barrier and cell permeability are more likely to be contributing factors. The difference in background labeling for the two types of glucosamine was probably due to the fact that a greater amount of D-[1-^{14}C]glucosamine was injected and this resulted in proportionately greater leakage into the circulation.

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Intravenous Injection in Man of Δ^9-Tetrahydrocannabinol and 11-OH-Δ^9-Tetrahydrocannabinol

Abstract. *A microsuspension of Δ^9-tetrahydrocannabinol and of its metabolic derivative 11-OH-Δ^9-tetrahydrocannabinol has been prepared with 25 percent human serum albumin as the vehicle. Intravenous infusion of this preparation to humans indicates that both tetrahydrocannabinols are equally potent in producing the typical marihuana-like pschological and physiological effects.*

There is controversy as to whether the active principle of marihuana is Δ^9-tetrahydrocannabinol (Δ^9THC) or its 11-hydroxylated metabolite (11-OH-Δ^9THC). Christensen *et al.* (*1*) found that 11-OH-Δ^9THC was twice as potent in producing specific neurologic and behavioral responses when injected intravenously to mice than its parent compound. Based on this finding, they suggested that the 11-OH-Δ^9-THC might be the active form of the compound. The scarcity of 11-OH-Δ^9-THC has prevented the study of its pharmacological activity in man. Having available only very small quantities of material (*2*), the intravenous route is mandatory because the oral administration of the drug would require larger amounts to produce significant effects. To our knowledge, the intravenous administration of Δ^9THC or any one of its metabolic derivatives in doses capable of producing physiological and psychological effects in humans has not been performed because of the lack of a suitable intravenous preparation. Thus, although Lemberger *et al.* (*3*) have injected ^{14}C-labeled Δ^9THC intravenously dissolved in absolute ethanol, they only used tracer doses (0.5 mg). Larger doses of the drug requiring larger volumes of absolute ethanol cannot safely be injected into man because they can cause vein irritation, hemolysis, and precipitation of the plasma proteins.

We hypothesized that, if tetrahydrocannabinols could be suspended in human serum albumin and if the particle size of such a suspension were to be smaller than 0.22 μm (the pore size of filters used to sterilize solutions), a safe and easy method for intravenous administration would be available. After experimentation with different proportions of the tetrahydrocannabinols and the human serum albumin, we found the following preparation satisfactory.

Ten milligrams of either Δ^9THC or 11-OH-Δ^9THC dissolved in 0.5 ml of absolute ethanol were pipetted into a beaker containing 50 ml of 25 percent human serum albumin being vigorously stirred magnetically. Since stirring produced considerable foaming of the serum albumin, the suspension was transferred to tubes and centrifuged for 30 minutes. The preparation was then placed in a 50-ml syringe and filtered through a 0.22-μm Millipore filter into a vial and stored until used. All of the glassware utilized was sterile. Empirically, we found that filtration at a rate of 0.92 ml/min with a Harvard constant infusion pump as a source of pressure was satisfactory.

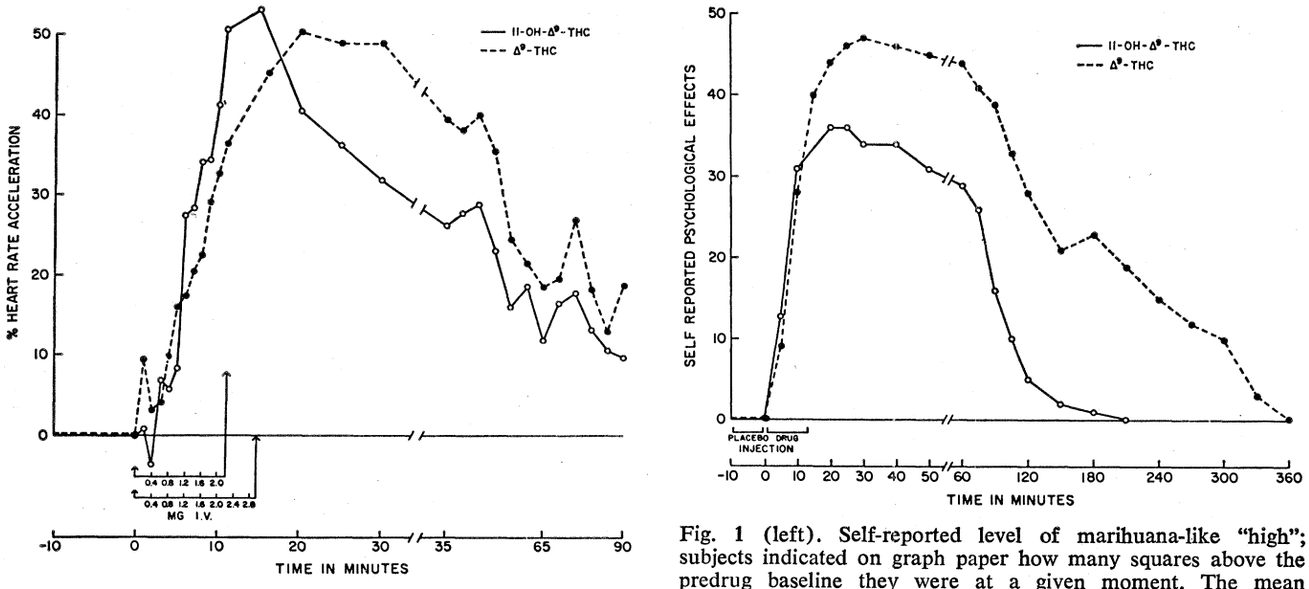

Fig. 1 (left). Self-reported level of marihuana-like "high"; subjects indicated on graph paper how many squares above the predrug baseline they were at a given moment. The mean values of the two groups are illustrated and indicate that the intravenous administration of Δ^9THC produced a more intense and long-lasting psychological experience ($P > .05$) than that produced by its 11-hydroxylated metabolite; *I.V.*, intravenous. Fig. 2 (right). Both Δ^9THC and 11-OH-Δ^9THC produced a significant increase in heart rate. The magnitude of the percentage of heart rate acceleration produced by either one of the compounds is statistically undistinguishable.

Table 1. Amount (micrograms per kilogram of body weight) of Δ^9THC or its 11-hydroxylated metabolite injected intravenously to obtain certain specific effects. Although observation of the values listed indicates that a lesser amount of the 11-hydroxylated metabolite was necessary to produce the specific effects, the difference between this compound and the parent compound was not statistically significant.

Subjects	Perception of "high"	Heart rate acceleration	Total dose
	Δ^9-tetrahydrocannabinol		
B.B.	19.54	19.54	32.57
S.J.	14.47	14.47	28.94
C.S.	16.00	16.00	40.00
J.B.	24.83	24.83	47.40
M.G.	26.39	26.39	46.92
R.F.	11.49	29.89	48.28
Mean	18.79	21.85	40.69
S.D.	5.39	5.60	7.59
	11-OH-Δ^9-tetrahydrocannabinol		
D.B.	15.95	19.14	25.52
J.L.	25.89	29.13	35.60
T.L.	20.53	14.66	23.46
N.C.	15.52	15.52	51.75
I.G.	14.18	8.51	31.21
J.B.	16.00	16.00	26.67
Mean	18.01	17.16	32.37
S.D.	4.03	6.22	9.54

Because the tetrahydrocannabinols were labeled with tritium (2.5 μc/mg), it was possible to ascertain the amount of the drugs that passed through the filter. The recovery of the total radioactivity after filtration varied between 85 and 100 percent. These data and the fact that no particles were observed under microscopic magnification ($\times$ 40) indicated that an adequate microsuspension had been obtained. The preparation was initially tested in anesthetized dogs. After intravenous administration of the preparation, the animals did not experience any cardiovascular or respiratory abnormalities and exhibited the typical effects of Δ^9THC which consist mainly of bradycardia and hypotension in the anesthetized animal. These results assured us of the safety and efficacy of the preparation for human experimentation.

Twelve normal, paid, male volunteers ranging in age from 21 to 24 years were tested. All of them were graduate students in good academic standing. Half of them received Δ^9THC intravenously while the other half received 11-OH-Δ^9THC metabolite. Subjects varied in their previous experience with marihuana from less than one cigarette a month to no more than two cigarettes per week and were equally distributed in both groups. In addition, all subjects had received Δ^9THC orally in a previous study and were familiar with the experimental conditions. The subjects were hospitalized at the Clinical Research Unit of the North Carolina Memorial Hospital, Chapel Hill. Respiration and heart rate were constantly monitored throughout the experiment by means of an Offner polygraph situated in a one-way screen room adjacent to the subject's room. Blood pressure was determined at intervals throughout the experiment by the clinical auscultatory method. To obtain the subjective evaluation of drug effects, that is, of a marihuana-like "high," we asked the subjects to rate themselves in a graph form provided for them.

Subjects were told that initially they would be intravenously infused with a drug-free solution of 25 percent human serum albumin and that, at some unspecified time, we would replace it by the preparation containing either Δ^9THC or its 11-hydroxylated derivative. Replacement of solutions without the subjects' awareness was possible because the Harvard constant infusion pump utilized for injection was located in the observation room. The subjects were instructed to report the moment they felt the action of the drug and to ask for the termination of the infusion as soon as they felt they had arrived at their desired level of "high." By giving the subjects control as to the amount of drug to be injected and by the constant recording of vital signs, we insured the safety of the volunteers. Subjective feelings were rated every 5 minutes regardless of whether the placebo or the drug was being injected. Variable times of placebo injection were used, ranging from 15 to 25 minutes, and the subjective ratings were always baseline. No discomfort of any kind or change in vital signs occurred with the infusion of the human serum albumin (0.46 ml/min). After the placebo injection, Δ^9THC or its 11-hydroxylated metabolite were infused at the rate of 0.2 mg/min (0.92 ml/min) until the subject decided that he had achieved his desired level. The doses necessary to achieve this level were 3.10 ± 0.90 mg of Δ^9THC and 2.27 ± 0.81 mg of 11-OH-Δ^9THC, and the difference in the values was not statistically significant.

Table 1 illustrates the doses necessary in micrograms per kilogram to perceive the action of the drug, to accelerate the heart rate more than 25 percent over the initial level, and to achieve the desired level of "high." The results suggest that the 11-hydroxylated metabolite is equally as potent as the parent compound.

In contrast, when we tabulate the self-reported psychological effects (Fig. 1), it appears that the Δ^9THC produces a larger and more long-lasting level of "high." This difference, which is statistically significant ($P > .05$), could be explained by the fact that the total dose of Δ^9THC injected was slightly greater than that of the 11-OH-Δ^9THC or on the basis of a faster metabolic inactivation of the 11-hydroxylated metabolite. On the other hand, both compounds seem to be equally as effective in producing acceleration of the heart (Fig. 1). Since changes in heart rate are involuntary, but perception and report of "high" is by definition subjective and influenced by extraneous and uncontrollable factors, we conclude that both compounds are not significantly different in pharmacological activity when injected intravenously to man.

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the Research Triangle Institute. The compound was prepared by rat microsomal hydroxylation of Δ^9THC; M. E. Wall, D. R. Brine, G. A. Brine, C. G. Pitt, R. I. Freudenthal, H. D. Christensen, *J. Amer. Chem. Soc.* **92**, 3466 (1970); M. E. Wall, *Ann. N.Y. Acad. Sci.* **191**, 23 (1971).

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4. These studies were conducted under contract HSM-42-71-95 between the Center for Studies of Narcotic and Drug Abuse of the Division of Narcotic Addiction and Drug Abuse, NIMH, and the Research Triangle Institute. We thank Drs. Monique Braude and Stephen Szara, Center for Studies of Narcotic and Drug Abuse, NIMH, for interest and encouragement. We thank Daynise Skeen for technical assistance.

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Synthetic Scotophobin in Goldfish: Specificity and Effect on Learning

Abstract. *Synthetic rat scotophobin was injected intracranially into common goldfish* (Carassius auratus) *which were then trained to avoid light or dark. The substance interacts with the learning process in goldfish in an apparently specific way, facilitating the acquisition of dark avoidance, a task homologous with that acquired by rats from which the natural peptide was isolated, while inhibiting acquisition of light avoidance.*

The isolation and characterization of scotophobin (*1*, *2*), a molecule formed in the brains of rats learning to avoid a darkened compartment, and its synthesis (*3*) and reported activity in mice (*4*) raise the question of the activity of this learning-linked rat peptide in other vertebrates.

We have studied the effect of synthetic scotophobin (*5*) in *Carassius auratus*, the common goldfish. In addition to our work with the goldfish (*6*), evidence that scotophobin may be active in goldfish has been reported by Guttman *et al.* (*7*), who found in fish screened for dark preference that scotophobin depressed a high preinjection level of time in the dark in a procedure not involving learning. Our work bears on the question of the specificity of the scotophobin effect in goldfish, using a procedure in which fish injected with scotophobin were trained to actively avoid light or dark. In this procedure, we have used normal fish rather than animals selected for dark preference, as in other reports (*1*, *7*). We have found that scotophobin appears to interact with the learning process in goldfish in a specific way, facilitating the acquisition of dark avoidance, a task homologous with that acquired by the rats from which the natural peptide was isolated, while inhibiting acquisition when the cues are reversed.

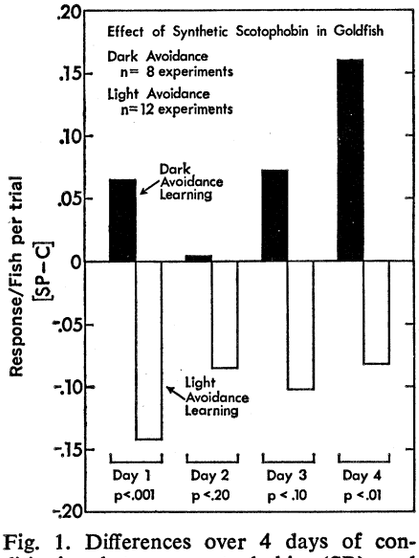

Fig. 1. Differences over 4 days of conditioning between scotophobin (SP) and control (C) fish in acquisition of active dark-avoidance responding (black bars), and between SP and C fish in acquisition of active light-avoidance responding (white bars). Positive values indicate faster learning in SP fish; negative values indicate slower learning in SP fish. Measure is the response per fish per trial.

Common goldfish 8 to 10 cm long were, before use, kept in the laboratory at least 1 week after arrival from Ozark Fisheries, Stoutland, Missouri. Fish were maintained in shallow home tanks under constant illumination. In groups of five to ten, fish were trained to avoid light or to avoid darkness in a large "fish shuttlebox" (*8*). The tank was divided by an opaque partition allowing a 4-cm clearance underneath. Electric current could be applied, as described by Fjerdingstad (*9*), across either end of the tank. Two clear stimulus lamps were mounted on the ends of the tank to permit either compartment to be lighted while the other was dark. The entire tank was under a lightproof cover.

Synthetic scotophobin [peptide D (*4*)] was injected in various nominal doses ranging from 12.5 to 120 ng. In all cases the intracranial route was used with an injection volume of 10 μl (*10*). Control fish received injections of the vehicle (*11*). All glassware was siliconized.

Training was begun, one session of ten trials per day, 48 hours after injection and was continued at least 4 days. The learning of scotophobin recipients was evaluated with respect to controls trained concurrently to avoid light or to avoid darkness. A trial for light-avoidance conditioning consisted of a 15-second presentation of the stimulus lamp in one end of the tank, followed by the addition of shock (in that end only) for 45 seconds, after which the lamp came on in the other end, beginning the next trial. The number of light-avoidance responses for a trial was recorded as the number of fish observed to swim under the barrier away from the light before the onset of shock. Only net avoidances were counted; if a fish swam away from the light but returned before the onset of shock, it was not counted as avoiding. Dark-avoidance training was identical with light-avoidance training except for the reversal of cue significance: to avoid shock the fish were required to swim out of the darkened compartment, not into it as in light-avoidance training (*12*).

Figure 1 presents the results of 20 experiments involving a total of 313 fish trained for 4 days to avoid light or to avoid dark (*13*). Negative values indicate slower learning in scotophobin

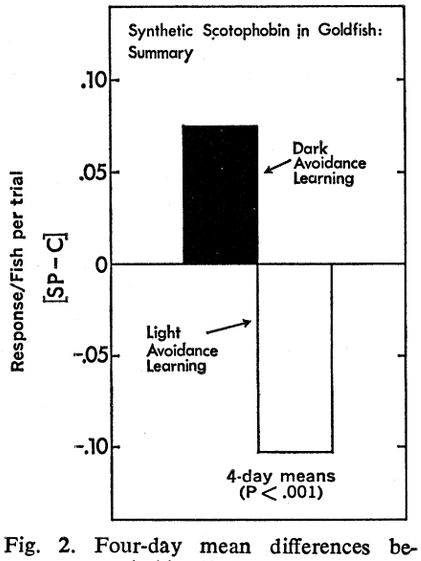

Fig. 2. Four-day mean differences between scotophobin (SP) and control (C) fish in acquisition of dark-avoidance responding (black bar) and light-avoidance responding (white bar).