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ORIGINAL INVESTIGATION

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Self-administration of Δ^9-tetrahydrocannabinol (THC) by drug naive squirrel monkeys

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Abstract *Rationale:* Interest in therapeutic activities of cannabinoids has been restrained by the fact that they are most often mediated through activation of cannabinoid CB_1 receptors, the same receptors that mediate the effects of Δ^9-tetrahydrocannabinol (THC) and are responsible for the abuse liability of marijuana. Persistent intravenous self-administration of THC by animals was first demonstrated in squirrel monkeys and shown to be mediated by CB_1 receptors, but monkeys in the study had a history of cocaine self-administration, raising the possibility that persistent neurobiological adaptations might subsequently predispose animals to self-administer THC. *Objectives:* To demonstrate persistent intravenous self-administration of THC in drug-naive squirrel monkeys. *Methods:* Monkeys with no history of exposure to other drugs learned to press a lever for intravenous injections (0.2 ml in 0.2 s) of THC under a 10-response, fixed-ratio schedule with a 60-s time-out after each injection. Acquisition of THC self-administration was rapid and the final schedule was reached in 11–34 sessions. Dose of THC was then varied from 1 to 16 μg/kg per injection with vehicle extinction following each dose of THC. *Results:* THC maintained significantly higher numbers of self-administered injections per session and higher rates of responding than vehicle at doses of 2, 4 and 8 μg/kg per injection, with maximal rates of responding at 4 μg/kg per injection. Response rates, injections per session and total THC intake per session were two- to three-fold greater in monkeys with no history of exposure to other drugs compared to previous findings in monkeys with a history of cocaine self-administration. *Conclusions:* THC can act as an effective reinforcer of drug-taking behavior in monkeys with no history of exposure to other drugs, suggesting that self-administration of THC by monkeys provides a reliable animal model of human marijuana abuse.

Keywords Cannabis · Δ^9 · -Tetrahydrocannabinol (THC) · Drug self-administration · Squirrel monkeys · Marijuana

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Introduction

A growing number of studies have shown that the endocannabinoid system is involved in many different physiological functions in the brain and in peripheral tissues (Wilson and Nicoll 2002). Consequently, endogenous, natural or synthetic cannabinoids acting directly on cannabinoid receptors or compounds that alter the function of the endogenous cannabinoid system (e.g. cannabinoid antagonists or blockers of endocannabinoid uptake or degradation) might possess therapeutic efficacy (Piomelli et al. 2000; Wilson and Nicoll 2002; Kathuria et al. 2003). Interest in the therapeutic activity of cannabinoids has been restrained, however, by a potentially harmful side effect, abuse liability, of Δ^9-tetrahydrocannabinol (THC), the active principle of *Cannabis sativa* (marijuana) (Gaoni and Mechoulam 1964). Unfortunately, most of the therapeutic activities attributed to cannabinoids are mediated through activation of CB_1 receptors (Devane et al. 1988; Matsuda et al. 1990), the same receptors that mediate the reinforcing effects of THC are responsible for the abuse liability of marijuana (Tanda et al. 1997, 2000). The possibility of finding a drug that shares the therapeutic efficacy of cannabinoids but avoids the

potential for abuse would be greatly facilitated by development of animal models for human marijuana abuse.

Animal self-administration behavior is the most direct method for studying the reinforcing properties of drugs and for predicting their abuse potential (for review, see Balster 1991). Past attempts to demonstrate intravenous self-administration of THC or of synthetic cannabinoid CB_1 receptor agonists by experimental animals were relatively unsuccessful (e.g. Harris et al. 1974; Mansbach et al. 1994; see review by Tanda and Goldberg 2003). Recently, there have been positive reports of intravenous self-administration of the synthetic CB_1 cannabinoid receptor agonists, WIN 55,212-2 (Martellotta et al. 1998), CP 55,940 and HU-210 (Navarro et al. 2001) in mice and of WIN 55,212-2 in rats (Fattore et al. 2001), but the experimental procedures employed in each of these studies limit the generality of the findings. For example, the studies with mice (Martellotta et al. 1998; Ledent et al. 1999; Navarro et al. 2001) employed 1-day experimental tests during which mice were severely restrained for acute intravenous administration through the tail vein. This procedure provides little, if any, information about acquisition, extinction and relapse to self-administration behavior. Also, self-administration of cannabinoids may have been maintained due to their known analgesic activity resulting in reductions in levels of pain or stress (Martin and Lichtman 1998; Kathuria et al. 2003) produced by restraint, rather than to direct positive reinforcement effects. The study by Fattore et al. (2001) did utilize unrestrained, freely moving rats as subjects and they were allowed to self-administer WIN 55,212-2 over repeated daily sessions. However, rats in their study were initially deprived of food for 1 day and subsequently their daily food ration (given as a single meal after the session) was restricted to maintain them at approximately 80% of normal body weight. Diet restriction has been repeatedly shown to increase a wide variety of appetitive behaviors, including self-administration of drugs from each of the major classes of abused drugs (see review by Carroll and Meisch 1984). For example, in one early series of studies (Takahashi and Singer 1979, 1980), THC self-administration behavior above placebo levels was found in diet-restricted rats (maintained at 80% of normal body weight), under conditions where a food pellet was automatically delivered every 1 min, but this self-administration behavior immediately decreased to placebo levels when food restriction was discontinued.

Persistent intravenous self-administration of THC itself was first demonstrated in squirrel monkeys by Tanda et al (2000). The study utilized monkeys with cocaine self-administration experience that were not food deprived and had access to THC only after at least 1 week of saline extinction. The dose-range of IV THC in this study was lower than that previously used in other THC self-administration studies and comparable to that contained in a single puff of a marijuana cigarette (Agurell et al. 1986; Tanda et al. 2000). Under these conditions, monkeys rapidly acquired THC self-administration behavior. Once acquired, self-administration behavior could be rapidly extinguished by substituting vehicle for THC or by administering the cannabinoid CB_1 receptor antagonist, SR 141716A, suggesting the behavior was mediated by CB_1 cannabinoid receptors. However, monkeys in the Tanda et al. (2000) study had a history of cocaine self-administration, raising the possibility that persistent neurobiological adaptations might subsequently predispose animals to self-administer THC (e.g. Maldonado 2002). Nonetheless, earlier attempts to obtain THC self-administration behavior in monkeys with a previous cocaine self-administration experience had been unsuccessful, even when THC was directly substituted for cocaine with no intervening vehicle extinction (Harris et al. 1974).

In the present report, squirrel monkeys that were experimentally and drug naive at the start of the study were subjects. As in the previous study by Tanda et al. (2000), low clinically relevant doses of THC were employed and THC was dissolved in a Tween-80, saline vehicle to produce a clear solution. Under these conditions, THC self-administration behavior was initiated and subsequently maintained at very high rates in monkeys with no history of exposure to other drugs.

Materials and methods

Subjects

Three adult male squirrel monkeys (*Saimiri sciureus*; 6754, 67D2, 67F4) weighing 0.9–1.1 kg were housed in individual cages in a temperature- and humidity-controlled room with unrestricted access to water. Monkeys were fed (approximately 2 h after the session) a daily food ration consisting of five biscuits of high protein monkey diet (Lab Diet 5045, PMI Nutrition International, Richmond, Ind., USA) and two pieces of Banana Softies (Bio-Serv, Frenchtown, N.J., USA) that maintained their body weights at a constant level throughout the course of the study. Fresh fruits or vegetables and environmental enrichment were provided daily. At the start of the present study all three monkeys were experimentally naive. The monkeys were surgically prepared with a chronic indwelling venous catheter (polyvinyl chloride), as described previously (Goldberg 1973), and wore nylon-mesh jackets (Lomir Biomedical, Canada) to protect the catheters.

The monkeys were maintained in facilities fully accredited by AAALAC and all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Intramural Research Program, NIDA, NIH, and the Guide for Care and Use of Laboratory Animals (National Research Council 1996).

Apparatus

The sound-attenuating isolation chambers (Model AC-3, Industrial Acoustics Co., Bronx, N.Y., USA) were equipped with a Plexiglas chair with a white house light and white noise for masking of external sound. The chair contained a response lever (No. 121-05; BRS/LVE Corp., Laurel, Md., USA) mounted on a transparent front wall; each press of the lever with a force greater than 0.2 N produced an audible click and was recorded as a response. Pairs of green and amber stimulus lights, mounted behind the transparent wall of the chair, could be illuminated and used as visual stimuli. The monkey's catheter was connected to polyethylene tubing, which passed out of the isolation chamber where it was attached to

a motor-driven syringe pump (model No. 57-6496; Harvard Apparatus, South Natick, Mass., USA). Operation of the experimental chambers and data collection was controlled by IBM computers using the MED Associates MED-PC software package (East Fairfield, Vt., USA).

Procedures

Experimentally naive squirrel monkeys were first placed on a food-restricted diet until weight dropped to about 90% of levels maintained during normal feeding condition levels (see above) and they then learned to press a lever for food pellets (Goldberg 1973). Once responding for food was well maintained, sessions with food were stopped and monkeys were returned to normal feeding conditions for several weeks. Intravenous catheters were then surgically implanted (Goldberg 1973) and THC self-administration sessions began 2–3 weeks after catheter implantation.

During daily 1-h sessions, conducted Monday to Friday between 0800 and 1200 hours, monkeys sat inside the experimental chamber, restrained in the seated position by a waist lock on the Plexiglas chair, and IV injections (0.2 ml in 0.2 s) were delivered from the syringe pump outside the chamber (as described above). Before the start of each session, one priming injection was delivered (calculated to fill the dead space of the IV catheter). At the start of each session, the white-house light was turned off and the green stimulus lights were turned on; five lever presses turned off the green light and produced a 2-s amber light paired with IV injection of 4 μg/kg THC (based on our previous study in which maximal rates of responding were maintained by doses of 2–4 μg/kg per injection THC; Tanda et al. 2000). There was a 30-s time-out period after each injection, during which the chamber was dark and lever presses had no programmed consequences. As monkeys learned to press the lever for THC injections, the number of responses required to produce each injection was raised to 10 and the time-out duration was raised to 60 s (a final ten-response, fixed-ratio schedule of IV THC injection with a 60-s time-out duration: FR10, TO 60 s).

When responding for 4 μg/kg injections of THC was stable for at least five sessions (less than 15% variability), self-administration behavior was extinguished by replacing THC with its vehicle for five consecutive sessions. Next, a THC dose-effect curve was evaluated by giving monkeys the opportunity to self-administer a range of THC doses (1, 2, 4, 8 and 16 μg/kg per injection). Each THC dose was studied for five consecutive sessions and each dose-condition was separated by five consecutive sessions of vehicle extinction.

Drugs

Δ^9-Tetrahydrocannabinol (THC) was provided by the National Institute on Drug Abuse, NIH (Rockville, Md., USA). THC was dissolved in a vehicle containing 0.4–1.0% Tween 80 and 0.4–1.0% ethanol in 0.9% saline solution to produce a clear solution (modification of previously described procedure; Olsen et al. 1973).

Data analysis

Cumulative records were obtained during all sessions in order to visually inspect patterns of responding. Rates of responding during self-administration sessions are expressed as responses per second averaged over the session, with responding during timeouts not included in calculations. Injections per session represent total number of injections delivered per one-hour session. Data for dose-effect curves are expressed as mean response rates and numbers of injections±SEM over the last three sessions for each dose of THC (vehicle values are the average of the last three sessions of vehicle extinction after each of five consecutive doses of THC). In addition, total intake of each THC dose per 1-h session was calculated. Statistical analysis was done using single-factor repeated measures ANOVA to assess difference between vehicle condition and different doses of THC. Significant main effects were analyzed further by subsequent paired comparisons to control values using Dunnett's test. Changes were considered to be significant when $P<0.05$. SigmaStat program (Jandel Scientific, USA) was used.

Results

Acquisition of THC self-administration was relatively rapid. It took 11–34 sessions for monkeys to reach the final schedule of reinforcement (FR10, TO 60 s). Under the final FR10 schedule, injections of 4 μg/kg THC (Fig. 1) maintained very high response rates (0.88±0.21 response/s; all data are presented as mean±SEM) and nearly the maximal possible number of injections per session was self-administered (48.89±2.61 injections/1-h session). When vehicle was substituted for THC, the number of injections self-administered per session decreased dramatically as compared with the last session with THC [$F(5,10)= 9.749$, $P=0.001$, one-way ANOVA for repeated measures main effect]. Self-administration of THC (4 μg/kg per injection) was immediately reacquired after vehicle extinction [$F(5,10)=24.866$, $P<0.001$].

Varying the THC dose per injection resulted in an inverted U-shaped dose-effect curve (Fig. 2a and b), as reported previously (Tanda et al. 2000). THC maintained significantly higher numbers of injections self-administered per session [$F(5,9)=12.563$, $P<0.001$; Fig. 2a] and higher rates of responding [$F(5,9)=8.101$, $P=0.004$; Fig. 2b] than vehicle at doses of 2, 4 and 8 μg/kg per injection. Maximal rates of responding were maintained at a dose of 4 μg/kg per injection, as revealed by post-hoc comparisons with vehicle self-administration levels after a significant one-way ANOVA for repeated measures main effect ($P<0.01$, Dunnett's test). Increasing the THC dose from 4 to 8 or 16 μg/kg per injection resulted in

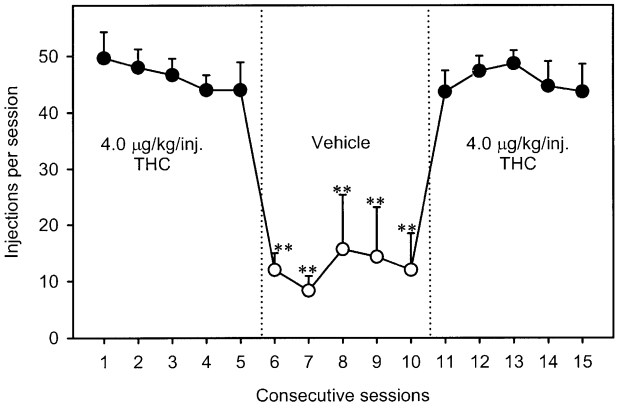

Fig. 1 Maintenance, extinction and reacquisition of self-administration behavior over consecutive sessions. Numbers of injections per session during THC (4 μg/kg per injection) and vehicle self-administration sessions are presented. *Symbols* represent the means (±SEM) of injections per session from three squirrel monkeys. $^{**}P<0.01$, post-hoc comparisons with the last THC session prior to vehicle extinction after significant one-way ANOVA for repeated measures main effect, Dunnett's test

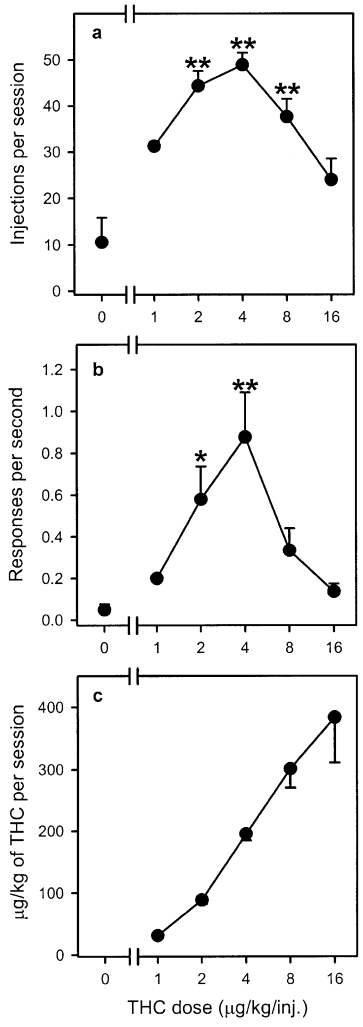

Fig. 2a–c THC dose-response curves in squirrel monkeys with no history of exposure to other drugs (n=3). Numbers of injections per session (**a**), overall rates of responding in the presence of a green light signalling THC availability (**b**) and total THC intake per session (**c**) are presented as a function of injection dose of THC. Each *symbol* represents the mean (±SEM) of the last three sessions under each THC injection dose condition and under a vehicle condition from three monkeys, with exception of the values for the 1 µg/kg per injection dose, which represent means from two monkeys. *P<0.05, **P<0.01 post-hoc comparisons with the vehicle conditions after significant one-way ANOVA for repeated measures main effect, Dunnett's test

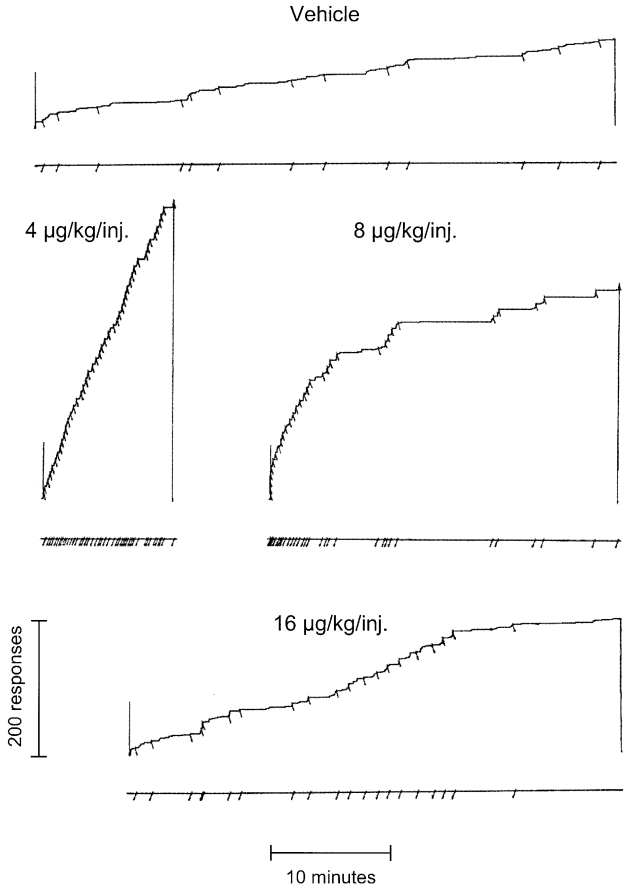

Fig. 3 Performance of a monkey (67D2) under the FR10 schedule of THC self-administration at different injection doses of THC. *Abscissae*: time; *ordinates*: cumulative lever-pressing responses. Short *diagonal marks* on the cumulative records indicate drug injections. After each injection of THC, there was a 60-s time-out during which the recorder did not operate. The length of each session including time-outs was 1 h

marked dose-related decreases in number of injections per session (Fig. 2a) and response rates (Fig. 2b), while total THC intake per session increased by 50% and 95%, respectively (Fig. 2c).

Patterns of responding with monkey 67D2 were representative of responding for THC injections in the other subjects under the same conditions (Fig. 3). At lower THC doses (2 and 4 µg/kg per injection) stable response rates were maintained throughout the session. Increasing the dose to 8 and 16 µg/kg per infusion resulted in lower response rates, particularly toward the end of the session. Rates of responding during vehicle substitution were very low throughout the session.

Discussion

The present study shows for the first time that low clinically relevant doses of THC, comparable to those in marijuana smoke inhaled by humans (Agurell et al. 1986), can initiate and sustain very high rates of intravenous self-administration behavior in animals without a history of exposure to other drugs. Under the present fixed-ratio schedule of intravenous drug self-administration, the optimum rates of responding maintained by THC were similar to or greater than the optimum rates of responding maintained by intravenous injections of cocaine, *d*-amphetamine, nicotine, methohexital or midazolam in previous studies using the same primate species and the same schedule of intravenous drug injection (Goldberg 1973; Spear et al. 1991; Sannerud et al. 1994; Munzar et al. 2001). Vehicle substitution for THC immediately

decreased self-administration behavior to very low levels, but self-administration responding was rapidly reinstated when THC was again made available. The present findings extend previous findings by Tanda et al. (2000) in cocaine-trained monkeys, by demonstrating that THC can serve as an effective reinforcer of drug-taking behavior in drug-naive as well as drug-experienced non-human primates, as it does in humans.

The present study also shows that the fixed-ratio patterns of responding maintained by THC injection are similar to patterns of responding for cocaine, amphetamine or nicotine injections or for food presentation previously found using the same schedule of reinforcement in squirrel monkeys (Goldberg 1973; Sannerud et al. 1994). Also, changes in injection dose of THC produced changes in the within-session distribution of responses similar to those previously reported with food or other drugs. Thus, in the presence of the green light signaling drug availability, a short pause was followed by steady, high-rate responding that culminated in drug injection and at the 4 μg/kg injection dose of THC regular responding at rates approaching one response/s was maintained throughout the session. When the injection dose of THC was increased from 4 to 8 μg/kg, monkeys showed a "satiation-like" effect (e.g. Sidman and Stebbins 1954), similar to that previously described in squirrel monkeys responding for cocaine or amphetamine injection or for food presentation under the same fixed-ratio schedule (Goldberg 1973). The monkeys responded at a high rate at the beginning of each session, but, as the session progressed, rates of responding decreased.

When injection dose of THC was increased to 16 μg/kg, 4-fold higher than the 4 μg/kg injection dose that maintained peak responding, rates of responding and numbers of injections per session decreased markedly but they still remained above vehicle substitution levels and the monkeys' total intake of THC per session continued to increase. Thus, there was no indication that higher THC doses and higher THC intake resulted in aversive effects of THC, as might be expected from previous findings from conditioned place-preference studies. In place-preference studies, the development of preferences or aversions for a compartment associated with previous THC injections is highly dose-dependent, with positive place preferences, when they do occur, usually being restricted to a single dose and increases in THC dose often resulting in conditioned place aversions (e.g. Lepore et al. 1995; Sanudo-Pena et al. 1997; Cheer et al. 2000; Valjent and Maldonado 2000; see review by Tanda and Goldberg 2003). In contrast, in the present study there was no indication that marked increases in injection dose or total session intake of THC resulted in suppression of responding below vehicle levels that would indicate the appearance of aversive (punishing) effects, as has been seen with other non-abused, psychoactive drugs such as chlorpromazine (e.g. Hoffmeister and Goldberg 1973).

Overall response rates, injections per session and total THC intake per session in the present study were 2- to 3-fold greater in monkeys with no history of exposure to other drugs compared to previous findings in monkeys with a history of cocaine self-administration. There were no obvious differences in age, source or laboratory conditions with monkeys in the present study and those in the previous study by Tanda et al. (2000). At the 4 μg/kg per injection dose of THC in the present study, mean rate of responding was 0.88±0.21 response/s, with a mean of 48.89±2.61 injections/session and a total session intake of THC of 195.6±10.45 μg/kg. Compare this to mean values of 0.22±0.07 response/sec, 29.92±5.32 injections/session and 119.67±21.27 μg/kg per session in the previous study by Tanda et al. (2000) in which monkeys had a history of cocaine self-administration. Rather than predisposing animals to self-administer THC (Maldonado 2002), a history of cocaine self-administration appeared to limit the intensity of subsequent THC self-administration behavior.

This limiting effect of contrasting drug history on subsequent drug self-administration has been previously noted with drugs from other pharmacological classes (Young and Herling 1986). For example, the antitussive agent dextrorphan maintained self-administration behavior by monkeys above vehicle levels when substituted for ketamine but not when substituted for codeine (Young and Woods 1981). In another study, diazepam maintained self-administration behavior by monkeys when substituted for pentobarbital but not when substituted for cocaine (Bergman and Johanson 1985). Ketamine and dextrorphan have been reported to share some discriminative-stimulus effects (Holtzman 1980; Herling et al. 1981), as have diazepam and pentobarbital (Colpaert et al. 1976; Shannon and Herling 1983), and this may serve as a mechanism for the observed effects. However, attempts to obtain THC self-administration by directly substituting THC in animals actively self-administering drugs thought to share stimulus characteristics with THC, such as phencyclidine phenobarbital or ethanol, have generally been unsuccessful (Harris et al. 1974; Mansbach et al. 1994).

The present preclinical findings with squirrel monkeys support previous conclusions that cannabis derivatives have a pronounced abuse liability comparable to other drugs of abuse. Moreover, the ability of THC to maintain drug-taking behavior in monkeys without a history of exposure to other drugs shows that this drug possesses reinforcing properties of its own that are not dependent on prior self-administration of other drugs. Thus, self-administration of THC by squirrel monkeys provides a reliable animal model of human marijuana abuse, suitable for studying the relative abuse liability of other natural and synthetic cannabinoids and for developing new therapeutic strategies for the treatment or prevention of marijuana abuse in humans.

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