and the same procedure was followed for all rats: A choice between water and hashish of a particular concentration was available for 2 days, with the positions of the water and hashish tubes alternated on the second day. If total intake of hashish comprised 35% or less of total fluid, the concentration presented to a rat in the descending group was lowered to the next level, where it remained for the next 2 days and so on. When intake of a particular concentration comprised 35% or less of total fluid for a rat in the ascending group, the concentration was fixed at that level and became the test concentration in Experiment 2b. If the 2-day intake of a given concentration of hashish comprised more than 35% of total fluid (as happened only in the ascending group), the concentration was raised to the next level and so on until a concentration was reached the intake of which comprised 35% or less of total fluid. The rats in the descending group were offered in order the following concentrations of hashish in mg/ml: 0.1000, 0.0750, 0.0500, 0.0250, 0.0100, and 0.0050. The ascending group was offered the following concentrations of hashish in mg/ml: 0.0025, 0.0050, 0.0075, 0.0100, 0.0150, 0.0200, 0.0250, 0.0300, 0.0400, 0.0500, 0.0600, 0.0700, 0.0900, etc. Six rats in the ascending group had a choice between hashish and the appropriate volume of vehicle in water for the entire duration of the experiment to determine if they were able to discrimate hashish + vehicle in water from vehicle alone in water.

Procedure of Experiment 2b. Since the rats in the ascending group exhibited a higher level of intake of hashish than did those exposed to the descending series, the former were retained for the second part of the experiment. The lowest concentration of hashish which each animal had consumed during exposure to the ascending series at 35% or less of total fluid intake was designated as that rat's test concentration and remained fixed for the rest of the experiment. Each rat was given a daily choice between water and hashish at the test concentration for 30 days, with the positions of the water and hashish tubes reversed every day. Since no change had occurred in hashish intake by the end of the 30-day period, the choice between water and hashish was next available on alternate days for 40 days, with 2 water tubes available on the intervening days. This presentation of the choice on alternate days

allows one to determine if position preferences play any role in the patterns of intake observed, since any such preferences should also be reflected on the intervening days when water alone is available.

Ten additional rats were offered an ascending series of hashish concentrations to determine a test concentration for each animal. These rats were then exposed to the same procedure as the above (first) ascending group except that the 30-day period of daily choice was omitted. Thus immediately after determination of the test concentration of hashish, each rat in the second ascending group was placed on the 40-day schedule of alternate-day presentation of water and hashish, with only water available on the intervening days.

Results

Experiment 2a. Two rats in the first ascending group died from unknown causes during the early phases of the experiment, and so the data reported here were obtained from the 10 rats in the descending group and 18 rats in the combined ascending groups. Exposure to a descending series caused a general suppression of the hashish intake of all rats in the descending group, which comprised a mean of 5% of total fluid across the 6 concentrations tested. The failure of the rats in the descending group to increase their intake as the hashish became more dilute is demonstrated by the fact that no significant difference existed in relative intake of hashish at the lowest as compared to the highest concentration (means of 9% as compared to 7% of total fluid, respectively: $P > 0.05$). On the other hand, the rats in the ascending groups clearly exhibited higher levels of intake of hashish than the rats in the descending group. Figure 2 shows the number of rats in the ascending group that ingested more than 35% of total fluid in hashish of each concentration. Note that some rats continued to ingest even high concentrations of the drug, and most ingested the low concentrations which had been rejected by the rats in the descending group (for example, 12 of 18 rats consumed the 0.0100 mg/ml concentration of hashish at more than 35% of total fluid, whereas all 10 rats in the descending group drank this concentration at less than 35%). Table 1 shows the marked variability in the concentrations of hashish which individual rats in the ascending group selected as their test concentration. During the early stages of the ascending series when the more dilute concentrations of hashish were offered, many rats in the ascending group exhibited preferences over water for these dilute suspensions of hashish and in some cases actually totally rejected water in favor of hashish. Since these preferred concentrations of hashish were each available only for 2 days, it is impossible to comment on the reliability or durability of this apparent hashish preference.

The rats offered a choice between the vehicle in water and hashish + vehicle in water were clearly able to detect the presence of even the

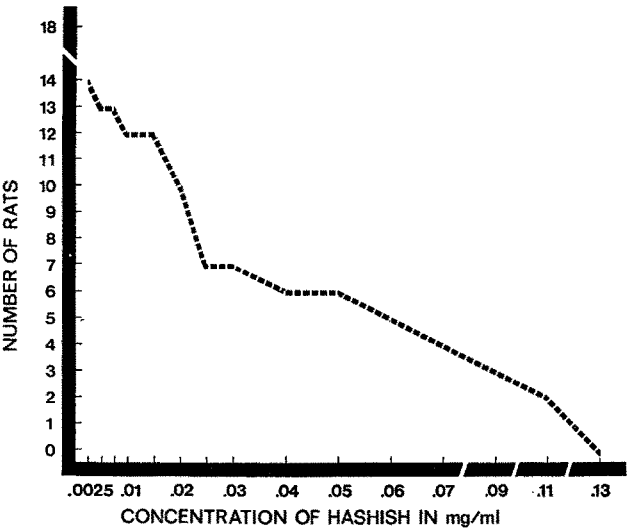

Fig. 2. Number of rats in the ascending group consuming more than 35% of total fluid in hashish suspension at each concentration

Table 1. The combined ascending groups' intake of hashish at test concentration during the alternate-day period of Experiment 2

Rat	Test concentration	$\bar{x}$ % of Total daily intake	$\bar{x}$ Absolute daily intake	$\bar{x}$ % of Total intake on final two sessions
	mg/ml	%	mg	%
1st Ascending group				
31[a]	0.0025	41	0.031	48
32	0.0900	5	0.153	3
33[a]	0.0100	48	0.144	55
34[a]	0.0400	9	0.194	6
35[a]	0.0025	61	0.047	70
36[a]	0.0025	26	0.022	39
38	0.0250	46	0.354	46
39[a]	0.0025	62	0.049	80
2nd Ascending group				
51	0.1300	7	0.234	5
52	0.0200	42	0.208	8
53	0.0200	18	0.101	8
54	0.1300	3	0.162	2
55	0.1100	11	0.248	0
56	0.0250	28	0.236	8
57	0.0250	53	0.321	71
58	0.0500	38	0.044	31
59	0.0700	8	0.186	2
60	0.0600	23	0.375	4

[a] Offered a choice between hashish and vehicle in water.

lowest (0.0025 mg/ml) concentration of hashish, since in several cases the vehicle was preferred to the hashish suspension early in the ascending series when the very dilute suspensions were presented. For example, one rat completely rejected 0.0025 mg/ml hashish on initial exposure, while another consumed 80% of its fluid in the vehicle solution. While it is not certain that all rats responded to the hashish rather than the vehicle or the combination of the two, it appears that at least some rats were capable of discriminating hashish + vehicle from the vehicle alone.

Experiment 2b. The first ascending group's intake of hashish was no higher on the last than on the first 2 days of the 30-day period of daily presentation, with mean intake of hashish in each case comprising 24% of total fluid. The intake of this group was also not significantly elevated by the end of the 40-day period of alternate-day presentation. Although each rat's mean intake of hashish was somewhat higher under the alternate-day condition than that under daily presentation, this difference was not significant (means of 37 and 31% of total fluid, respectively; $P > 0.05$). The intake of the second ascending group actually decreased during the period of alternate-day presentation from a mean of 43% on the first 2 choice days to 14% on the last 2 choice days ($t = 2.56$, $df = 9$, $P < 0.025$). Yet while the overall consumption of hashish during the alternate-day period either remained constant or decreased, some individual rats did increase their intake of the drug by the end of this period. For example, rat no. 35 of the first ascending group drank 70% of total fluid in hashish on the last 2 choice sessions of the period, while rat no. 39 of the same group consumed 80% of total fluid in hashish on the last 2 days as shown in Table 1. Both rats had selected the most dilute test concentrations of hashish (0.0025 mg/ml), and their intake of the drug at these preference levels reached approximately 300—320 μg/kg body weight. These two rats were not exceptional, since across all animals in the ascending groups the test concentration was negatively correlated with both the mean hashish intake of the entire 40-day period and the hashish intake on the last 2 days of the period ($r = -0.76$, $r = -0.81$, respectively, each $P < 0.01$). This negative correlation indicates that the rats with the more dilute test concentrations were likely to drink more hashish during the period than the rats that had selected more concentrated suspensions. The fact that the rats that had selected the more dilute test concentrations tended to increase their intake with time (also suggested by Table 1) provides an explanation for the finding that the rats in the second ascending group tended to drink less hashish by the end of the period while those in the first maintained a fairly constant intake: The latter selected significantly less concentrated test suspensions of hashish than the former ($U = 14$, $P < 0.025$).

Discussion

The results of this experiment indicate that rats are generally reluctant to drink a hashish suspension when water is also available, although drug intake sometimes occurs if the hashish is sufficiently dilute. Exposure to a descending series of hashish concentrations resulted in a markedly lower level of intake than exposure to an ascending series, similar to the effect Myers and Carey (1961) obtained with ethanol solutions. The rats in the ascending groups were more likely to consume hashish, although only those rats that had selected the most dilute test concentrations showed any tendency to develop any intake of the drug at indifference levels (50% of total fluid) or above. This effect of an ascending series upon drug consumption has also been observed by Veale and Myers (1969) with ethanol. There was considerable variability in the concentrations of hashish which rats in the ascending group selected as their particular test concentration. These values ranged from 0.0025 mg/ml to 0.1300 mg/ml in different rats. Similar variability has been described in the rat's preference-aversion response to ethanol solutions (e.g., Amit *et al.*, 1970; Cicero and Myers, 1968; Myers, 1966).

Experiment 3

The two preceding experiments indicate that rats are generally reluctant to drink concentrated hashish in aqueous suspension when water is available as an alternative. The present experiment describes an attempt to induce a preference over water for aversive concentrations of hashish by exposing rats to a schedule of electrical stimulation of the lateral hypothalamus. This technique has previously been used successfully to induce a preference for aversive ethanol solutions in rats (Amit, Stern, and Wise, 1970; Amit and Stern, 1971). Amit *and colleagues* determined a concentration of ethanol for each rat, the initial rejection concentration (IRC), which that rat totally rejected for 3 days in a choice with water. The rats were then offered a free choice between water and ethanol at the IRC on alternate days, with water alone available on the intervening days. This alternate-day treatment even in the absence of hypothalamic stimulation has a profound effect on ethanol intake, since the nonstimulated control rats in these experiments eventually consumed as much as 40% of total fluid in the previously rejected ethanol solutions. Beyond the effect of this alternate-day schedule, however, imposition of a 30-day schedule of daily sessions of hypothalamic stimulation further induces a home-cage preference for ethanol at IRC, with some stimulated rats eventually consuming as much as 100% of their total fluid intake on the choice days in ethanol solutions as concentrated as 35%. This acquired preference for previously aversive concentrations of ethanol is not a function of con-

summatory behavior that might be generated by the stimulation, since the effect has been obtained in "stimulation-bound drinkers" offered ethanol (Amit *et al.*, 1970), water (the WD group of Amit and Stern, 1971), or no fluids (Amit and Stern's NCR group) during the stimulation sessions, and in rats that did not exhibit stimulation-bound drinking in preliminary testing (NCR group of Amit and Stern). Therefore the development of a preference for ethanol solutions at IRC must result from some change in the brain, presumably chemical (Amit and Stern, 1972), caused by electrical stimulation of the lateral hypothalamus.

In the experiment described below, an aversive concentration of hashish suspension (the IRC) was determined for each rat, and this suspension was then available in a free choice with water on alternate days. The rats were subjected to a 30-day schedule of daily sessions of hypothalamic stimulation identical to that administered to the NCR group of Amit and Stern (1971). In fact the only difference in the procedures of this experiment and Amit and Stern's experiment is that most rats in the present study were offered aversive hashish suspensions rather than ethanol solutions.

Method

Subjects. A total of 36 adult male Wistar rats, housed as in the previous experiments, were used. The rats had unrestricted access to chow, and fluids were presented via 2 Richter tubes mounted at the front of the cage.

Apparatus. Intracranial stimulation was delivered via a 60-Hz constant-current sine wave stimulator. Current was adjusted with a calibrated potentiometer and monitored on a microammeter. The on-off pattern of stimulation was programmed automatically.

The rats were stimulated in wooden boxes which measured approximately 30 cm long, 23 cm wide, and 38 cm deep and had a one-way-vision glass as the long front panel. During preliminary testing with brain stimulation, three water-filled Richter tubes were available in the cage and Purina chow pellets were scattered about the cage floor. No such goal objects were present in the test boxes during the formal stimulation sessions.

Surgery. The rats were anesthetized with 50 mg/kg sodium pentobarbital intraperitoneally and fixed in a stereotaxic instrument. A 250 μ stainless-steel wire bared of insulation at the tip was implanted into the lateral hypothalamus in the left cerebral hemisphere of each rat. To serve as anchors for the cranioplast cement, three stainless-steel surgical screws were mounted on the skull anterior, lateral, and posterior to the electrode. The anterior screw served as the indifferent electrode. Following surgery the rats were given an intramuscular injection of 0.25 ml Fortimycin—$^1/_2$ (50000 U penicillin G procaine plus 0.06 g streptomycin sulfate).

Procedure. A few days after surgery an aversive concentration of ethanol (IRC) was determined for 7 of the rats (the ethanol group) using the technique of Amit and Stern (1971). Briefly, the rats were offered a choice between water and a 3% (v/v) ethanol solution for 24 h, after which the ethanol concentration was increased by 1% each day until the solution was rejected completely (i.e., less than 1 ml was consumed). If this solution was rejected for 72 consecutive hours, it became the test solution (IRC) for the individual rat. The lowest IRC for ethanol in this group was 9% and the highest 15%.

An aversive concentration of hashish was then determined for all 36 rats using the same method except that an aqueous suspension of hashish was offered rather than an ethanol solution. The rats were offered a choice first between water and a 0.0025 mg/ml hashish suspension for a 24-h period. If more than 1 ml of this concentration of hashish was consumed, the concentration in mg/ml was raised to the next level in the following progressions: 0.0025, 0.0050, 0.0075, 0.0100, 0.0150, 0.0200, 0.0250, 0.0300, 0.0400, 0.0500, etc. The concentration ascended to the next higher level each day until a level was reached which for 1 day was rejected (i.e., less than 1 ml consumed), so that the total 24-h fluid intake consisted of water. If this concentration continued to be rejected for the next 48 h, it became the test concentration (IRC) for the individual rat. Thus while the concentration of hashish at IRC varied from subject to subject, the concentration presented to each rat remained fixed throughout the experiment. The hashish IRCs ranged from 0.0025 to 0.5100 mg/ml. The positions of the Richter tubes containing water and drug were alternated daily during determination of the hashish IRC for all rats as well as during determination of the ethanol IRC for the rats in the ethanol group.

Following determination of the hashish IRC, each rat was exposed to brain stimulation in order to select an appropriate nondisruptive level of current to be used later during the formal stimulation schedule. The rats were placed in stimulation boxes containing 3 water-filled Richter tubes and Purina chow scattered on the floor. After 15—30 min habituation to the box, the rats were exposed to a schedule of intermittent low-intensity hypothalamic stimulation consisting of 20-sec stimulation periods alternating with 20-sec nonstimulation periods. Intensity was initially set a 2 μA, and the current was raised 2 μA after each stimulation until a level was reached at which forced movements, aversive responses, or stimulation-bound behavior (feeding, drinking, gnawing, or locomotor activity) were obtained. One rat exhibited forced movements and two others aversive responses at low levels of stimulation; these subjects were assigned to the hashish control group and received no further brain stimulation. Thus the control group was intended to control for any effects on hashish intake of anesthetization and surgery, the presence of a hypothalamic electrode, and brief exposure to various levels of brain stimulation. Four other animals displayed stimulation-bound feeding and were assigned to the hashish experimental group. None of the 7 rats already assigned to the ethanol group exhibited any stimulation-bound ingestive behavior. Five rats were randomly chosen from the other 22 rats and placed in the hashish control group, and the remaining 17 rats completed the hashish experimental group.

All 36 subjects were then maintained for 60 days in their home cages with free access to food and water, and on alternate days were offered a choice between water and hashish at IRC. The rats in the ethanol group were offered a choice between water and ethanol at IRC on the days hashish was not presented, while the rats in the hashish groups had access to 2 tubes containing only water on those days. For the first 30 days the rats in the hashish experimental group and in the ethanol group underwent a daily 30-min session of electrical stimulation of the lateral hypothalamus consisting of 20-sec periods of stimulation alternating with 20-sec nonstimulation periods. The current levels used were those which in preliminary testing had elicited either stimulation-bound feeding or nondisruptive locomotor activity typical of hypothalamic stimulation (e.g., Christopher and Butter, 1968). In keeping with Amit and Stern's (1971) procedure, the current intensity was twice raised by 2 μA steps, 10 min and then again 20 min into the session. During the stimulation sessions no goal objects were present in the stimulation boxes, thus preventing the rats from engaging in stimulation-bound ingestive behavior. The exposure of the hashish experimental group but not the hashish control group to 30 days of hypo-

thalamic stimulation in the only difference in the treatment of the two groups. The only difference in the treatment of the hashish experimental group and the ethanol group is that the latter had a choice between water and ethanol at IRC on the days hashish was not available while the former was offered only water on those days.

Stimulation sessions were discontinued after day 30 of the experiment, and the rats continued to have a choice between hashish and water in their home cages on alternate days (with a choice between ethanol and water available to the ethanol group on the intervening days) for the next 30 days. Following day 60 of the experiment the rats were killed with pentobarbital and perfused intracardially with physiological saline followed by 10% formal saline. The brains were removed and frozen, and sections 40 μ thick were stained with the Weil method for myelinated axons.

Results

Hashish groups: As shown in Fig. 3 neither hashish group displayed any marked intake of, or preference for, hashish throughout the experiment. The hashish control group ingested a median of 6% of total fluid in the hashish suspension for the duration of the study. Only one control rat exhibited any regular preference for hashish, and this animal's IRC was 0.0025 mg/ml, the lowest IRC of any subject in the study. Electrical stimulation of the lateral hypothalamus failed to induce the hashish experimental group to acquire a preference for hashish over water; the hashish intake of this group comprised a median of 4.5% of their total fluid for the entire 60-day period. As Fig. 3 indicates, the level of intake of both water and hashish was quite similar for the hashish groups. The median water intake of the hashish experimental and hashish control

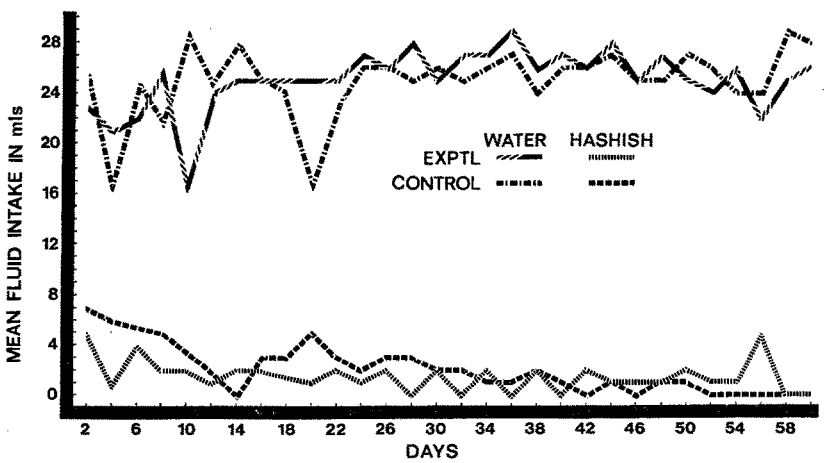

Fig. 3. Intake of water and hashish suspension of the rats in the hashish experimental and hashish control groups; note that hypothalamic stimulation in the experimental group terminated on day 30

groups for the entire experiment was 25 ml and 25.5 ml, respectively, and the median hashish intake was the same for both groups, 1.5 ml.

Ethanol group: None of the rats in the ethanol group developed a preference over water for the hashish suspensions, and after the first few days of testing the rats seldom drank more than 1 ml of hashish on a given day. On the other hand, 4 of the 7 rats did acquire a preference over water for the ethanol solutions offered on the days hashish was unavailable. During the last 30 (poststimulation) days of the experiment the mean and median preference for ethanol of the 4 ethanol-preferring rats was 90 and 100%, respectively, while their intake of hashish during the same period comprised a mean and median, respectively, of 2 and 0% of total fluid. The three rats that did not acquire a preference for ethanol consumed a mean and median, respectively, of 32 and 22% of total fluid in ethanol solution during the last 30 days of the experiment; their intake of hashish during this period comprised a mean and median of 1 and 0% of total fluid, respectively. The ethanol and hashish intake of the four ethanol-preferring rats and of the three nonpreferring rats is shown in Fig. 4.

A reconstruction of the electrode placements of the rats in the two hashish groups is shown in Fig. 5 and that of the rats in the ethanol group in Fig. 6. Fig. 5 indicates that the great majority of electrodes of the rats in the hashish groups was scattered throughout the perifornical lateral hypothalamus, the area stimulated by Amit and Stern (1971). Fig. 6

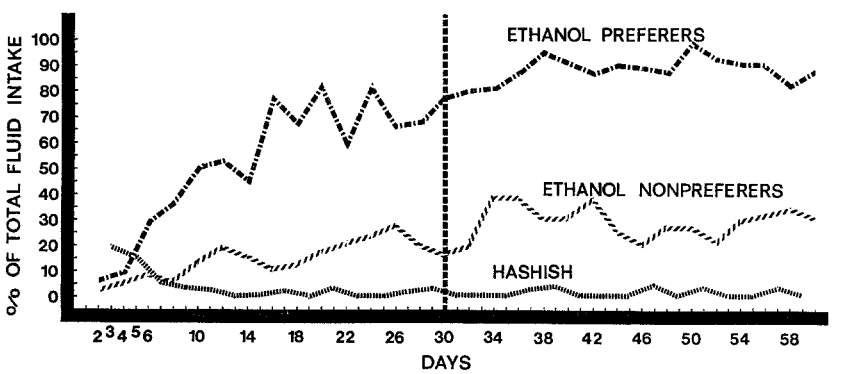

Fig. 4. Intake of aversive ethanol solution and aversive hashish suspension of the rats in the ethanol group, expressed as percent of total fluid; note that hypothalamic stimulation terminated on day 30, indicated by the vertical line. The rats had a choice between ethanol and water on the even-numbered days, and between hashish and water on the odd-numbered days. The relative hashish intake of all 7 rats is shown in a single curve, whereas the relative ethanol intake of the 4 ethanol-preferring rats is presented separately from that of the 3 nonpreferring rats

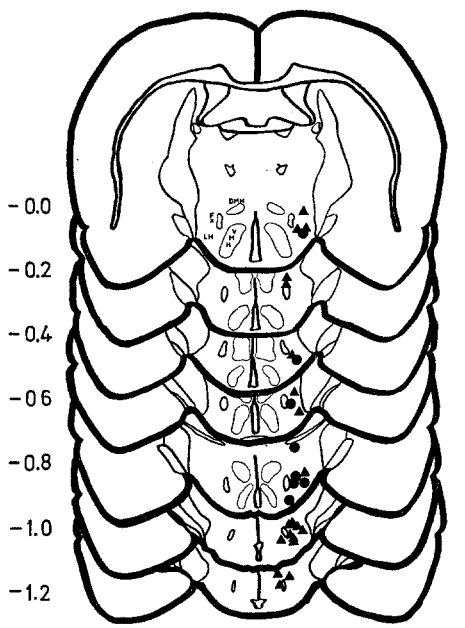

Fig. 5. Reconstruction of electrode placements of rats in the hashish experimental (triangles) and hashish control (circles) groups. Numbers to the left of the sections represent mm posterior to bregma. *DMH* dorsomedial hypothalamus; *FX* fornix; *LH* lateral hypothalamus; *VMH* ventromedial hypothalamus

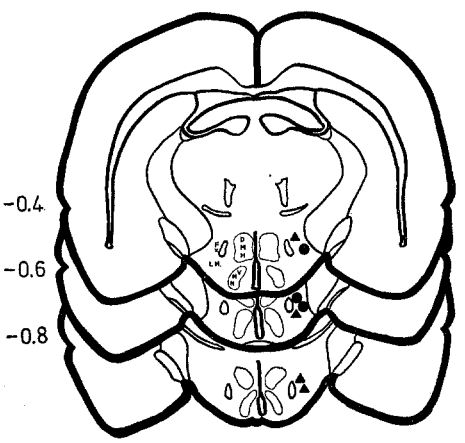

Fig. 6. Reconstruction of electrode placements of ethanol-preferring (triangles) and nonethanol-preferring (circles) rats in the ethanol group. Same abbreviations as in Fig. 5

indicates that the electrodes of all the rats in the ethanol group were lodged in the perifornical lateral hypothalamus; in accordance with Amit and Stern's observation, it is not possible on the basis of gross electrode placement to differentiate between rats that acquired a preference for ethanol and those that did not.

Discussion

Several conclusions can be drawn on the basis of the results of this experiment: First, rats offered the drug every other day show little tendency to begin to drink an aversive concentration of hashish when water is available simultaneously. This is in marked contrast to the gradual increase in the consumption of previously rejected ethanol solutions that occurs when the ethanol is offered in a choice with water on a schedule of alternate-day presentation [see the ethanol intake of the nonstimulated control groups in the reports of Amit *et al.* (1970) and Amit and Stern (1971)]. Second, a schedule of lateral hypothalamic stimulation which is capable of inducing rats to develop a preference over water for previously rejected ethanol solutions has no such effect upon the rats' rejection of aversive concentrations of hashish suspension. Thus although this finding does not provide a clear understanding of the mechanisms underlying the induction of a preference for ethanol by hypothalamic stimulation, it does demonstrate that the ethanol effect is not the result of a tendency aroused by the stimulation to ingest *any* fluid with distinctive sensory or pharmacological properties.

General Discussion

The present experiments reveal that rats are generally reluctant to drink concentrated aqueous suspensions of hashish when an alternative fluid is available. This reluctance is presumably in part due to aversive sensory qualities of the drug preparation, as suggested by the avoidance of the concentrated hashish suspension exhibited in a free choice situation by the rats in the pilot study of Experiment 1. Aversive sensory qualities are also known to influence the rat's consumption of other drugs such as ethanol (e.g., Amit and Stern, 1969; Kahn and Stellar, 1960) and morphine (e.g., Khavari and Risner, 1972). Acute or chronic aversive pharmacological (postingestional) effects of hashish may also have contributed to the general avoidance of the drug in these experiments: rats learn to avoid saccharin and other novel solutions whose tastes have been paired with parenteral injections of hashish (Corcoran, 1973) or Δ^1-THC (Elsmore and Fletcher, 1972), indicating that the pharmacological properties of cannabinoid drugs can have aversive effects independent of any generated by their sensory properties. It is not clear, of course, that the

rats in the present experiments displayed reluctance to drink hashish because of any such aversive pharmacological effects, since it is possible that they distributed their bouts of drinking over each 24-h period so that a quantity of the drug sufficient to produce an unpleasant interoceptive state was never ingested at any one time. This point can only be resolved by further research.

Preference for the hashish suspension over water was noted only in two phases of this series of experiments: during screening with the ascending series of concentrations of hashish (Experiments 2a and 3), when the majority of rats exhibited substantial consumption of and in many cases preference for the more dilute concentrations of hashish; and during the 40-day period of alternate-day presentation in Experiment 2b, when only a few rats developed a pattern of regular intake of the drug. The only promising approach to inducing oral consumption of hashish by rats in a free-choice situation therefore seems to rely on offering sufficiently dilute concentrations of the drug. However it remains to be seen whether the high levels of hashish consumption usually observed during the early stages of the ascending series will be maintained if these initially preferred concentrations are offered repeatedly to the rats. Even if a consistent preference for hashish can be obtained under these circumstances, it will still be necessary to determine what role in motivating the self-administration is played by the drug's sensory effects and by it pharmacological effects (assuming that the dilute concentrations consumed produce detectable pharmacological effects).

The results of these experiments help clarify the nature of the effects of hypothalamic stimulation upon the intake of ethanol by rats (Amit *et al.*, 1970; Amit and Stern, 1971). Two aspects of that phenomenon are illuminated: the generality of the effects of presenting the drug on alternate days, and the generality of the hypothalamic stimulation effect itself. As mentioned above, rats offered a free choice between aversive ethanol solutions and water on alternate days will increase their intake of ethanol, even in the absence of electrical stimulation, until as much as 40% of total fluid is consumed in ethanol solutions. Thus the alternate-day regimen alone results in a profound increase in the consumption of concentrated solutions of ethanol, with intake reaching levels not usually reported in the literature (e.g., Kahn and Stellar, 1960; Richter and Campbell, 1940; Veale and Myers, 1969). Although Wayner, Greenberg, Tartaglione, Nolley, Fraley, and Cott (1972) have claimed that a schedule of alternate-day presentation results in enhanced consumption of other unpalatable substances such as quinine solution, Experiments 2b and 3 of the present paper demonstrate that the alternate-day schedule produces no reliable increase in the consumption of concentrated hashish

suspensions. Together with the fact that a similar schedule fails to affect the rejection of aversive diazepam solutions by rats (Amit and Cohen, 1974), this clearly indicates that a schedule of alternate-day presentation does not produce a nonspecific increase in the consumption of any unpalatable substances offered.

The last experiment of the series demonstrated that rats with electrodes in the perifornical hypothalamus fail to begin drinking aversive hashish suspensions during or after a schedule of electrical stimulation through the electrodes. Two facts argue against the possibility that this negative result is an artifact of incorrect electrode placement or variation from the procedure of Amit and Stern (1971): First, examination of the histology verified that the majority of electrodes were placed accurately in the lateral hypothalamus, the area stimulated by Amit and Stern. Second, four of seven rats in the ethanol group acquired a preference over water for previously aversive ethanol solutions, while persisting in their rejection of hashish. Since stimulation induced a preference for ethanol but not hashish *in the same animal*, it must be concluded that the stimulation was affecting the neural systems critical for the ethanol effect, and further that activation of these systems is not a sufficient condition to generate a preference for hashish over water. It might be argued that the acquired preference for ethanol displayed by these rats in some way interfered with the development of a similar preference for hashish. This hypothesis can be rejected since rats that did not acquire a preference for ethanol (the three other rats in the ethanol group) or rats that did not have access to ethanol solutions (the hashish experimental group) also failed to begin to drink aversive hashish suspensions during or after the schedule of hypothalamic stimulation. Taken together, then, these results indicate that hypothalamic stimulation identical to that used by Amit and Stern to induce an ethanol preference is incapable of inducing a similar increase in the intake of aversive hashish suspensions. Therefore, although we cannot conclude that the effect of hypothalamic stimulation upon ethanol consumption is specific to ethanol, these results at least allow us to conclude that the ethanol effect is not entirely nonspecific.

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