Hashish smoke interfers with Sidman avoidance in mice

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Summary. Hashish smoke has been proved to be active in the Sidman avoidance. Its activity is similar to that of hallucinogens.

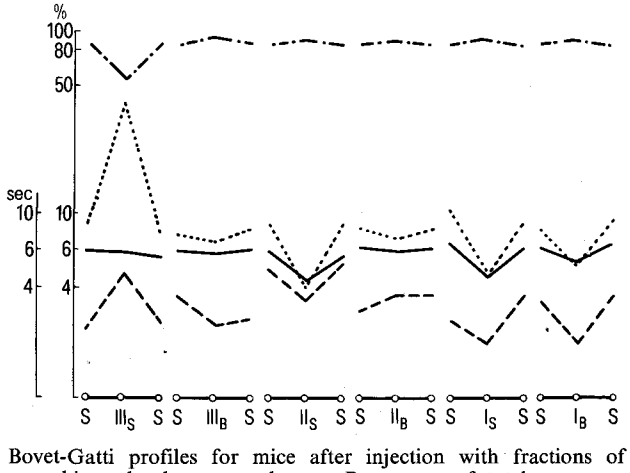
Characterization of a drug as hallucinogenic involves animal methods in which a behavioral reaction is affected in a specific way by all known hallucinogens and is not affected in this way by other drugs. These tests named 'behavioral models of psychosis'[1] include the DRL, the discriminate-Sidman avoidance schedule and the nest-building. Δ^9-THC, a major component of cannabis, which has been shown to be hallucinogenic in animals and man[2,3] has been found to be active in the Sidman schedule[4,5] and in nest-building[6] and not active in the DRL[4]. However, the action of Δ^9-THC differs both qualitatively and quantitatively from that of marijuana extracts[7-9]. Possible explanations for these actions might be: a) the pharmacological interaction between the known active cannabinoids[8,10-12] or b) the activity of some unknown substances. It was felt that seeking mechanisms responsible for cannabis action should involve more near to life cannabis products. It is the scope of this paper to show that cannabis pyrolysates (like the ones normally consumed) are active in the Sidman-avoidance to an extent not related to their Δ^9-THC content.

Materials and methods. 8 DBA male mice, 2 months of age at the beginning of the experiment, were trained in the Sidman-discriminate up to the asymptote. The animals were kept in an animal house at constant temperature (19–21 °C). An automatic timer provided a 12 h light-12 h dark day.

The discriminate Sidman avoidance operates as follows: The mouse is placed in a Skinner box the floor of which is an electrified grid. For the 1st 20 sec nothing happens. Then a light comes on for 10 sec. At the end of this time a shock of 0.5 sec duration is delivered every 5 sec. The mouse may avoid or terminate shock at any time by pressing a lever. This sets back the schedule to the beginning. The test gives a number of variables to measure: 1. Late responses, i.e. responses made too late to avoid shock. 2. Premature responses, i.e. responses made before conditioned stimulus (CS) comes on. 3. Reaction time, i.e. the time between the onset of the CS and the animal's response. 4. Efficient responses, i.e. those made while the CS is on and before any shock has been delivered. This is usually a very stable index of animal's performance (the mice in our experiment had a mean performance of 88% ±2 prior to injection).

All animals received 120 h of training (1 h daily sessions) up to the plateau. All animals were then injected with each of the several fractions of pyrolysates. These fractions were obtained by smoking hashish and tobacco through a water pipe, a procedure currently used by Greek hashish smokers. 3 types of material were collected in this way[13]: a) The particulate material of the smoke (fraction III_s) comprises what is called 'sublimate' and is normally inhaled by the smoker. The percentage of the main cannabinoids present in this material were as follows: Δ^9-THC 7.18%, CBD 3.29% and CBN 25.28%. b) Water soluble components of cannabis and tobacco and their pyrolysis products, free of any known cannabinoids (fraction I_s). c) Water insoluble components of cannabis and tobacco and their pyrolysis products. This material was non-volatile and the percentages of the known cannabinoids present in it were as follows: Δ^9-THC 2.37%, CBD 0.89% and CBN 38.66% (fraction II_s). For comparison purposes analogous types of material (I_B, II_B, III_B) have been obtained by smoking only tobacco. All drugs were injected i.p. as suspensions in saline-Tween 80 mixture (1 drop of tween-80 in 8 ml of saline), in a dose of 60 mg/kg. The volume of the liquid injected was 10 ml/kg.

Results. The figure shows modified Bovet-Gatti profiles. The profile obtained for fraction III_s is the only one that matches well with those reported for other hallucinogens by Smythies et al.[1]. Namely an increase is observed in both late and premature responses resulting in a deterioration of the animal's performance (table) which is more pro-



Bovet-Gatti profiles for mice after injection with fractions of cannabis and tobacco pyrolysates. Percentage of total responses made in the various intervals are given on the ordinate following a logarithmic scale, premature interval (———), late interval (· · ·), efficient (— · — · —). Reaction time (——) is also given in sec on the ordinate. S stands for saline injections before and after drug administration.

The effects of cannabis and tobacco pyrolysates on mice efficiency (percent of total responses ±SEM) and reaction time (±SEM) in the Sidman discrimination avoidance

Drugs		S_1	D	S_2
III_S	Efficiency	87.85±1.95	56.09±19.1 a_1,b_1	88.93±1.23
	Reaction time	6.57±0.48	6.44± 0.57	5.59±0.52
III_B	Efficiency	88.69±3.38	90.59± 4.88	88.99±3.57
	Reaction time	6.49±0.38	6.09± 0.81	6.45±0.22
II_S	Efficiency	86.21±4.62	92.69± 9.78 a_2	86.25±5.10
	Reaction time	6.12±0.64	4.23± 0.35 a_1,b_1	5.95±0.56
II_B	Efficiency	88.47±2.51	88.97± 1.75	88.15±2.35
	Reaction time	6.64±0.38	6.49± 0.49	6.61±0.48
I_S	Efficiency	86.85±1.51	93.19± 2.58 a_1	87.37±4.3
	Reaction time	6.92±0.37	4.66± 1.01 a_1,b_2	6.49±0.34
I_B	Efficiency	87.34±3.02	92.69± 2.58 a_2	87.21±2.71
	Reaction time	6.62±0.63	5.49± 0.21 a_2	6.84±0.97

S_1, Saline control before drug test; D, drug test; S_2, Saline control after test; a, differs significantly from S_1 (a_1: $p<0.01$, a_2: $p<0.05$, Mann-Whitney U-test) and b, differs significantly from analogous fraction of tobacco pyrolysates (b_1: $p<0.01$, b_2: $p<0.05$, Mann-Whitney U-test).

nounced during the 1st 120 min of the session. The profiles of the other fractions (figure) are similar to that reported for nicotine by Bovet and Gatti[16], i.e. they show an amelioration in performance and a fall in reaction time (table).

Discussion. In our experiment, the Bovet-Gatti profile obtained for III_s, i.e. the smoke inhaled by the cannabis consumer, conforms with the one previously reported for hallucinogens[1]. Similar results obtained for Δ^9-THC were interpreted as indicating a hallucinogenic effect of the drug[4]. We do not interpret our results in the same way. The animal displays a highly complex behavior associating painful and visual stimuli while distributing its responses in time in the most profitable way. Cannabis products or extracts might interfer at any one of these levels, since besides psychogenic effects they induce analgesia in mice and alter the perception of time in man[14,15].

It is interesting to note that the profile of II_s conforms with that reported for nicotine[16]. Taking into consideration the concentration of Δ^9-THC in II_s, we have calculated that the dose of Δ^9-THC injected with it was 1.5 mg/kg. A similar dose of Δ^9-THC given by Webster et al. in the rat caused a significant decrease in the animal's efficient responses[4,5]. This confirms the hypothesis of Karniol and Carlini according to which the effects of several samples of cannabis sativa are not related only with their Δ^9-THC content. These investigators explained their results as due to the existence of various quantities of other cannabinoids in the marijuana extracts they used. In our experiment the relative concentration of Δ^9-THC:CBD:CBN was 2.2:1:7.7 for III_s and 2.7:1:43.5 for II_s.

On the other hand, the existence of some unknown substances, possibly pyrolysis products of the known cannabinoids, may be responsible for this modification of the action of Δ^9-THC in II_s. The hashish smoke (III_s) may be devoid of such substances. We are trying at present to evaluate the validity of this hypothesis. Similar research involving marijuana smoke might provide us with certain very interesting data in the view of the different filters used in the 2 modes of cannabis consumption.

Our results do not agree with those of H. Savaki et al. where both II_s and III_s were equally effective[17]. The specificity of their tests is questioned since fractions of tobacco were shown to be quite active as well. On the other hand, the usefulness of their results are undeniable in view of the difficulty of using the Sidman schedule as a routine method in screening drugs obtained in a serial fractionation of the highly complex cannabis pyrolysates. Consecutive testing of certain key drugs among the active ones, in the Sidman avoidance, may permit us a more reliable characterization of the drugs as active or nonactive.

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Possible stimulatory effect of retinoic acid on pulmonary macrophages

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Summary. Retinoic acid was administered to hamsters suffering from N-nitroso-N-methylurethane-induced fibrosing alveolitis. A significant increase in macrophage numbers was seen in the lungs of retinoid-treated animals as compared to the unsupplemented group.

Retinoic acid and its synthetic analogues (retinoids) have been shown to possess a number of biological activities. Among these are maintenance of normal epithelial differentiation[1], reversal of epithelial metaplasia[2,3], and inhibition of chemical carcinogenesis[4-6]. Recently, the role of retinoids in immunological reactions has come under investigation. A number of studies suggest an enhancement of humoral and cell-mediated immunity following retinoid administration[7-10]. In particular, Leutskaya and Fais[11] report stimulation of antibody synthesis and Dennert and Lotan[12] observe increased activity of killer T cells with retinoic acid. This study suggests an additional effect of retinoic acid on the immune system, that of increasing macrophage activity. Hamsters suffering from fibrosing alveolitis, a lung disease thought to be immunologically mediated[13], which were fed retinoic acid, showed significantly increased numbers of pulmonary macrophages as compared to animals on unsupplemented diets.

Fibrosing alveolitis represents a class of diseases better known as 'pulmonary fibrosis'. The disease is often asso-