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# Very low doses of $\Delta^8$-THC increase food consumption and alter neurotransmitter levels following weight loss

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**Abstract**

We have investigated the effect of 0.001 mg/kg $\Delta^8$-tetrahydrocannabinol (THC) on food consumption, cognitive function, and neurotransmitters in mice. Sabra mice were treated with vehicle, THC, or THC+CB1 antagonist (SR141716A). The mice were fed for 2.5 h a day for 9 or 50 days. In the 9-day schedule, THC-treated mice showed a 16% increase in food intake compared with controls ($P<.001$). This effect was reversed by the antagonist ($P<.01$). In the long-term schedule a 22% increase in intake ($P<.05$) was recorded. During the course of the 9- and 50-day experimental protocol, all mice lost about 20% and 10% of their original weight, respectively, to reach approximately the same weights, which were not significantly different between the different treatment groups. In addition, THC caused an increase in activity ($P<.05$). Cognitive function showed a tendency to improve ($P<.06$) in the THC-treated mice, which was reversed by the antagonist for Days 4 and 5 of the maze ($P<.01$, and $P<.05$, respectively). Significant decreases in dopamine and serotonin (5-HT) levels were found both in the hypothalamus ($P<.01$) and the hippocampus ($P<.01$, $P<.05$), respectively, while norepinephrine (NE) levels showed tendency to increase in both the hypothalamus and hippocampus. $\Delta^8$-THC increased food intake significantly more ($P<.05$) than did $\Delta^9$-THC, while performance and activity were similar. Thus, $\Delta^8$-THC (0.001 mg/kg) caused increased food consumption and tendency to improve cognitive function, without cannabimimetic side effects. Hence, a low dose of THC might be a potential therapeutic agent in the treatment of weight disorders.


*Keywords:* THC; Food consumption; Cognitive function; Neurotransmitter levels; Weight loss; Activity

## 1. Introduction

Anorexia nervosa (AN) is a disorder of unknown etiology, characterized by pathological eating behavior expres sed as self-starvation. With the discovery of the endogenous cannabinoids, anandamide (ANA; Devane et al., 1992), a fatty acid amide, and 2-arachidonoyl glycerol (2-AG; Mechoulam et al., 1995), a fatty acid ester, it became possible to examine the effects of these brain constituents on appetite. Low dose ANA (0.001 mg/kg ip) was found in our laboratory to improve food intake and cognitive function and to reverse some of the neurotransmitter changes caused by diet restriction (DR) in two murine brain areas associated with appetite (hypothalamus) and learning (hippocampus; Hao et al., 2000).

Two kinds of cannabinoid receptors, termed CB1 and CB2, have been characterized and cloned (Devane et al., 1988; Matsuda et al., 1990; Munro et al., 1993). Tetrahydrocannabinol (THC) binds to both cannabinoid receptors. The CB1 receptor is mostly distributed in several brain regions such as the cortex, hippocampus, basal ganglia and cerebellum (Herkenham et al., 1990), while the CB2 receptor is found in immune tissues such as the spleen, thymus and tonsils (Pertwee, 1997) but not in the brain. Specific antagonists include SR141716A for the CB1 receptor and SR144528 for the CB2 receptor.

In the cerebrospinal fluid of subjects with AN, levels of tyrosine, dopamine, serotonin (5-HT) and metabolites are all decreased (Kaye et al., 1984) and returned to normal with weight restoration (Garner et al., 1984). The enhancement effect of THC on appetite led to the initiation of a clinical trial with AN patients; the results were negative

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(Gross et al., 1983). The high doses employed (7.5 up to 30 mg/day) caused cannabimimetic effects and tolerance. However, THC is an approved drug in the treatment of cancer- and AIDS-associated cachexia (Mechoulam et al., 1998), (Berry and Mechoulam, 2002). Cannabinoid agonists are well known to have a biphasic mode of action (Sulcova et al., 1998). Thus, ANA has a general stimulatory effect at low doses and a sedative effect at high doses. The known effects of cannabinoid agonists on appetite and the established changes on neurotransmitter levels in AN led us to evaluate the effect of low doses of THC (0.001 mg/kg), which does not produce cannabinommimetic side effects (Sulcova et al., 1998), on food intake, cognitive function and brain neurotransmitters in a model of DR in mice, as potential treatment for AN. We used $\Delta^8$-THC rather than $\Delta^9$-THC because the latter is easily oxidized to cannabinol by air, while $\Delta^8$-THC is stable (Mechoulam et al., 1998). The synthesis of $\Delta^8$-THC is much more simple (and hence less expensive) than that of $\Delta^9$-THC, and the price may be of economic importance if THC is ultimately used as a drug in eating disorders.

## 2. Materials and methods

The experimental protocol was approved by the Institutional Committee for the Use of Animals No. 10021. Cannabinoids and antagonist were kindly supplied by Prof. R. Mechoulam.

### 2.1. Food intake study

Food intake measurements were performed as described by Hao et al. (2000). Female Sabra mice were divided randomly to cages, with two mice per cage. The cages contained wood-chip bedding and were placed in a temperature-controlled room at 22 °C, on a 12-h light/dark cycle. The food was given as cakes, containing 30 g gelatin, 1 l water and 700 g of dry food, with the following nutrition values: 54.9% carbohydrate, 21.1% protein and 4.7% fat. The food was given for 2.5 h a day for 9 or 50 days. Food was weighed before and after feeding. Food intake was calculated by the difference divided by two, and then in the following way: $(\text{day}_{n+1}+\text{day}_{n+2}+\ldots+\text{day}_{n+9})-8\times\text{day}_n$. $\text{Day}_n$ is the first day of the food consumption. This formula gives the area under the food intake curve. During the first 2 days of the experiment, the mice were on food restriction only and, as of the third day, injections were started.

To find the effect of $\Delta^8$-THC on food intake, initially, five different doses of $\Delta^8$-THC were tested: 0.001, 0.01, 0.1, 1 mg/kg including vehicle (explained below). This experiment lasted for 10 days, and the mice were fed in a 2.5-h feeding schedule. An additional experiment was conducted to see whether there are differences between $\Delta^8$- and $\Delta^9$-THC, which has been approved for medicinal use.

There were three experimental groups: (a) controls which received daily vehicle (emulphore:ethanol:saline, 1:1:18); (b) THC group (0.001 mg/kg); (c) THC plus antagonist (THC: 0.001 mg/kg and SR141716A: 2.5 mg/kg). In Groups b and c, both materials were dissolved in the vehicle mixture. THC and antagonist were first dissolved in emulphore and ethanol, and then, saline was added gradually. The injections (0.1 ml ip) took place 10 min before food consumption was allowed, a timing that was based on a previous experiment conducted by our group (Hao et al., 2000).

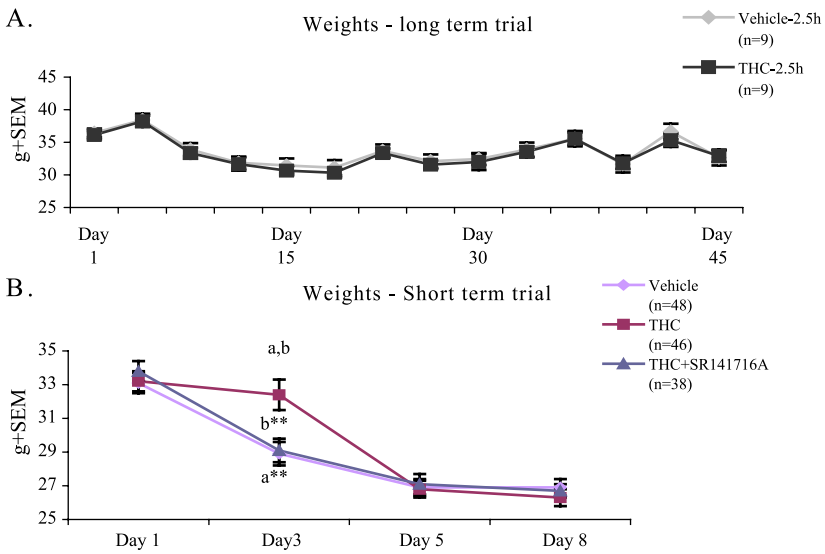


Fig. 1. (A) Changes in the average body weight of the mice in a long-term experiment (50 days), during the 2.5-h feeding schedule, (vehicle ◆, THC ■), nine mice in each group. (B) Changes in the average body weight of the mice in a short-term (9 days) experiment. All three groups (vehicle ◆, $\Delta^8$-THC ■, $\Delta^8$-THC+SR141716A △) were fed in a 2.5-h feeding schedule. $^{**}P<.01$. Letters represent pairs that are statistically different. There are 38–48 mice in each group.

### 2.2. Eight arm maze

Eight-arm maze testing was performed 5 days after the beginning of the experiments. The eight-arm maze is a scaled-down version of that developed for rats (Olton and Samuelson, 1976). We used water deprivation and a reward of 50 μl of water presented at the end of each arm (Steingart et al., 2000). Water deprivation was achieved by limiting water consumption overnight. The mice were tested for 5 days. Ten minutes after injection, each mouse was evaluated for its performance in the maze (Hao et al., 2000). The mice were observed until they made entries into all eight arms or until they completed 24 entries (whichever came first);

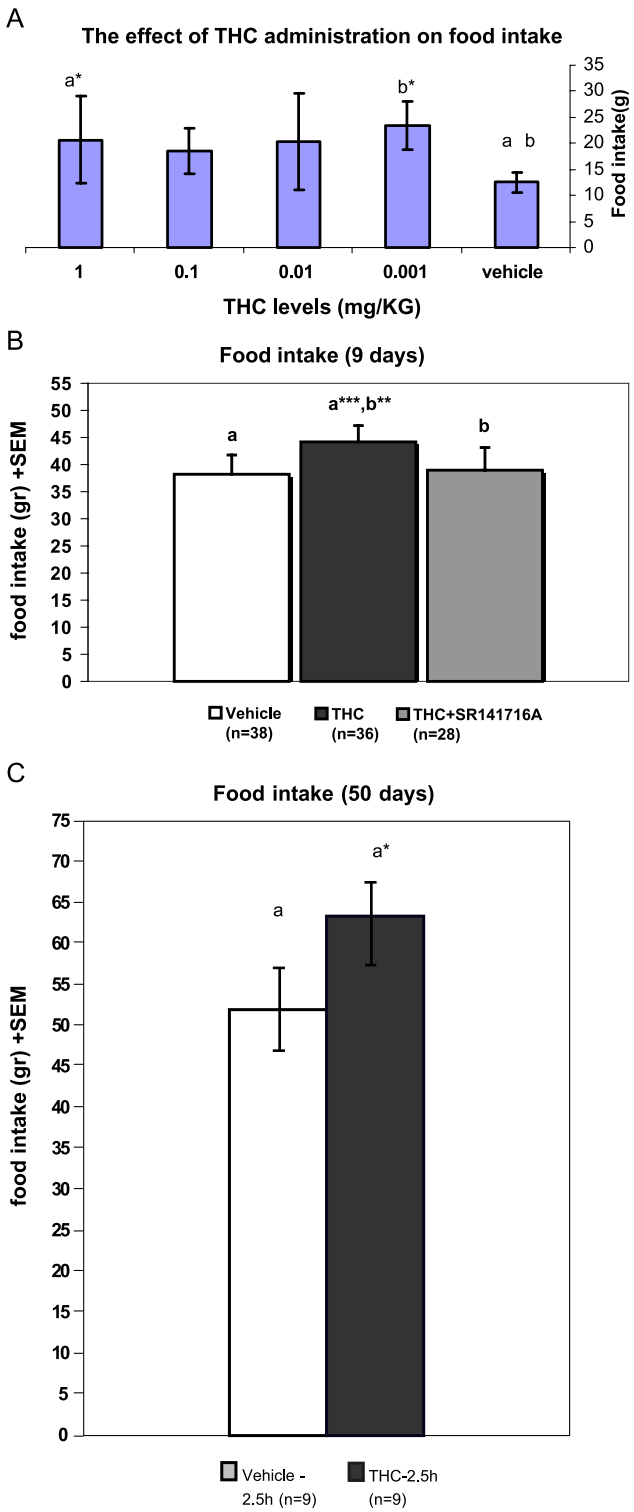


Fig. 2. (A) Biphasic effect of $\Delta^8$-THC on food intake. There was a biphasic effect of $\Delta^8$-THC on food intake, a significant increase in the very low (0.001 mg/kg) and high doses (1 mg/kg; $P<.05$; a and b) and a nonsignificant increase in 0.01 and 0.1 mg/kg towards vehicle (12.52±0.65 g); 10 mice in each group. (B) Effect of $\Delta^8$-THC on food intake in a short-term experiment (9 days). $^{**}P<.01$ and $^{***}P<.001$. Letters represent pairs that are statistically different (vehicle □, THC ■, THC+SR141716A ▨). There are 28–38 mice in each group. (C) Effect of $\Delta^8$-THC on food intake in a long-term experiment (50 days). $^{*}P<.05$ (vehicle □, THC ■). Letters represent pairs that are statistically different. There are nine mice in each group.

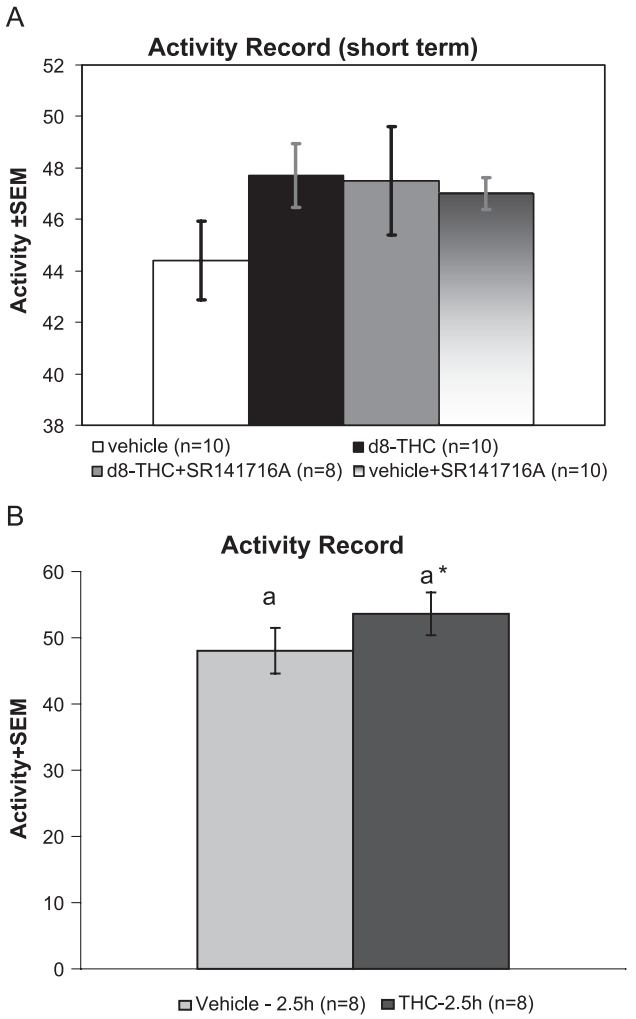


Fig. 3. (A) Activity record: The effect of DR and $\Delta^8$-THC with and without SR141716A on activity, presented as the mean number of crossing in 5 min/min/mouse (vehicle □, THC ■ THC+SR141716A ▨). Letters represent pairs that are statistically different. There are 8–10 mice in each group. (B) Effect of $\Delta^8$-THC on mice activity. Activity was measured for three consecutive days, 5 min each day. The $\Delta^8$-THC groups were more active than the vehicle groups were. $^{*}P<.05$ (vehicle □, THC ■). Letters represent pairs that are statistically different. There are eight mice in each group.

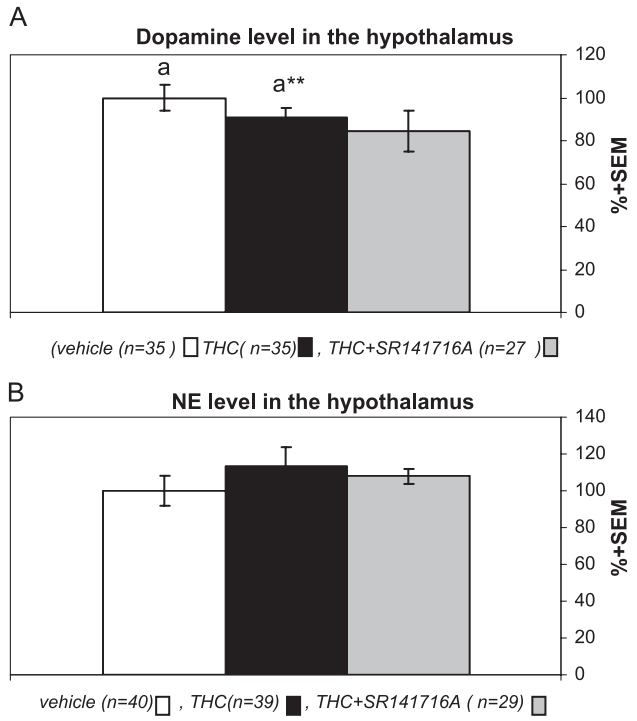


Fig. 4. (A) The level of dopamine in the hypothalamus. **$P$<.01, 27–35 mice in each group. (B) The level of norepinephrine in the hypothalamus. There are 29–40 mice in each group. Letters represent pairs that are statistically different (vehicle □, THC ■, THC+SR141716A ▨). The statistics is based on the Mann–Whitney two-sample test performed on each experiment, separately, and the $P$ values combined, as described in the Materials and methods section, to the composite $P$ value.

statistically, after 24 entries, the mice should have visited all arms. Food was given at the completion of the test. Maze performance was calculated per each day during the 5 days of the test.

### 2.3. Activity

To check whether mice injected with THC were more active than those injected with vehicle, we used an activity apparatus, which consists of a cylindrical chamber (60 cm in diameter) with crossing infrared beams. The activity test took place in the last 3 days of the experiment. Locomotor activity is recorded by a counter, which is attached to the apparatus, that counts the number of beam crossings by the mice at 1-min intervals. The activity of two mice was measured simultaneously for a 5-min period, 10 min after injections. Two mice were tested together to lower their stress to the minimum, and to save time. The mice received food after they had completed the test. Activity is presented as the mean number of crossings in 5 min during 3 days of the test, per minute per mouse.

### 2.4. Brain extraction

The mice were killed by decapitation 10 min after the injections of vehicle, THC or THC+antagonist. Brains were rapidly removed, and the hypothalamus and hippocampus were dissected out. The tissues were weighed and homogenized, and the homogenate was then centrifuged for 15 min. Of the suspension, 50 μl was measured for protein using the Bradford reagent (Sigma). This method was based on Avraham et al. (1996) and Hao et al. (2001).

### 2.5. Neurotransmitters evaluation

Neurotransmitters were measured as described by Avraham et al. (1996). The assays for dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), norepinephrine (NE) and

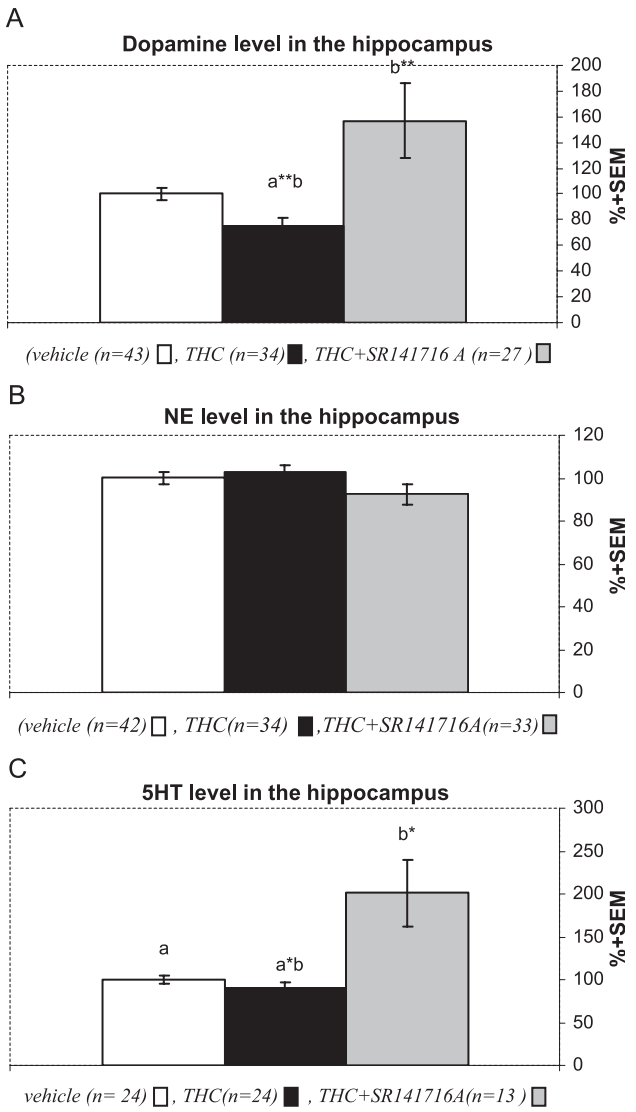


Fig. 5. (A) The level of dopamine in the hippocampus. **$P$<.01, 27–43 mice in each group. (B) The level of norepinephrine in the hippocampus, with 33–42 mice in each group. (C) The level of 5-HT in the hippocampus. *$P$<.05 (between THC and THC+CB1: $P$=.05), 13–24 mice in each group. Letters represent pairs that are statistically different (vehicle □, THC ■, THC+SR141716A ▨). The statistics is based on the Mann–Whitney two-sample test performed on each experiment, separately, and the $P$ values combined, as described in the Materials and methods section, to the composite $P$ value.

methoxyhydroxyphenylglycol (MHPG) were performed by HPLC separation and detection using HPLC-ECD. 5-Hydroxytryptamine (5-HT) was measured by HPLC-ECD as well. Neurotransmitter levels are presented as the percentage of ng/g tissue weight. The analysis was done on single hippocampus or hypothalamus.

### 2.6. Statistical analysis

The data are presented as means and standard errors. All of the results were analyzed by the Mann–Whitney test, except of the results of Fig. 2A and C and the activity trial that were analyzed by *t* test. When necessary, *P* values from similar experiments were combined by a method described previously to increase the samples size, their dispersion and, thus, the power of the experiments (Berry et al., 1998; D'Agostino and Stephen, 1986; Sokal and Rohlf, 1981).

## 3. Results

### 3.1. Body weight (Fig. 1A and B)

All groups had identical body weight at the beginning of the experiment. During the course of the 9-day experimental protocol, all mice lost about 20% of their original weight to reach the approximately same weights, which were not significantly different between the vehicle, THC or THC+SR141716A groups. The decrease in body weight in the THC group was, at first, slower than in the other two groups ($P<.01$). Because we did not find a difference in body weight in the short-term experiment, despite the significant increase in food consumption, we conducted a long-term experiment that lasted for 50 days. The mice were either injected with THC in its solvent mixture or in the controls, with the solvent mixture alone. In the long-term experiment, mice on the 2.5-h feeding schedule with vehicle lost about 11% of their weight, while the THC group lost about 9%. The difference was not significant. Fig. 1.

### 3.2. Food intake (Fig. 2A, B and C)

There was a biphasic effect of $\Delta^8$-THC on food intake: a significant increase in the very low (0.001 mg/kg) and high doses (1 mg/kg; $P<.05$; symbols a and b) and a nonsignificant increase in 0.01 and 0.1 mg/kg towards vehicle (Fig. 2A).

During the 9-day feeding period, the 0.001 mg/kg $\Delta^8$-THC-treated mice consumed 44.2 g food, which was significantly higher than the consumption by the groups treated with either the 38.2-g vehicle or the 39.0-g $\Delta^8$-THC+SR141716A ($P<.001$, $P<.01$, respectively; Fig. 2B).

In the long-term experiment, the $\Delta^8$−THC-treated mice consumed significantly more food (63.4±4.3 g) than did the vehicle-treated mice (52±5.12 g; Fig. 2C).

### 3.3. Activity (Fig. 3A and B)

There was a significant increase in activity ($P<.05$) in the $\Delta^8$-THC-treated group compared with the vehicle-treated group in the long-term experiment, while in the short-term experiment, it showed tendency to increase, which was not reversed by the antagonist. Fig. 3.

### 3.4. Effect of $\Delta^8$-THC on neurotransmitters in the hypothalamus (Fig. 4)

In the hypothalamus of the $\Delta^8$-THC-treated mice, there was a significant decrease in dopamine ($P<.01$; Panel A) and a nonsignificant increase in NE (Panel B). Treatment with the CB1 antagonist did not reverse the dopamine levels, but an insignificant decrease in the changes of NE levels was observed. In the $\Delta^8$-THC treated mice, there was

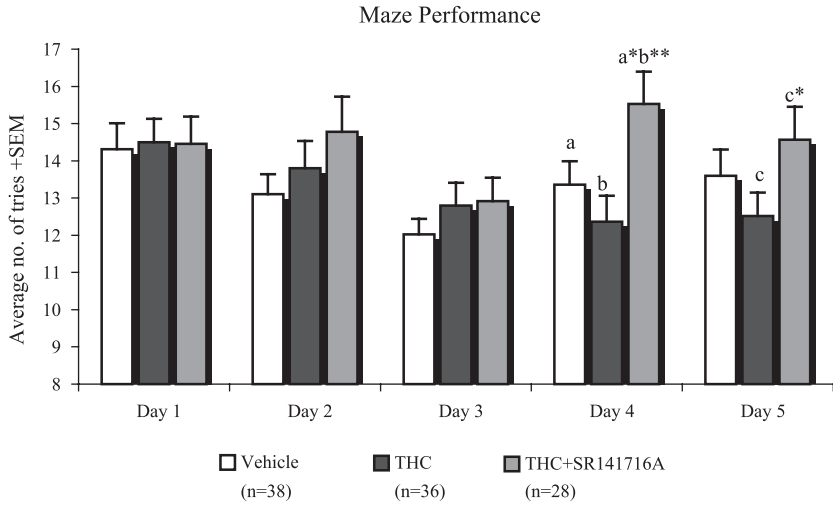


Fig. 6. Effect of $\Delta^8$-THC on maze performance. Maze testing lasted for five consecutive days and is presented as the average number of entries in each group (vehicle, $\Delta^8$-THC, $\Delta^8$-THC+SR141716A). *$P<.05$ and **$P<.01$. Letters represent pairs that are statistically different (vehicle □, THC ■, THC+SR141716A ▨).There are 28–38 mice in each group.

a significant decrease in 5-HT levels ($P<.01$). The CB1 antagonist reversed the level of $\Delta^8$-THC to 130.5 ($P<.05$; data not presented). Fig. 4.

### 3.5. Effect of $\Delta^8$-THC on neurotransmitters in the hippocampus (Fig. 5)

In the $\Delta^8$-THC-treated mice, there was a significant decrease in dopamine levels ($P<.01$; Panel A) and a small increase in NE (Panel B). CB1 antagonist treatment reversed the effect of $\Delta^8$-THC in the hippocampus: an increase of dopamine to 156.9% ($P<.001$) and a decrease of NE to 92.4%. In the $\Delta^8$-THC-treated mice, there was a significant decrease in 5-HT in the hippocampus from 100% to 90.75% ($P<.05$; Panel C). The CB1 antagonist reversed the effect of $\Delta^8$-THC in the hippocampus and enhanced the level up to 200.9% ($P<.05$). Fig. 5.

### 3.6. Maze performance (Fig. 6)

Maze performance was evaluated by the number of entries used to complete the eight arms of the maze. The lower the number of entries, the better the performance. The performance of the $\Delta^8$-THC treated mice on Day 4 was almost significant ($P=.06$) in comparison with the vehicle group. On the same day, the $\Delta^8$-THC+SR141716A group performed poorly in the maze, both in comparison with the $\Delta^8$-THC and the vehicle groups ($P<.01$ and $P<.05$, respectively). A similar result was found on the fifth day, compared with the THC group ($P<.05$). These results indicate that the cannabinoid might improve performance, while the antagonist significantly impairs it. Fig. 6.

### 3.7. $\Delta^8$-THC versus $\Delta^9$-THC (Fig. 7, Table 1)

In an experiment that examined the effects of both substances in means of food consumption, maze behavior and activity, we found that the average body weight of vehicle, $\Delta^8$-THC with and without antagonist and $\Delta^9$-THC was about the same during the experiment, but $\Delta^8$-THC increased food consumption more then $\Delta^9$-THC did ($P<.05$), while activity and performance were the same. Fig. 7, Table 1.

Table 1
The effects of $\Delta^8$- and $\Delta^9$-THC on food consumption, activity and cognitive function

| | $\Delta^8$-THC | $\Delta^9$-THC |
|---|---|---|
| Food consumption (g±S.E.M.) | 97.10±0.54a (10) | 95.12±0.66a* (10) |
| Activity (mean no. of crossing/mouse/min) | 47.7±1.24 (10) | 50.47±2.36 (10) |
| Performance entries—AUC (area under the curve) | 0.33±0.50 (10) | −0.28±0.42 (10) |

Food consumption was evaluated as the sum of food consumed during the experiment, activity as the mean no. of crossing/mouse/min, and performance in the eight-arm maze was calculated by the area under the curve (AUC).
* $P<0.05$.

## 4. Discussion

The main findings of this study were that a very low dose of $\Delta^8$-THC (0.001 mg/kg) significantly increased food consumption, without the elevation of body weight, and decreased dopamine and 5-HT levels in the hypothalamus and the hippocampus, and showed tendency to increase NE levels in both the hypothalamus and hippocampus.

A nonsignificant tendency in an improvement of cognitive function was noted, which was significantly reversed by the CB1 antagonist. $\Delta^8$-THC increased food consumption more then $\Delta^9$-THC did ($P<.05$), while activity and performance were the same.

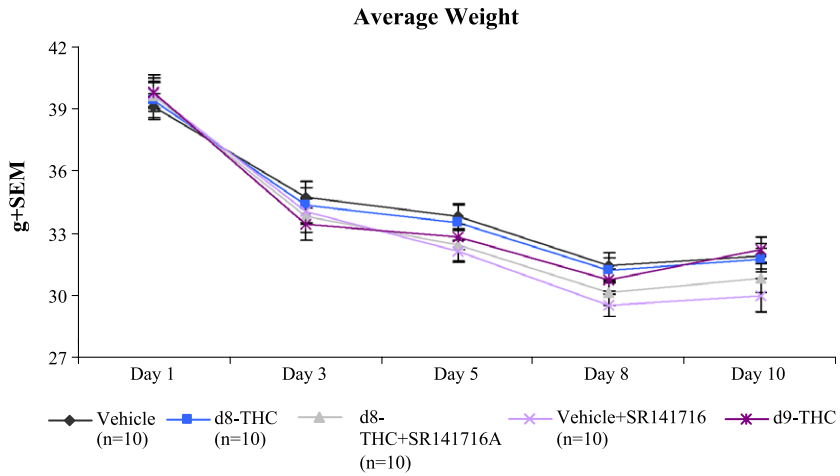


Fig. 7. The effect of DR and $\Delta^8$-THC with and without antagonist and $\Delta^9$-THC on mean body weight (vehicle ◆, THC ■, THC+SR141716A △ vehicle+SR141716A X, $\Delta^9$-THC *). There are 10 mice each group.

### 4.1. Dosage for effects on food intake

We have found that the effect of THC on food intake was biphasic and that both 0.001 and 1 mg/kg THC significantly increased food intake, while intermediate doses of 0.1 and 0.01 mg/kg did not cause any significant changes. Such biphasic responses have been observed previously (Poddar and Dewey, 1980) and are perhaps related to a differential involvement of a Gs and Gi protein, activated at low and high doses, respectively, or to an allosteric modulation of the cannabinoid, and to an activation of presynaptic cannabinoid receptors by low doses of the cannabinoid (Sulcova et al., 1998), suggesting effects on satiety as a mirror image to Fig. 1A. Therefore, to prevent possible cannabimimetic side effects of THC, we chose, for these experiments, the lower dose of THC—0.001 mg/kg. Although cannabis is widely used as recreational drug in humans, only a few studies have studied the orexigenic (appetite) potential of cannabinoid agents in animals (Williams et al., 1998; Williams and Kirkham, 1999; Chaperon and Thiebot, 1999; Kirkham and Williams, 2001). Leptin regulates endocannabinoids in maintaining food intake (Di Marzo et al., 2001), and there is a hypersensitization of the orexin 1 receptor by the CB1 receptor, which is blocked by the CB1 antagonist (Hilairet et al., 2003).

In the THC group, there was a significant increase in food intake compared with the vehicle and the CB1 antagonist treatments. However, despite the significant increase in food consumption, no differences in body weight were observed during the short schedule (9 days) of the experiments. A significant increase in food intake was also found in the long-term (50 days) experiment, again, without differences in body weight. We assume that the absence of increased weight in the treated group may have been due to the significant enhancement ($P<.05$) of the activity observed. These results can explain the discrepancy between food consumption and weight. The activity experiment was done also in the presence of an antagonist; however, there was no significant difference between the THC group and THC+CB1 antagonist group. It is possible that the effect of THC on activity is not CB1 mediated and that other receptors or mechanisms may be involved.

Low doses of $\Delta^9$-THC could stimulate motor activity, as indicated by hyperlocomotion, and induce ipsilateral turning behavior in rats with unilateral 6-hydroxydopamine lesion of the substantia nigra (Sakurai et al., 1985). Chaperon and Thiebot (1999) have also recorded that cannabinoid agonists, such as $\Delta^9$-THC, may induce hyperactivity at low doses.

Microdialysis studies have shown that THC facilitates dopamine release in the striatum and enhances the firing of dopaminergic neurons in the substantia nigra (Ng Cheong Ton et al., 1988). The hyperactivity is mediated by the activation of the nigrostriatal dopamine pathways, presumably through the stimulation of CB1 receptors. These results indicate that cannabinoids may differentially affect the activity of the extrapyramidal dopaminergic system depending on the dose and the ongoing activation of the different elements of the basal ganglia. Rodriguez de Fonseca et al. (1998) have proposed that endocannabinoids could function as local regulators of neurotransmission processes within the basal ganglia.

The CB1 antagonist (SR141716A) decreased food intake in mice by antagonizing the putative endogenous cannabinoid tone that normally stimulates feeding (Arnone et al., 1997; Chaperon et al., 1998; Colombo et al., 1998). The increase in food intake was associated with the changes in neurotransmitter concentrations—a decrease in both dopamine and 5-HT in the hypothalamus and a nonsignificant increase in dopamine turnover. Changes in transmitter levels may indicate some aspects of functional activity. The situation is complex, especially because of the nonsignificant difference in turnover. Thus, the differences in concentration may act by affecting the relative balance or tone between neurotransmitters (NE, dopamine) and modulators (endocannabinoids). Preliminary measurements of both NE and dopamine turnover have been performed, but they were not significantly different because of higher S.D. and, therefore, were not presented. While the physiological role of cannabinoids on feeding behavior is not known, these results are compatible with an effect of stimulating appetite or inhibiting satiety. These neurotransmitters (NE and 5-HT) are manipulated in the opposite direction to promote weight loss in the treatment of obesity (James et al., 2000), as are also cannabinoid antagonists (Le Fur et al., 2001). It has been reported that neurons in the solitary tract nucleus, which respond to increases in glucose concentrations, are sensitive to THC. Himmi et al., 1996 have suggested that such neurons may mediate cannabinoid effects on feeding behavior.

We have found that the THC administration caused significant increase in food consumption; however, possibly due to increased activity, it was not expressed in the weight. AN patients are not necessarily highly active, and their activity could be controlled (for example, there are clinics which hold low-weight girls during treatment on wheelchair).

### 4.2. Behavior

Several observations can explain the tendency ($P<.06$) for improved performance results after 0.001 mg/kg THC.

There are several pathways that affect learning and memory abilities, which can be changed by nutritional state (Avraham et al., 1996; Wurtman et al., 1981; Schweiger et al., 1985a,b; Philipp and Pirke, 1987; Collier et al., 1988; Haleem and Haider, 1996). We have found that THC-treated mice performed better compared with the vehicle- and antagonist-treated groups. The hippocampus is the brain area with the largest concentration of cannabinoid receptors (Di Marzo, 1998, Di Marzo et al., 1998). Cannabinoids may affect memory directly (Lichtman et al., 1995) or as neuro-

modulators (Kimura et al., 1998). The serotonergic system plays an important role in learning and memory: It was found that the use of 5-HT1B receptor antagonist impaired memory and learning (Buhot et al., 2000). In addition to the decrease in 5-HT, there was an increase in NE in the hippocampus. NE has a role in memory improvement (Friedman et al., 1999), and we have reported that following tyrosine injections, there was an increase in NE in the hippocampus and an improvement in cognitive function (Avraham et al., 1996). Thus, both a decrease in 5-HT and an increase in NE levels could explain the behavioral improvement.

### 4.3. Neurotransmitters

DR caused changes in the neurotransmitter levels in the hypothalamus and hippocampus. These changes were reversed by THC treatment. The most significant changes were in NE, dopamine and 5-HT. The neurotransmitters level decreased both in the hypothalamus and in the hippocampus (except the NE level). Measurements of both NE and dopamine turnover have been done in this study; however, they were not significant and, therefore, were not presented. The decreased level of 5-HT and the tendency to increase the levels of NE in the hypothalamus after the administration of THC, to increase food consumption, may have caused the increase in food intake.

Bowers and Morton (1994) showed that THC administration to rats increased DOPAC in the olfactory tubercle and prefrontal cortex, without affecting dopamine levels, suggesting increased dopamine release, while the effects on NE and MHPG were not evident. Navarro et al. (1993a) fed male rats with $\Delta^9$-THC and showed that there were no changes in dopamine, DOPAC or tyrosine hydroxylase in the striatum. Navarro et al. (1993b) treated male rats with oral THC and showed an increase in DOPAC and its turnover in the limbic forebrain. Bloom (1982) found that THC affected tyrosine uptake and the conversion of tyrosine to dopamine and NE in synaptosomes isolated from the whole brain or the corpus striatum. His conclusion was that the decrease in the precursor uptake might be the cause of the decrease in NE and dopamine synthesis that was found after high THC dosages. He suggested that $\Delta^9$-THC might have a direct effect upon catecholamine-containing neurons in the brain. However, the concentrations used in this trial were higher than in ours and, in addition, the brain areas that were examined were different, hence, it is difficult to compare these experiments with this study. When the effect of THC was examined on catecholamines in the hypothalamus (Poddar and Dewey, 1980), it was found that the dopamine and NE uptake was stimulated in low doses of THC ($1\times10^{-7}$ and $2\times10^{-7}$ M), while in high doses ($1\times10^{-5}$ and $1\times10^{-4}$ M), both $\Delta^9$-THC and its $\Delta^8$ isomer inhibited the uptake of dopamine and NE. These results demonstrate that both $\Delta^9$- and $\Delta^8$-THC produce a biphasic effect on the uptake and release of dopamine and NE in the corpus striatum and hypothalamic regions of brain, and that $\Delta^8$-THC is more potent than $\Delta^9$-THC on both the uptake and release of dopamine and NE. It is seen from these results that THC has a biphasic effect on the neurotransmitter uptake. In conclusion, the effects of THC on neurotransmitters depend on many factors including the dosage, route of administration, brain areas and, probably, also the animal strain under study.

### 4.4. $\Delta^8$-THC versus $\Delta^9$-THC

As was mentioned in the Methods section, we used $\Delta^8$-THC rather than $\Delta^9$-THC because the latter is easily oxidized to cannabinol by air, while $\Delta^8$-THC is more stable, and its synthesis is much more simple. Our results supported this observation because $\Delta^8$-THC increased food consumption more than did $\Delta^9$-THC ($P<.05$), while weight curve, activity and performance were about the same. Poddar and Dewey (1980) claimed that $\Delta^8$-THC is more potent than $\Delta^9$-THC on both uptake and release of dopamine and NE.

Thus, $\Delta^8$-THC is much more recommended for treatment of eating disorders.

### 4.5. Comparison between THC and ANA

Although both substances bind to the cannabinoid receptors, they come from different origins: THC is derived from plant sources while ANA is formed endogenously in the brain from arachidonic acid. Both increased food intake: ANA by 44% (Hao et al., 2000), THC by 16%. Between concentrations of 0.001, 0.7 and 4 mg/kg, only 0.001 mg/kg ANA increased food intake by the same degree, while between 0.001, 0.01, 0.1 and 1 mg/kg, only 0.001 and 1 mg/kg THC increased food intake to the same level. ANA increased catecholamines in both the hippocampus and the hypothalamus and increased 5-HT in the hypothalamus but decreased it in the hippocampus. THC caused the opposite effects: It decreased dopamine and 5-HT in both the hypothalamus and hippocampus and showed tendency to increase NE in both areas. Despite these opposite effects on catecholamines and 5-HT, they both improved maze performance. These contradicting effects may be related to the differences in experimental design and feeding protocols. In the ANA study, we used 40% DR, and the time of feeding was for 24 h, while in this article, we used a 2.5-h feeding regimen, during which the animals ate the equivalent to weight-maintaining (100%) requirements, and yet, still lost weight. They lost weight because they where transferred from ad libitum to 2.5-h feeding to facilitate measurement of food consumption. In addition, food administration was different: dry food as opposed to wet food, which contained gelatin. These different schedules might be expected to have very different effects on neurotransmitters. THC is known to be an inhibitor of tyrosine hydroxylase, while ANA can act as an inducer (Romero et al., 1995a,b; Walters and Carr, 1988). Tyrosine hydroxylase activity was significantly de-

creased in offsprings exposed to $\Delta^8$-THC. Patel et al. (1985) showed that $\Delta^9$-THC (3 mg/kg) administered to male rats decreased significantly in the plasma and mediobasal hypothalamus (MBH) levels of NE, E, and significantly increased in the MBH levels of dopamine and DOPAC. Dalterio et al. (1985) repeated the oral administration of cannabinol: 5 or 50 mg/kg significantly reduced the concentration of NE in the median eminence.

Another possibility might be that ANA is an endocannabinoid, and by injecting it to the mice, its level increases in a more significant way to affect the neurotransmitter levels. The administration of THC may have caused competition with the endogenous ANA, thereby reducing neurotransmitter production. Most of the effects caused by THC were reversed by the CB1 antagonist, while the effect of the antagonist on ANA effects was not investigated and remains to be tested.

In conclusion, a very low dose of $\Delta^8$-THC (0.001 mg/kg) caused increased food consumption and tendency to improve cognitive function, without cannabinomimetic side effects. Thus, low dose THC might be a potential therapeutic agent in the treatment of cachexia, in general, and AN, in particular.

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