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Cannabinoid receptors and reward in the rat: a conditioned place preference study

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Abstract *Rationale:* We wished to investigate further the hypothesis of an endogenous cannabinoid 'aversive counter-rewarding system', as the rewarding properties of cannabinoids using standard procedures remain ambiguous. *Objectives:* The purpose of this study was to confirm the behavioural effects of a highly potent synthetic cannabinoid agonist (HU210) and the selective cannabinoid antagonist SR141716A using conditioned place preference (CPP). *Methods:* HU210 (20, 60 and 100 $\mu\text{g kg}^{-1}$), SR141716A (0.25, 0.5, 2 and 3 mg kg^{-1}), cocaine (15 mg kg^{-1}) and Δ^9 -THC (1.5 mg kg^{-1}) were given to male Lister hooded rats using an unbiased CPP design. *Results:* SR141716A and cocaine produced place preference at all doses tested, whereas HU210 and Δ^9 -THC produced aversion as expressed by time spent in the drug-paired compartment of the CPP apparatus. *Conclusions:* The aversive effects of cannabinoid agonists and the rewarding effect of the cannabinoid antagonist are suggestive of a cannabinergic tone in the rat brain. Further research is needed to determine the precise relationship of that tone with the reward pathways of the brain.

Key words HU210 · SR141716A · Conditioned place preference · Lister hooded rat · Cannabinoid

Introduction

Marijuana is one of the oldest drugs of abuse known to man, and the past decade has seen significant advances in our knowledge and understanding of the way marijuana exerts its effects via its primary psychoactive component – Δ^9 -tetrahydrocannabinol or Δ^9 -THC (Mechoulam et al. 1970). It is now evident that an endogenous cannabinoid signalling system exists based mainly on the dis-

covery of cannabinoid receptors (Matsuda et al. 1990; Munro et al. 1993) and endogenous cannabinoid ligands (Devane et al. 1992). Moreover, several synthetic cannabinoids (such as the analogue HU210; Mechoulam et al. 1988) have been designed that have higher affinities for cannabinoid receptors. These compounds can be used to assess the possible therapeutic potential of cannabinoids and to identify unwanted side effects stemming from the abuse profile of cannabinoids. Abuse potential and liability of a drug can be tested using a variety of behavioural tasks, including the conditioned place preference (CPP) paradigm.

Habit-forming drugs are believed to interact with motivational drive in animals by an action on certain brain areas collectively known as the brain reward pathways. The circuitry of the system consists of synaptically interconnected tracts closely associated with the median forebrain bundle (MFB). The originating neurons in this pathway are located posteriorly within the MFB and then synapse onto mesolimbic dopaminergic neurons in the ventral tegmental area (VTA). From there, dopamine neurons project anteriorly to the forebrain at the level of the nucleus accumbens. The pathway then reaches its terminal areas diffusely within the prefrontal cortex (Cox and Werling 1991).

In human users, marijuana can produce opposite effects depending both on environmental cues (social setting) at the time of administration and on the concentration of the psychoactive ingredient itself. Thus, marijuana is known to produce euphoria (the 'high' profile), but dysphoria, dizziness and anxiety are also elicited (the 'stoned' profile; Zuardi et al. 1982; Hollister 1986; Fishman et al. 1988). As expected, results from rodent studies support the notion that cannabinoids appear atypical as drugs of abuse. Cannabinoid agonists fail to lower the threshold for electrical self-stimulation (Stark and Dews 1980; but see Gardner et al. 1988a, 1988b) and only one study reports self-administration with mice (Martelotta et al. 1998). They also produce conditioned place and taste aversion (while the antagonist SR141716A produces place preference) and can have

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anxiogenic properties as assessed by the elevated plus maze (McGregor et al. 1995; Sañudo-Peña et al. 1997; Mallet and Beninger 1998; Onaivi et al. 1990).

In vivo evidence of a reinforcing effect of cannabinoids derives from VT. An extracellular single-unit recordings, as neurons increase their firing rate when rats are given cannabinoid agonists (French et al. 1997). In order to assess the in vivo hedonic effects of the cannabinoid agonist HU210 (Mechoulam et al. 1988), the present study used the CPP paradigm. We have also examined the reinforcing properties of the competitive cannabinoid receptor antagonist SR141716A to test whether endogenous cannabinoids are indeed part of an internal 'aversive counter-rewarding system' (Sañudo-Peña et al. 1997). Finally, we have studied several somatic signs of SR141716A-induced withdrawal from HU210 as a control measure to test whether the chosen dose regime was indeed having a centrally mediated dependence effect.

Methods

Subjects

Male Lister Hooded rats ($n=83$; Biomedical Services Unit, University of Nottingham) weighing 250–300 g served as subjects. The rats were group housed in large plastic tubs with wire lids (six rats per tub) in a temperature-regulated (22–23°C) room. Food and tap water were available ad libitum during the duration of the experiment. Artificial lighting was provided from 0700 hours to 1800 hours. All experiments were performed during the light cycle. All experiments were performed under UK Home Office regulations (Project License PP 40/1955).

Drug preparation and doses

Δ^9 -THC (Sigma; 1.5 mg kg⁻¹) and SR141716A (Gift from Sanofi; 3, 2 and 0.5 mg kg⁻¹ and 0.25 mg kg⁻¹) were suspended in an ethanol/triacetin (50/50) solution and incorporated in the commercially available fat emulsion Intralipid. Briefly, the solutions were filtered into the required volume of Intralipid (10%) using a 26-gauge needle. An aliquot of 0.2 ml was added at a time and the emulsion was sonicated after each addition using a Soniprep 150 Ultrasonic Disintegrator (MSE Scientific Instruments, Sussex, England) fitted with a probe assembly with a 9.0-mm tip. The probe was operated at an amplitude of 14 μ m for 30-s bursts. During sonication, the emulsion was kept on ice to avoid inactivation of the compounds due to the rise in temperature. The emulsion was made up to the desired volume of Intralipid and injected at the desired dose.

HU210 (Tocris; 20, 60 and 100 μ g kg⁻¹) and cocaine HCL (Sigma; 15 mg kg⁻¹) were dissolved in saline. All drugs were given i.p.

Apparatus and behavioural procedures

Place conditioning

The CPP apparatus has been described elsewhere (Sañudo-Peña et al. 1997). Briefly, the apparatus consisted of two Perspex compartments (30×30×40 cm each) separated by a guillotine door. Each compartment had different visual and textural cues in the form of thick (2.5 cm) or thin (1.0 cm) horizontal black and white lines and rough or smooth Perspex floors. Before the start of the experiment, animals were handled once daily for a week. During the pre-test phase, animals were placed in the middle of the apparatus without the guillotine door and allowed to explore it for 10 min; this was done for

three consecutive days for all animals and the amount of time spent in each compartment was averaged for this phase. The animals showed no significant preferences for either of the two compartments (average values $\pm$ SEM for the first pre-test sessions for the HU210- and SR141716A-treated animals were smooth/thick 297.73 $\pm$ 1.49 and rough/thin 301.66 $\pm$ 1.39. This allowed for unbiased designs).

The conditioning phase consisted of three pairings, with one of the distinctive compartments with either cocaine ($n=6$), Δ^9 -THC ($n=6$), HU210 ($n=18$) or SR141716A ($n=24$) alternated with three pairings of the other compartment with the corresponding vehicles ($n=24$). Each animal was assigned to a randomly chosen compartment and treatment order (Δ^9 -THC, HU210, SR141716A and cocaine), both of which were counterbalanced. Rats were placed in their respective compartments with the guillotine door in place for 10 min, 10 min after injection (during which time, animals were placed in a neutral tub).

Test sessions were separated by 48 h to allow clearance of the drugs. During the testing phase, the guillotine door was removed and the animals were placed at the intersection of the two compartments of the apparatus and left there for 10 min. All sessions were recorded by means of a camera mounted on the ceiling perpendicularly to the apparatus. The time spent in each compartment during the pre-test and test sessions was analysed by means of a PC using licensed copies of Ethovision and Videotrack behaviour-al software packages.

Withdrawal

Rats were injected with HU210 once a day at 1100 hours for 4 days at a dose of 100 μ g kg⁻¹ (i.p.). Control animals received saline ($n=2$) at the same time of day. Rats were challenged with the cannabinoid antagonist SR141716A at a dose of 1 mg kg⁻¹ (i.p.) 48 h after the final HU210 ($n=3$) and saline injections.

The behavioural assessment of cannabinoid-induced withdrawal was similar to the one used by Hutcheson et al. (1998). Rats were placed in a circular observation arena for 45 min, immediately after injection of the antagonist. Withdrawal signs (scratching, front paw tremor, wet-dog shakes, hunched posture and face rubbing) were videotaped from a camera mounted on the ceiling perpendicularly above the arena for subsequent quantification analysed by a trained observer blind to the treatment protocol. After the observation period, rats were transferred to Perspex observation cages with which they had no prior experience. The movement of each rat from one side of the box to the other resulted in an infra-red (IR) beam being broken, and an incremental count was recorded by a PC. The IR beam in the middle of the box measured locomotor activity, whereas the beam at the top of the box recorded rears. Activity was recorded every 5 min for 45 min.

Data analysis

Mann-Whitney U tests were used to compare saline or vehicle versus drug values for the time spent in each compartment for each dose. A one-way analysis of variance (ANOVA) was performed to compare differences between groups of animals treated with either SR141716A or HU210 followed by a post-hoc Newman-Keuls test, for which obtained raw data were utilised.

Withdrawal signs and locomotor activity counts were compared using Mann-Whitney U tests. All data were analysed using the computer programme Prism 2 (Graph Pad, Calif.).

Results

Conditioned place preference

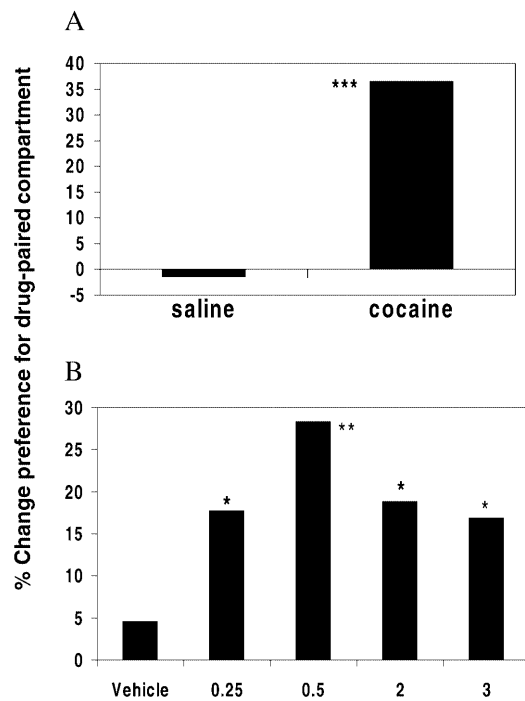
Cocaine (15 mg kg⁻¹) produced a significant preference for the drug-paired compartment ($P<0.01$). SR141716A also induced significant preference for the drug-associated

Table 1 SR141716A-induced place preference

Dose (mg/kg)	Paired compartment before SR ($\pm$ SEM)	Paired compartment after SR ($\pm$ SEM)	Percentage change preference (%)
3	342.41 $\pm$ 19.6	400.36 $\pm$ 25.17	16.85
2	328.81 $\pm$ 9.23	390.52 $\pm$ 13.94	18.94
0.5	311.64 $\pm$ 8.52	400.71 $\pm$ 24.26	28.29
0.25	299.58 $\pm$ 5.77	351.92 $\pm$ 17.79	17.5
Vehicle	320.61 $\pm$ 8.82	385.87 $\pm$ 10.33	4.51

Table 2 HU210-induced place avoidance. Averaged data for all animals per dose. Because of the way the acquisition software conveyed behavioural information – producing large standard deviations – it was necessary to normalise the data for graphical representation

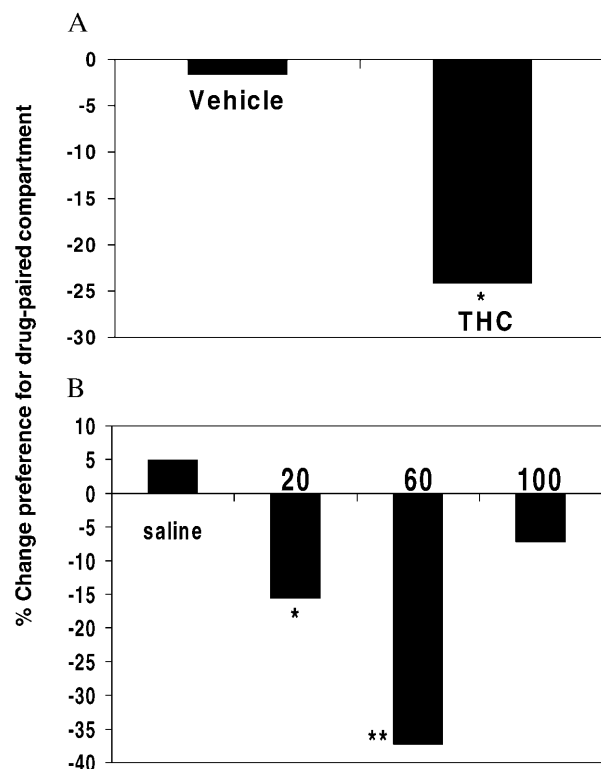
Dose (μ g/kg)	Paired compartment before HU210 ($\pm$ SEM)	Paired compartment after HU210 ($\pm$ SEM)	Percentage change preference (%)
100	401.32 $\pm$ 9.81	372.56 $\pm$ 26.22	-7.14
60	357.01 $\pm$ 15.84	219.77 $\pm$ 4.17	-37.7
20	355.57 $\pm$ 15.33	301.92 $\pm$ 15.15	-15.03
Saline	310.62 $\pm$ 10.38	326.17 $\pm$ 13.51	4.97

**Fig. 1** Effects of cocaine (**A** 15 mg kg⁻¹) and SR141716A (**B** 0.25, 0.5, 2 and 3 mg kg⁻¹) treatments in the conditioned place preference paradigm. Data are expressed as percentage change in seconds spent in the treatment-paired compartment during pre-test and test sessions. * $P<0.05$ ($n=18$), ** $P<0.01$ and *** $P<0.001$ ($n=6$), Mann-Whitney U test

ed compartment at all doses tested (0.5 mg kg⁻¹, $P<0.01$; 0.25, 2 and 3 mg kg⁻¹, $P<0.05$, one-way ANOVA **A**; Fig. 1A and B, respectively and Table 1).

Conditioned place avoidance

By contrast, the groups treated with Δ^9 -THC (1.5 mg kg⁻¹, $P<0.05$) and HU210 (20 μ g kg⁻¹, $P<0.05$; 60 μ g kg⁻¹, $P<0.01$, one-way ANOVA) all showed a significant

**Fig. 2** Δ^9 -THC (**A** 1.5 mg kg⁻¹; * $P<0.05$) and HU210 (**B**)-induced place aversions. Data are expressed as percentage change in seconds spent in the treatment-paired compartment during pre-test and test sessions. * $P<0.05$ for 20 μ g kg⁻¹ and ** $P<0.01$ for 60 μ g kg⁻¹ one-way analysis of variance followed by a Newman-Keuls post-hoc multiple comparison test ($n=12$); no significant effect was observed for 100 μ g kg⁻¹ ($n=6$)

avoidance (decreased preference) for the drug-paired side. HU210 at the highest dose (100 μ g kg⁻¹) produced a marked and long-lasting catalepsy which prevented meaningful behavioural measurements (Fig. 2A and B, respectively, and Table 2). None of the vehicles used (saline; T/I emulsion at 0.2 ml per animal) had an effect on CPP.

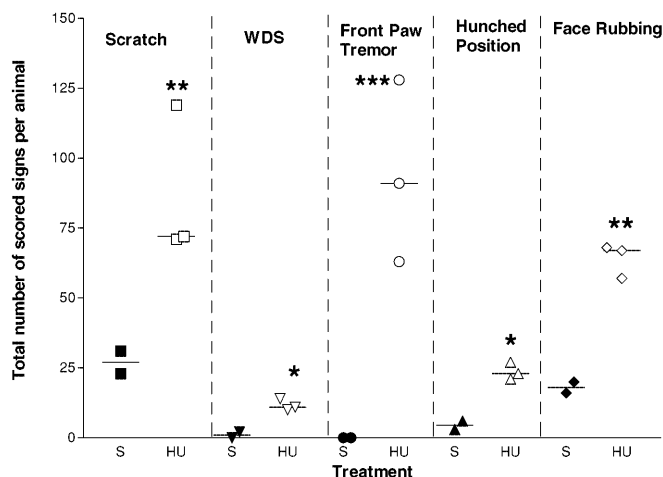


Fig. 3 SR141716A precipitates withdrawal in rats treated chronically with HU210. Data are expressed as total number of counted somatic signs of withdrawal per animal [filled symbols indicate saline/SR treated animals (S); open symbols represent HU210/SR-treated rats (HU); median is indicated by the horizontal bar] that occurred during the 45-min observation period after the injection of the cannabinoid receptor antagonist ($n=5$). * $P<0.05$, ** $P<0.01$ and $P<0.001$ (Mann-Whitney U test)

Withdrawal

A significantly higher incidence of all the quantified somatic signs of withdrawal was found in animals that received the cannabinoid antagonist after chronic HU210 treatment than in animals that were injected with SR141716A after chronic saline (Fig. 3).

Administration of the antagonist to rats treated with HU210 significantly increased exploration, but not rears, compared with saline controls (Fig. 4).

Discussion

This work confirms a number of previous findings that natural and synthetic cannabinoids produce place aversion and is consistent with the notion that cannabinoids can induce aversive motivational states. Our findings are also in agreement with the observation that laboratory animals will not self-administer cannabinoids (Corcoran and Amit 1974; Harris et al. 1974; Leite and Culini 1974). Yet, these aversive effects of cannabinoids seem to disagree with the fact that marijuana has been used for recreational purposes by humans since ancient times (Mechoulam 1986).

In this study, the potent cannabinoid agonist HU210 produced conditioned place avoidance at the two lower doses tested ($20 \mu\text{g kg}^{-1}$ and $60 \mu\text{g kg}^{-1}$) as measured by a decrease in time spent in the drug-paired compartment from the conditioning to the test phases. When the animals were given the highest dose ($100 \mu\text{g kg}^{-1}$), HU210 induced a profound hypoactivity, which disrupted the expression of other behaviours, possibly preventing the animals from associating their motivational state with the

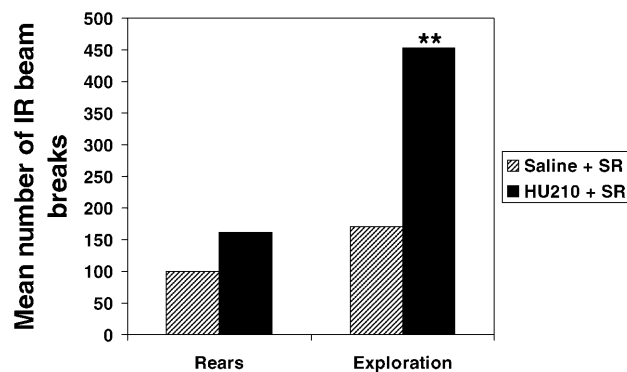


Fig. 4 Effects of withdrawal on locomotor activity in the rat. The cannabinoid receptor antagonist injection increased the number of middle infra-red beams being broken (exploratory activity) but had no effect on the number of top infra-red beams (rears) ($n=5$, same animals used in the previous withdrawal study). ** $P<0.01$ (Mann-Whitney U test)

spatial cues of the conditioning chambers. There is extensive evidence proving the disruptive effects of cannabinoids in both laboratory animals and humans (Abel 1971; Zimmerberg et al. 1971; Grilly et al. 1973; Dornbush 1974; Heyser et al. 1993; Molina-Holgado et al. 1995).

Δ^9 -THC (1.5 mg kg^{-1}), like HU210, produced place avoidance at a dose reported to be aversive by Mallet and Beninger (1998). Thus, the stimulus properties of HU210 appear to be similar to those of Δ^9 -THC as they both elicit the same type of operational response in the CPP paradigm.

However, cannabinoids seem to share some physiological characteristics ascribed to most other drugs of abuse, namely, an enhancement of basal neuronal firing and an increase in basal neurotransmitter release in reward-relevant pathways (French et al. 1997; Tanda et al. 1997; Gardner and Vorel 1998). These physiological characteristics do not seem to correlate well with the use of behavioural paradigms, as these yield conflicting results in terms of the motivational effects of cannabinoids. The impact of strain differences on the outcome of the paradigm is one of the major sources of discrepancy among reports. The present data obtained with Lister hooded rats support Sprague-Dawley, Lewis and Wistar and mice cannabinoid-induced place aversions (McGregor et al. 1995; Parker and Gillies 1995; Sañudo-Peña et al. 1997; Hutcheson et al. 1998). Thus, the only report of cannabinoid-induced place preference to date used Long-Evans rats (Lepore et al. 1995) under particular experimental conditions. Nonetheless, the discrepancy appears not to be due entirely to genetic differences between the strains as Δ^9 -THC in Lewis (Gardner et al. 1988b) but not Long-Evans rats lowers the threshold for intra-cranial self-stimulation (ICSS). Δ^9 -THC also produces an increase in dopamine release in the nucleus accumbens (Lepore et al. 1996) in the "drug-preferring" Lewis rat. Furthermore, the only species that has been reported to support positive cannabinoid (WIN55,212-2 but not Δ^9 -THC)-induced i.v. self-administration is the

mouse (Martelotta et al. 1998). Therefore, it appears that the differences may not only depend on the species but also the paradigm used.

It is well known that, with high doses, Δ^9 -THC can cause dysphoria and even panic in human subjects (Raft et al. 1977; Laszlo et al. 1981). In laboratory animals, cannabinoids also possess an anxiogenic profile and stimulate the hypothalamo–pituitary axis in a manner similar to foot shock with a concomitant *c-fos* expression in stress-related loci (central and basolateral nuclei of the amygdala and periaqueductal grey in particular; Herkenham and Brady 1994). One possible explanation for the aversive effects of the cannabinoids when assessed by means of the CPP paradigm is that the paradigm is stressful per se and the cannabinoids have an additive effect in terms of anxiogenesis. This would perhaps mask a rewarding effect of the cannabinoids in the strains where the compounds are producing aversive effects. However, the absence of reward might be associated with the long-lasting pharmacokinetics of HU210 (McGregor et al. 1995) such that the reward itself is expressed on return to the home cage, thus confounding the operational response in the CPP apparatus itself. Valjent and Maldonado (2000) seem to share this view, as their study with mice produced both place aversion and preference depending on the dose used and the context in which cannabinoid administration was paired.

Our data on the cannabinoid antagonist confirm and extend the findings of Sañudo-Peña et al. (1997). SR141716A produced, like cocaine, a place preference for the drug-paired compartment. This could be an indication for a cannabinergic tone in the rat brain. Again, controversy also seems to be the rule in this case, as two other studies have failed to reproduce the rewarding effects of the cannabinoid antagonist (Chaperon et al. 1998; Hutcheson et al. 1998). In one of these two reports however, SR141716A effectively antagonised the place aversion elicited by the cannabinoid agonist WIN55,212-2. This is a good indication that the place aversion is indeed CB1-receptor mediated; however, it would remain to be determined whether the antagonism is due to an action on the CB1 receptor alone or is also partly due to a reinforcing effect of the antagonist.

The antagonist's rewarding effects might be directly associated with an antagonising effect of SR141716A on endogenous cannabinoids at any of the brain structures linked with reward. The abrupt withdrawal elicited by SR141716A provides evidence for physical dependence to HU210. It is nevertheless worth mentioning that it is likely that the diversity of somatic signs accompanying SR141716A-induced withdrawal might be mediated by other neural systems that ultimately lead to the behaviours observed upon cessation of cannabinoid receptor coupling.

These results are somewhat at odds with the finding that anandamide has no effect on CPP (indicating that anandamide may not play a role in reward mechanisms; Mallet and Beninger 1998). Other endogenous cannabinoids such as 2-arachidonyl-glycerol that are present in higher brain concentrations than anandamide (Mechoulam

et al. 1995) might be possible candidates for the modulation of an endogenous counter-reward state.

Taken together, these findings suggest that the rat model used in this study does not reproduce the cognitively complex effects that give rise to marijuana use in humans, especially social setting and context learning. Whether SR141716A is self-administered or lowers the threshold for brain stimulation reward is a question that remains to be determined. Δ^9 -THC in general, however, does not seem to share the ability to induce self-administration, place preference or lower the threshold for ICSS, which are characteristics of other drugs of abuse, but shares other physiological properties of “harder” drugs. This could be an explanation of why marijuana does not produce obsessive drug-seeking and compulsive drug-taking behaviour.

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