tion to school than Keenleyside's rudd since (i) it had been trained previously to swim around the tank, (ii) the school met the test fish once a minute at each circuit of the tank, and (iii) one test saithe had 25 normal schooling fish to respond to. If we allow for the different experimental procedures, Keenleyside's findings do not seem incompatible with our own. Parr (*2*) found that seven chub mackerel, *Scomber colias*, temporarily blinded with a mixture of lamp black and Vaseline applied to both eyes, failed to join a milling school of normal fish and collided repeatedly with them.

We do not know whether the differences between our results and those of Parr are consequences of technique or whether they reflect real differences between the two species; the extent of arousal may also be important. Parr's fish showed panic reactions which increased the more they collided, whereas the test saithe recovered very gradually from handling and anesthesia while being repeatedly presented with the school. Finally, none of our blind saithe showed any reaction to the school within the time scale of Parr's experiment. It is therefore likely that the present technique allows more chance for a blind fish to school.

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Cannabinoid Induced Behavioral Convulsions in Rabbits

Abstract. *A population of New Zealand White rabbits has been found to exhibit behavioral convulsions when given low intravenous doses of psychoactive cannabinoids of marijuana. The behavioral convulsions decrease in severity and then disappear after the long-term administration of* Δ^9*-tetrahydrocannabinol. The extreme sensitivity of these rabbits to the stimulant action of cannabinoids suggests that the population might serve as a model in studies of the stimulant action of cannabinoids.*

Although the anticonvulsant properties of Δ^9-tetrahydrocannabinol (Δ^9THC) have been well established in experimental animals (*1*) there is some evidence that this major psychoactive component of marijuana possesses convulsant properties. Electroencephalographic (EEG) patterns of convulsive-like activity, for example, polyspikes, spike and slow waves, have been reported in rodents, dogs, cats, rabbits, and monkeys, but these EEG manifestations occur in the absence of behavioral convulsions (*1*). While behavioral convulsions occasionally have been reported in rats, dogs, and monkeys, these seizures generally occur only with lethal or near-lethal doses of Δ^9THC (*1*).

Recently, we have found that some rabbits of the New Zealand White breed exhibit behavioral convulsions when given an intravenous dose of Δ^9THC as low as 0.05 mg per kilogram of body weight (*1–3*). These rabbits were subsequently inbred and the resultant offspring were tested with additional naturally occurring and synthetic cannabinoids of marijuana. Here, we describe the behavioral effects of various cannabinoids in these rabbits, and demonstrate that the behavioral convulsions decrease in severity after the long-term administration of Δ^9THC.

The lineage of this population of New Zealand White rabbits is shown in Fig. 1. The first four rabbits found to be susceptible to convulsions induced by Δ^9THC were Nos. 20, 21, 22, and 24 (*2*). The parents of this litter were tested and the dam was found to be seizure susceptible while the sire was not. Among the litters bred from this single pair of rabbits, about 90 percent of the individuals exhibit convulsions after the intravenous administration of Δ^9THC in doses of 0.5 mg/kg or less. The seizures induced by Δ^9THC are apparently due to an autosomal recessive mutation in the rabbits, similar to that found in rabbits that are susceptible to audiogenic seizures (*4*), but in the New Zealand White population it is the high degree of penetrance that confers susceptibility to Δ^9THC. Standard genetic tests are being made to define clearly the mode of inheritance.

In the present experiments, the rabbits used ranged in weight from 2.5 to 3.3 kg. A catheter was implanted in the left external jugular vein so that drugs could be administered intravenously without disrupting the animal's behavior during subsequent testing. Surgery was performed with the animals under chlorpromazine and subsequently pentobarbital anesthesia. All cannabinoids except 4-morpholino-butyrate-Δ^9-THC (SP-111A) were prepared in a vehicle of 10 percent polysorbate (Tween 81) and saline. Since SP-111A is water soluble, distilled water was used as the vehicle for this synthetic derivative of Δ^9THC.

During testing, subjects were observed through a one-way vision window in a sound attenuated chamber measuring 82 cm square by 70 cm high. As previously described (*2*), the frequency and duration of the following behaviors were measured by an experimenter-operated digital event recorder: limb clonus; extension of front or hind limbs, or both; head tuck; body torsion; ears down; mydriasis; and nystagmus. Latency to convulsion (that is, the first onset of clonus or tonus, or both) was also mea-

sured. There was a minimum of 4 days between injections of drugs to any one animal.

Table 1 shows the effects of a number of cannabinoids on these rabbits. The compounds Δ^9THC, Δ^8THC, SP-111A, and 11-hydroxy-Δ^9THC, which are all psychoactive in man (*5, 6*), produced behavioral convulsions in the rabbits when given in low doses (0.25 to 1 mg/kg). Although the convulsions were qualitatively similar, that is, consisting mainly of head tucks, body torsion, or front and hind limb extension, the latency to convulsion differed among Δ^9THC, SP-111A, and 11-hydroxy-Δ^9THC. The mean latencies in seconds (± standard error) were: Δ^9THC, 129.7 ± 20.02; SP-111A, 461.8 ± 46.08; 11-hydroxy-Δ^9THC, 20.9 ± 3.81. There was a significant difference among the latencies of these drugs ($F = 35.05$; d.f. = 2,8; $P < .01$) and the latency for 11-hydroxy-Δ^9THC was significantly shorter than the latencies for Δ^9THC ($P < .001$) or SP-111A ($P < .001$). The onset of convulsive activity for SP-111A was consistently longer than that for Δ^9THC although the differences in latencies only approached statistical significance ($.05 < P < .10$).

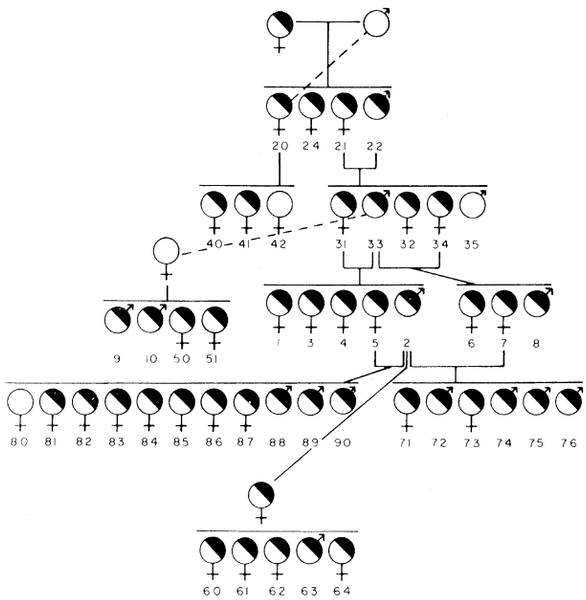

Fig. 1. Lineage of New Zealand White rabbits sensitive (half-filled circle) and nonsensitive (open circle) to behavioral convulsant effects of Δ^9THC given intravenously in doses of 0.5 mg/kg. All progeny that were raised to an age at which they could be tested are shown. The dam of litter 9–51 was a normal New Zealand White rabbit unrelated to the rest of the pedigree; the dam of litter 60–64 was seizure susceptible but her relationship to the rest of the pedigree is unknown.

Table 1. Effects of intravenous injections of cannabinoids on a population of New Zealand White rabbits. Rabbit identification numbers are given in Fig. 1.

Drug	Dose (mg/kg)	Identification numbers of rabbits tested	Ratio of rabbits convulsed to number tested
Δ^9-Tetrahydrocannabinol (Δ^9THC)	0.5	1, 2, 3, 4, 5, 6, 7	7/7
Δ^8-Tetrahydrocannabinol (Δ^8THC)	0.5	1, 2, 3, 4, 5, 6, 7	7/7
SP-111A	1.0	1, 9, 10, 33, 34, 50	6/6
Cannabinol (CBN)	10.0	1, 3, 4, 5, 6, 7	4/6
Cannabinol	15.0	3, 8, 9, 10, 33, 34	6/6
Cannabidiol (CBD)	10.0	1, 2, 3, 4, 5, 6, 7	0/7
Cannabidiol	15.0	3, 9, 10	0/3
Cannabidiol	20.0	1, 3, 8	0/3
11-Hydroxy-Δ^9THC	0.25	9, 10, 33, 34, 50	5/5
Cannabicyclol	8.0	3, 8	1/2
Cannabichromene	8.0	3, 8	0/2
Control experiments: rabbits injected with vehicle only			
Tween 81-saline*	1.0	1, 2, 3, 4, 5, 6, 7	0/7
γ-Morpholinobutyric acid hydrobromide	1.0	1, 9, 10, 33, 34, 50	0/6

*Tween 81-saline was the vehicle used for all the cannabinoids except SP-111A. Gamma-morpholinobutyric acid hydrobromide dissolved in distilled water was the vehicle for SP-111A.

These differences in latencies may reflect differences in the metabolism or distribution of these cannabinoids. The onset of action of SP-111A is longer than that of Δ^9THC, presumably because SP-111A must first be hydrolyzed to Δ^9THC in the liver before it becomes active (*6*). The larger dose required for effectiveness of SP-111A (1.0 mg/kg of SP-111A as opposed 0.5 mg/kg Δ^9THC) may be reflected by the fact that the inactive moity of the Δ^9THC derivative, that is, gamma morpholinobutyric acid hydrobromide, accounts for nearly half of the molecular weight of SP-111A. In a previous study, molar equivalent doses of SP-111A and Δ^9THC produced similar effects (*6*). Likewise, in the present study, molar equivalents of SP-111A (1.0 mg/kg) and Δ^9THC (0.5 mg/kg) produced similar behavioral effects. Gamma-morpholinobutyric acid hydrobromide, dissolved in distilled water, was given to six rabbits as a control for SP-111A, and no convulsions were observed; similarly, injection of 10 percent Tween 81-saline failed to cause seizures (Table 1).

11-Hydroxy-Δ^9THC, a purported active metabolite of Δ^9THC, has been reported to have a shorter onset and greater potency than the parent cannabinoid (*7, 8*). These observations are consistent with our findings that convulsions induced by 11-hydroxy-Δ^9THC have a much shorter latency than those induced by Δ^9THC or SP-111A, and that only half the dose of this metabolite (compared with Δ^9THC) is needed for the convulsion to occur.

Cannabinol (CBN) and cannabidiol (CBD) have not been found to be psychoactive in man (*5*). However, both drugs do produce some cortical EEG synchronization and corneal areflexia in rabbits (*9*). In addition, CBN and CBD are effective anticonvulsants in mice (*10*). As shown in Table 1, CBN at a dose of 10 mg/kg produced convulsions in four out of six rabbits tested, while increasing the dose to 15 mg/kg produced convulsions

Table 2. Development of tolerance in a strain of New Zealand White rabbits during the long-term administration of Δ^9THC. Asterisks indicate that no convulsion occurred; convulsions occurred at other times of Δ^9THC administration.

Rabbit No.	Dose (mg/kg) on day										1 week after last injection
	1	2	3	4	5	6	7	8	9	10	
3	0.5	0.5*	1.0*	2.0	2.0	2.0	2.0*			0.5*	0.5
9	0.5	0.5	0.5	0.5	0.5	0.5	0.5*	1.0	1.0	1.0*	0.5
10	0.5	0.5	0.5	0.5*	1.0	1.0*					0.5
33	0.5	0.5*	1.0	1.0*			0.5*				0.5
34	0.5	0.5	0.5*	1.0	1.0	1.0*					0.5

in all six rabbits. However, CBD in doses of 10, 15 and 20 mg/kg did not produce convulsions in the rabbits we tested. These findings suggest that CBN, and not CBD, has stimulant properties in our paradigm and may explain the observation that CBD has greater potential for anticonvulsant activity than CBN or Δ^9THC (*11*).

We also tested the effects of cannabicyclol and cannabichromene, two naturally occurring constituents of marijuana (Table 1). Cannabichromene (8.0 mg/kg) produced no convulsions or other observable effects in either of the rabbits tested. When cannabicyclol (8.0 mg/kg) was given to rabbit No. 3 no convulsions or observable change occurred. However, approximately 7 minutes after rabbit No. 8 had been given cannabicyclol (8.0 mg/kg) the rabbit began to sprawl, then went into a brief convulsive-like thrashing, and died. A necropsy of this rabbit showed that death was due to a pulmonary hemorrhage which may explain this unusual finding. Finally, rabbit Nos. 81 through 86, 89, and 90 did not convulse with relatively high doses of the following hallucinogens: lysergic acid diethylamide (100 μg/kg), mescaline (40.0 mg/kg), psilocybin (3.0 mg/kg), phencyclidine (2.0 mg/kg), and methamphetamine (0.5 mg/kg), suggesting that convulsions are specific to the cannabinoids.

A preliminary study in our laboratory (*2*) suggested that tolerance may develop to the convulsant inducing properties of Δ^9THC in this population of rabbits. Therefore, a more extensive study of the long-term effects of Δ^9THC was undertaken. We gave five rabbits one injection per day of 0.5 mg/kg Δ^9THC until no convulsion occurred. The following and subsequent days (if necessary) the dose of Δ^9THC was doubled until a convulsion was again elicited. This higher dose then was given daily until the rabbit again became tolerant to the cannabinoid induced convulsion. As shown in Table 2, although there was some individual variation, all rabbits exhibited behavioral tolerance at Δ^9THC doses of 0.5 mg/kg. An increase in dose to 1.0 mg/kg was sufficient to elicit behavioral convulsions in four out of the five rabbits, and each of these four rabbits became tolerant to this higher dose. One tolerant rabbit (No. 3) exhibited convulsions after an increase of dosage to 2.0 mg/kg, and as with the other four subjects, tolerance subsequently was demonstrated with this higher dose. Two days after becoming tolerant to convulsions with the highest dose, rabbit Nos. 3 and 33 were injected with 0.5 mg of Δ^9THC per kilogram in an effort to reinstate seizures, but neither convulsed. One week after becoming tolerant to convulsions with the last administered dose of Δ^9THC, each subject was injected with 0.5 mg/kg. In all five rabbits, convulsions were again elicited, indicating that tolerance had been lost.

These findings suggest the occurrence of an animal model that is uniquely and differentially sensitive to the (extreme) stimulant action of cannabinoids. This is especially interesting because CBD, which perhaps has the greatest anticonvulsant potential in experimental animals, did not elicit convulsions. These findings suggest that a model for testing the effects of marijuana, its congeners, and potential antagonists might be provided by this population of New Zealand White rabbits.

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Nitrous Oxide "Analgesia": Resemblance to Opiate Action

Abstract. *Nitrous oxide produced a dose-related "analgesia" in mice (median effective dose, 55 percent). The analgesia was evaluated by means of a phenylquinone writhing test. Narcotic antagonists or chronic morphinization reduced nitrous oxide analgesia. Either nitrous oxide releases an endogenous analgesic or narcotic antagonists have analgesic antagonist properties heretofore unappreciated.*

Nitrous oxide has a long history of use as a euphoriant and analgesic (*1*). The purpose of our study was to characterize the nature of nitrous oxide analgesia. To estimate the "analgesia" in mice we used the phenylquinone writhing test (*2*). Mice were injected intraperitoneally with phenylquinone and placed in a clear plastic enclosure and exposed to mixtures of nitrous oxide and oxygen (*3*).

Nitrous oxide produces an "analgesic" response in mice, that is, an inhibition of writhing. This analgesia was dose-related with 50 percent inhibition of writhing occurring in the presence of 55 percent nitrous oxide (Fig. 1). Naloxone hydrochloride (5 mg/kg, subcutaneously) administered immediately before the phenylquinone had no significant effect on writhing. However, as can be seen in Fig. 1, naloxone administration did reduce the analgesic efficacy of nitrous oxide. In additional experiments, the average analgesia produced by 80 percent nitrous oxide in six groups of mice (five mice per group) was 84 ± 6 percent (Fig. 2). Naloxone reduced the analgesia caused by 80 percent nitrous oxide to 37 ± 4 percent. With 60 percent nitrous oxide, analgesia averaged 54 ± 6 percent, whereas 60 percent nitrous oxide plus naloxone produced only 12 ± 11 percent analgesia. Lower doses of naloxone were not effective in reversing nitrous oxide analgesia.

Naltrexone is also a narcotic antagonist (*4*), and this drug also antagonized nitrous oxide analgesia. If naltrexone was given alone in a dose of 5 mg/kg subcutaneously, it had no effect on the phenylquinone-induced writhing. It did, however, reduce the analgesia produced by 70 percent nitrous oxide from 64 percent to 21 percent, and also reduced the analgesic effects of 60 percent nitrous oxide from 45 percent to 12 percent.

The analgesic effects of nitrous oxide were also reduced in mice that had received morphine for several days prior to the test. In these experiments, morphine hydrochloride (*5*) was given subcutaneously in a dose of 30 mg/kg twice on day 1; 50 mg/kg twice on day 2; and 60 mg/kg three times on day 3. A paired group of mice received only saline injections and served as controls. On day 4, no morphine was given and the mice were tested for analgesia.

Both the saline- and morphine-injected mice reacted to the phenylquinone in a similar manner. Two groups of saline-treated mice writhed a total of 53 and 57 times, whereas the morphine-treated mice writhed 60 and 56 times. Thus, no