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Effects of chronic Δ^9-tetrahydrocannabinol on rat midbrain dopamine neurons: an electrophysiological assessment

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Abstract

Delta-9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive ingredient in marijuana elicits a variety of physiological effects in animals and humans, and with repeated exposure tolerance develops to most of its effects. However, studies in humans found that tolerance did not occur to the pleasurable marijuana "high". Since ventral tegmental dopamine neurons play a pivotal role in drug reinforcement and reward, and possibly in the euphorigenic quality of marijuana, the present study sought to determine whether tolerance develops to the neurophysiological response elicited in these neurons by Δ^9-THC. Using single-unit extracellular recordings the activity of midbrain ventral tegmental (VTA) and substantia nigra pars compacta (SNpc) dopamine neurons was measured in animals that had received twice-daily injections of 5 mg/kg Δ^9-THC for 14 days. Cannabinoid-induced changes in body temperature, locomotion, and catalepsy were also assessed in the same animals. After 2 weeks tolerance had developed to Δ^9-THC-induced hypothermia, catalepsy and reduction in locomotor activity. In naive animals and in animals that had received twice-daily vehicle injections for 14 days, Δ^9-THC increased VTA neuronal firing by 52% and 46%, respectively, while SNpc neurons showed increases of 23% and 30%, respectively. Following chronic cannabinoid treatment, however, SNpc neurons were significantly less responsive to Δ^9-THC with a maximum increase in rate of only 3%, while VTA neurons continued to show a robust increase in firing rate (+45%) when challenged with THC. These results suggest that VTA and SNpc dopamine neurons develop a differential response to Δ^9-THC following long-term cannabinoid exposure. This finding may be relevant to the observation that in humans tolerance occurs to many of marijuana's physiological effects but not to its euphorigenic actions.

Keywords: Marijuana; Delta-9-tetrahydrocannabinol; Ventral tegmental area; Substantia nigra; Electrophysiology

1. Introduction

Marijuana is the most widely used illicit drug and the incidence of its abuse continues to increase (Cargo, 1998). While marijuana is generally consumed for its psychotropic effects, it is also known that its principal psychoactive ingredient, delta-9-tetrahydrocannabinol (Δ^9-THC) also affects a number of other physiological systems, including those involved in motor control, temperature regulation, cardiovascular and immune function, and nociception. Nevertheless, the sites and mechanism(s) by which Δ^9-THC produces these effects have not been fully delineated. In the CNS Δ^9-THC alters several neurotransmitter systems, and the fact that it increases dopaminergic activity suggests that dopamine neurons could be involved in cannabinoid-evoked behaviors (French et al., 1997; Sakurai-Yamashita et al., 1989). For example, substantia nigra pars compacta (SNpc) neurons provide dopamine innervation to basal ganglia structures which are involved in the control of motor activity. Thus, SNpc neurons may be a target for the cataleptogenic actions of cannabinoids (Gough and Olley, 1978; Pertwee and Wickens, 1991). On the other hand, ventral tegmental (VTA) dopamine neurons innervate limbic and cortical areas which are important for motivation and cognition. In fact, VTA–mesolimbic pathways have been recognized as principal sites of action for the positive reinforcing and abuse liability properties associated with drugs of abuse (Koob et al., 1998).

Although the magnitude of many of the behavioral

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effects produced by Δ^9-THC and other synthetic cannabinoids lessens with repeated exposure, there are data to suggest that tolerance does not develop to marijuana's euphorigenic activity, or the "high" (Dewey, 1986; Perez-Reyes et al., 1991). Thus, the response to Δ^9-THC of those neurons which play a role in the marijuana "high" might not be expected to diminish with chronic treatment. However, a reduced response might be expected from neurons in those structures associated with behaviors for which tolerance does develop. For example, tolerance occurs to Δ^9-THC-induced catalepsy, an alteration of motor function which is mediated through nigrostriatal dopamine pathways (Gough and Olley, 1978).

Recently we reported that the acute administration of Δ^9-THC activated both SNpc and VTA dopamine neurons. This effect was mimicked by the selective cannabinoid-like drug WIN 55,212-2, but neither by its inactive enantiomer nor the non-psychoactive cannabidiol, and it was blocked by the selective cannabinoid CB_1 receptor antagonist SR141716A (French, 1997; French et al., 1997). To date, however, there is no evidence to suggest that tolerance occurs to the electrophysiological effects of cannabinoids on dopamine neuronal activity in either the substantia nigra or VTA. Assuming that SNpc neurons mediate the cataleptogenic effects to which tolerance develops, and that VTA neurons mediate the rewarding effects for which tolerance does not develop, we sought to test the hypothesis that these two populations of midbrain dopamine neurons would show a differential response to Δ^9-THC in animals chronically exposed to cannabinoids.

2. Methods

Adult male Sprague-Dawley rats (Harlan Sprague Dawley Inc.) weighing 250–350 g were used in all experiments. All animals were housed on a light–dark schedule (07:00–19:00 h) with constant room temperature and free access to food and water. Rats in the chronic cannabinoid group received intraperitoneal (IP) injections of 5 mg/kg Δ^9-THC two times per day for 14 days. A control group of animals received IP vehicle (1 ml/kg) injections on the same schedule. The daily injections were made in the home cages at approximately 08:00 h and 16:00 h. All experiments were conducted in accordance with National Institute of Health guidelines for the care and use of laboratory animals and approved by the IACUC of the University of Arizona.

The sequence for the administration of each of the behavioral and electrophysiological assessments described below is shown in Fig. 1.

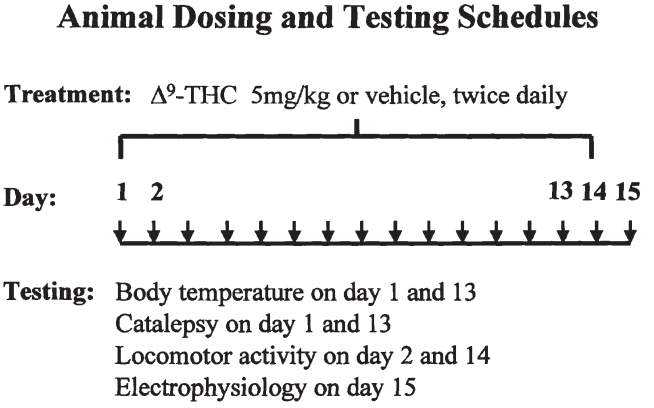

Fig. 1. Dosing and testing schedules for measurements of catalepsy, locomotor activity, body temperature and electrophysiology following acute and chronic Δ^9-THC.

2.1. Electrophysiology

The VTA assessments were divided into the following three treatment groups: acute (N=10), chronic vehicle (N=5) and chronic Δ^9-THC (N=7). For SNpc the groups were composed of: acute (N=10), chronic vehicle (N=5) and chronic Δ^9-THC (N=12).

Approximately 20–22 h after the last Δ^9-THC injection the animals were prepared for the electrophysiological portion of the study. Chloral hydrate (350 mg/kg, IP) was used for the induction and maintenance of anesthesia throughout the recording period. The preparation of the animals and the methods for extracellular recording from single dopamine neurons in the VTA and SNpc have been detailed elsewhere (French et al., 1993). Presumptive dopamine neurons were identified according to the following criteria:

1. biphasic (or triphasic) positive–negative waveform action potentials;
2. action potential duration $>$2 ms;
3. firing rates $<$10 spikes/s;
4. regular to bursting patterns of firing (Bunney et al., 1973).

Once a neuron meeting these characteristics was found, the activity was monitored for approximately 5 min before injections were begun. An intravenous (IV) bolus of saline (1 ml/kg) was then administered to control for possible effects from the injection procedure itself. This was followed by Δ^9-THC which was given IV and separated by inter-injection intervals of 2–4 min using the following schedule of doses: 0.125, 0.25, 0.5, 1, and 2 mg/kg for a total dose of 3.875 mg/kg. After completing the Δ^9-THC injections, apomorphine was administered in doubling dose amounts beginning with 10 μg/kg until the firing rate was reduced by 50%. The exquisite sensitivity of dopamine neurons to apomorphine was used as an additional indicator of the identity of the cells being

recorded. Only one cell per animal was tested. Light microscopic verification of electrode placement was also made at the conclusion of the experiment (French et al., 1993).

Interspike-interval histograms were constructed off-line to determine firing rates and patterns of action potential generation. The analysis program quantified firing rate (spikes/s), number of bursts, events in bursts (with a range of 2–18 spikes/burst), and percent burst firing (ratio of action potentials in bursts to total number of action potentials×100). The criteria used for computer recognition of bursts included (1) an interspike interval of 80 ms or less for the first pair of spikes in a train and (2) termination when the interval between two spikes exceeded 160 ms (Grace and Bunney, 1984).

2.2. Body temperature

Measurements of body temperature following Δ^9-THC or vehicle were made on day 1 and day 13. Core body temperature was obtained from a lubricated thermometer probe inserted rectally. Measurements commenced 30 min following vehicle or Δ^9-THC with four subsequent recordings made at 60, 120, 180 and 240 min.

2.3. Catalepsy

Catalepsy measurements on days 1 and 13 were made after the last temperature reading or 240 min following Δ^9-THC or vehicle. The presence of catalepsy was determined using a modification of the bar test (Costall and Olley, 1971). The apparatus consisted of a 6 mm diameter bar inserted into two wooden blocks separated by 20 cm. The distance from the top of the bar to the counter top measured 10 cm. Three determinations were made of the length of time the animal remained with its forelimbs on the bar. The timing clock was stopped when the animal removed one or both forelimbs from the bar or a maximum time of 300 s had elapsed. The median value of the three determinations was recorded and the seconds of immobility divided by 300 and multiplied by 100 to yield a value of percent maximum catalepsy.

2.4. Locomotor activity

Activity measurements were made on day 2 and day 14 of Δ^9-THC or vehicle treatment. Forty minutes following an IP injection of vehicle or 5 mg/kg Δ^9-THC in the homecage, the locomotor activity of each animal was measured in a photocell-equipped cage (French and Vantini, 1984). Photocell beam interruptions were recorded every 20 min for a total of 100 min. Since cannabinoids have been shown to reduce locomotor activity in rats, we maximized the conditions in which a reduction in activity could be adequately measured by not acclimating the animals to the test cages prior to injection.

2.5. Statistics

Differences in the electrophysiological responses to Δ^9-THC were analyzed by two-way ANOVA with dose as the repeated measure. Catalepsy scores between vehicle days 1 and 13 and chronic THC days 1 and 13 were compared by paired *t*-test. Locomotor activity comparisons between vehicle and chronic THC treatments were made by unpaired *t*-test. Body temperature comparisons between vehicle and chronic THC treatments were made using a two-way ANOVA with time as the repeated measure with Bonferroni's Multiple Comparison Test used to determine where differences occurred between the two treatment groups. In all cases a value of $P<0.05$ was considered significant.

2.6. Drugs

Δ^9-THC was prepared in a vehicle of 10% Tween 80, 20% DMSO and 70% distilled water (vol/vol). An aliquot of stock Δ^9-THC (100 mg/ml ethanol) was placed in a vial and evaporated to dryness under a stream of argon gas. DMSO was then added and vortexed, followed by Tween-80 and vortexed, and lastly water and vortexed. Solutions were prepared fresh for each experiment. Previous studies found the vehicle to be devoid of electrophysiological effects (French et al., 1997; French, 1997).

3. Results

3.1. Electrophysiology

Δ^9-THC produced dose-dependent changes in the firing rate of VTA dopamine neurons with average maximum increases of 52% and 46% in the naive and vehicle treated rats, respectively (Fig. 2). After 14 days of twice-daily injections of 5 mg/kg Δ^9-THC the response of VTA dopamine neurons to Δ^9-THC remained elevated (+44%).

In SNpc dopamine neurons acute injections of Δ^9-THC also produced a dose-dependent increase in firing rate with a maximum change of +33% in the vehicle control group, and +23% in the naive animals (Fig. 2, bottom panel). This difference between naive and controls, however, was not found to be significant. Also, the magnitude of the stimulation produced by Δ^9-THC was smaller in the SNpc than VTA. Furthermore, the response of SNpc dopamine neurons to THC became significantly diminished (+3%, $P<0.01$) following 14 days of Δ^9-THC exposure.

An analysis of bursting activity found that baseline

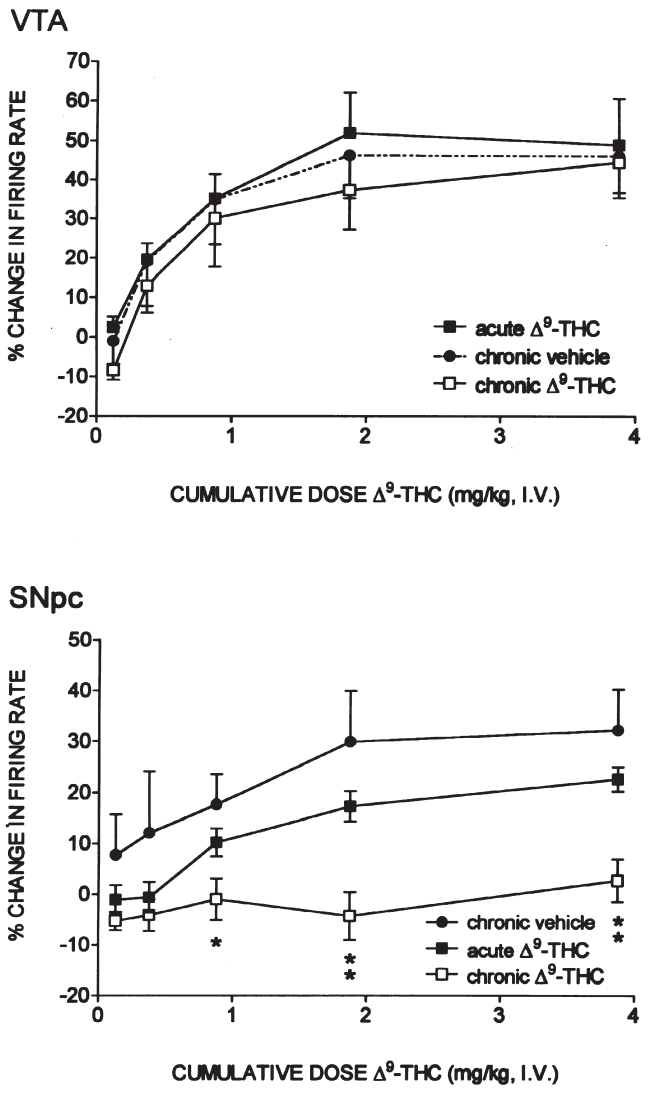

Fig. 2. Dose–response effects of Δ^9-THC on firing rates of VTA and SNpc dopamine neurons in naive and chronic cannabinoid treated animals. The chronic treated groups received twice-daily injections of 5 mg/kg Δ^9-THC or vehicle for 14 days with the last injection occurring 20–22 h before commencing the electrophysiological measurements. The acute group consisted of naive animals. Firing rates are presented as percent change over the baseline rates. The data are based upon complete dose–response determinations obtained in 5–12 cells per treatment group in VTA (top panel) and SNpc (bottom panel). A two-way ANOVA with dose as the repeated measure was used to analyze for differences between treatments. In the VTA there were no significant differences between the naive (non-treatment) and vehicle dose–responses ($F(1,13)=0.04$) or between the chronic Δ^9-THC and vehicle responses ($F(1,10)=0.22$). A similar analysis of the SNpc data also found no significant difference between the non-treated naive and vehicle dose–response curves ($F(1,13)=2.2$). However, a significant reduction in SNpc responses was found between the chronic Δ^9-THC and the chronic vehicle treated animals ($F(1,15)=9.1$), as well as between chronic Δ^9-THC and acutely injected animals ($F(1,20)=12.3$).

(pre-Δ^9-THC) values for percent bursts was 14% in VTA and 19% in SNpc of the control animals (Table 1). Acute Δ^9-THC elicited significant increases in VTA bursting activity from 14% to 38%, and from 19% to 22% in SNpc. After chronic Δ^9-THC treatment, basal levels of percent bursts in VTA were slightly larger at 17%, but not significantly different from control basal levels. Furthermore, after 14 days of Δ^9-THC exposure, VTA neurons still responded with an increase in percent bursts similar to that seen following first challenge (40% vs. 38%). In SNpc neurons, however, the number of action potentials contained in bursts was reduced from 19% to 5% ($P<0.05$) with chronic Δ^9-THC exposure. Also, Δ^9-THC did not significantly increase the amount of bursting in the SNpc neurons in the chronically exposed animals.

3.2. Body temperature

The administration of 5 mg/kg Δ^9-THC to naive rats elicited a hypothermic response with a mean maximum change in body temperature of −1.1°C at the 30 min timepoint (Fig. 3). Body temperature remained depressed for another 90 min, but by 3 h it had returned to pre-cannabinoid levels with a small overshoot of +0.5°C at 4 h. After 13 days of twice-daily cannabinoid injections, Δ^9-THC no longer produced a hypothermic response. In fact, over the 4 h observation period, core body temperature actually increased 1.1°C. The response to vehicle on day 1 did not differ from the response to vehicle following 13 twice-daily vehicle injections.

3.3. Locomotor activity

The activity of rats following 5 mg/kg Δ^9-THC was significantly lower than those receiving vehicle only ($P<0.001$) (Fig. 4). Total photocell beam interruptions over the course of the 100 min observation period averaged 299±27 counts in the controls versus 111±16 counts following Δ^9-THC on day 2. However, after 14 days of treatment the locomotor response to Δ^9-THC was no longer reduced in the chronic cannabinoid treated animals (251±26 counts vs. 287±34 counts in the controls).

3.4. Catalepsy

The amount of catalepsy that occurred upon first exposure to Δ^9-THC averaged 57% of the maximum of the 300 s cut-off, or 170 s of immobility on the horizontal bar (Table 2). After 13 daily injections of Δ^9-THC the duration of immobility was 110 s, or 37% of maximum, a significant reduction ($P<0.05$). The amount of immobility in the control animals increased slightly but not significantly over the course of chronic vehicle treatments (31 s on day 1 vs. 49 s on day 13).

4. Discussion

A number of studies have clearly established that tolerance develops to many of the physiological effects pro-

Table 1
Effects of acute and chronic Δ^9-THC on dopamine neuron electrophysiology[a]

		Percent bursts		Firing rate	
		Basal	Δ^9-THC	Basal (spikes/s)	Δ^9-THC (%Δ)
VTA	Controls (15)	14.1±4.8	38.4±7.0 ($P<0.01$)	3.6±0.4	54.1±7.5%
	Chronic Δ^9-THC (7)	17.4±7.9	40.4±9.5 ($P<0.01$)	3.9±0.5	48.7±11.4%
		(ns)	(ns)	(ns)	(ns)
SNpc	Controls (15)	19.0±6.0	22.6±6.4 ($P<0.05$)	3.4±0.4	25.3±3.5%
	Chronic Δ^9-THC (12)	5±2	9±3 (ns)	3.2±0.3	-3.7±5.5%
		($P<0.05$)	(ns)	(ns)	($P<0.01$)

[a] For the purposes of this table the control values are the means from the combined data of the vehicle control animals and naive animals, as these were found to be not significantly different from each other. Also, when calculating the percent bursts and firing rates (spikes/s), the point of maximum change in firing rate was used for analysis, since it is a more accurate indicator of the sensitivity of each animal to the drug, rather than the dose at which the mean change in firing rate had occurred for the group (French et al., 1993). In all groups tested some animals showed peak responses (increases or decreases) on either side of the group maximum mean. Thus, these values may be slightly above or below the values given for dose-dependent average changes in firing rates presented in Fig. 2.In analyzing percent bursts, the differences between basal values and those after Δ^9-THC across columns were compared using the *t*-test for paired data as each animal served as its own control. However, differences between basal and THC values within columns were compared using the *t*-test for paired data, which was also used for comparing changes in firing rates (given as percent change from baseline activity).

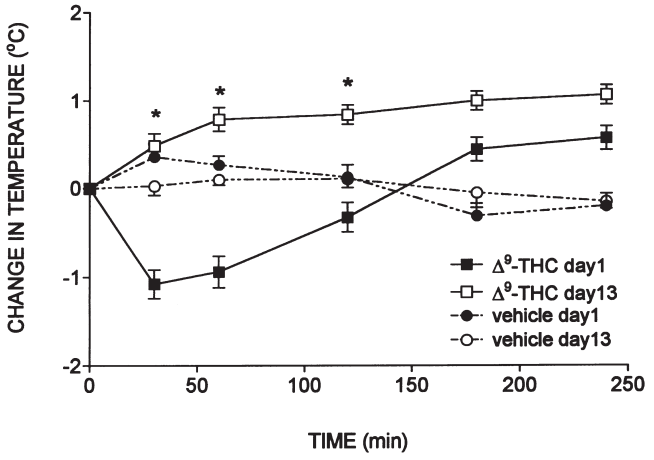

Fig. 3. Effects of Δ^9-THC on body temperature after acute or chronic treatment with Δ^9-THC. Two-way ANOVA with time as the repeated measure found a significant treatment effect between temperature responses to the first challenge with Δ^9-THC and to the last challenge following 12 days of twice-daily injections of the cannabinoid ($F(1,42)=18.4$, $P<0.01$). No differences were found in the temperature response to vehicle in acute or chronic vehicle treated animals ($F(1,20)=0.1$). The hypothermic effects of Δ^9-THC on first challenge were also significantly different from the vehicle control response ($F(1,31)=5.2$, $P<0.05$), as was the hyperthermic effect on day 13 ($F(1,31)=27$, $P<0.01$). The average basal body temperature in the naive animals averaged 37.9±0.1°C and 37.5±0.1°C on day 1 and day 13, respectively, and 38.4±0.1°C and 38.3±0.1°C on day 1 and day 13 in the chronic vehicle group, respectively.

duced by cannabinoids in laboratory animals (Dewey, 1986). Although studies in human subjects have reported that tolerance occurrs to the peripheral effects of once per day smoking of marijuana, the marijuana-induced "high" in these same subjects was found not to have diminished (Perez-Reyes et al., 1991). In order to explain how chronic marijuana administration produces

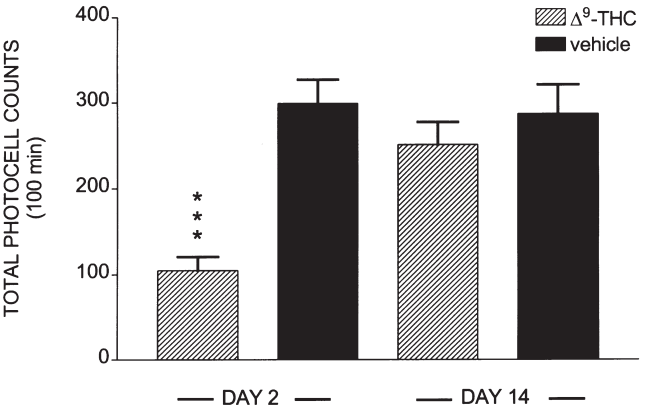

Fig. 4. Locomotor activity following the second and fourteenth injections of Δ^9-THC. The activity of the saline injected animals was measured before the first injection of Δ^9-THC on day 1. The asterisk denotes a significant difference ($P<0.001$) between Δ^9-THC and vehicle on day 2 ($N=22$).

Table 2
Catalepsy induced by acute and chronic exposure to Δ^9-THC[a]

		Catalepsy	
		Seconds	Percent of maximum
Δ^9-THC	Day 1	170±24	57
	Day 13	110±22*	37
Vehicle	Day 1	31±7	10
	Day 13	49±9	16

[a] Differences between day 1 and day 13 means were compared by *t*-test for paired data ($N=10$ for chronic vehicle group, $N=25$ for Δ^9-THC group). The percentage of the maximum was calculated by dividing seconds of catalepsy by the maximum 300 s cutoff×100. *$P<0.05$.

tolerance to some of its effects but not to others, it seemed a reasonable postulate that cannabinoids bring about a differential response in the neuronal population(s) subserving the euphorigenic properties of the drug from those subserving its other effects. However, given the absence of data from animal models that are traditionally used for studying the reinforcing properties of drugs, there is no evidence whether or not tolerance develops to Δ^9-THC's rewarding properties. Therefore, the aim here was to assess the response of VTA dopamine neurons to Δ^9-THC in behaviorally tolerant animals in order to ascertain the impact of repeated cannabinoid exposure on the activity of those dopamine neurons comprising central reward pathways.

In this study we measured the electrophysiological responses of SNpc and VTA dopamine neurons following incremental IV doses of Δ^9-THC in rats rendered behaviorally tolerant to the cannabinoid. These two midbrain neuronal populations were chosen for study because neurons comprising the striatonigral pathway are believed to be involved in the cataleptogenic effects of cannabinoids (Gough and Olley, 1978; Navarro et al., 1993), a behavioral response for which tolerance has been shown to develop (De Fonseca et al., 1994). In contrast, VTA dopamine neurons have been implicated in the positive reinforcing and likely the euphorigenic effects or the "high" experienced with marijuana use, a response for which tolerance does not appear to develop (Perez-Reyes et al., 1991; Gardner and Lowinson, 1991; French et al., 1997).

Our data show that acute Δ^9-THC increased the activity of dopamine neurons both in the VTA and SNpc in a dose-dependent manner, although the VTA response was of greater magnitude overall. However, in animals that were made behaviorally tolerant to Δ^9-THC through a course of chronic cannabinoid exposure, Δ^9-THC's effects on VTA neuronal firing rate and the amount of bursting activity were not diminished. The latter electrophysiological parameter may be particularly relevant since it has been shown that action potentials generated in a bursting pattern result in greater synaptic levels of transmitter than that which would occur from the same number of action potentials generated singly (Gonon and Buda, 1985). Since dopamine release in limbic and cortical structures is considered to play an important role in the rewarding aspects of several drugs of abuse, including marijuana (Gardner and Lowinson, 1991), the sustained level of stimulation of the mesolimbic dopamine neurons seen here upon repeated exposure to Δ^9-THC suggests a possible mechanism by which tolerance does not develop to the marijuana-induced "high". This, of course, is predicated on the assumption that VTA–mesolimbic pathways in human brain play a similar role to that in animals in their response to drugs of abuse.

In contrast to the effects of chronic cannabinoid treatment on midbrain reward pathways, the magnitude of change in firing rate and amount of bursting evoked in SNpc neurons by Δ^9-THC was significantly smaller following 14 days of cannabinoid exposure. This attenuation of the response in the nigrostriatal circuit correlated with the decline in catalepsy we and others have observed following a course of chronic cannabinoid treatment. Thus, our data suggest that following chronic THC exposure there develops a clear divergence in the electrophysiological responses of these two distinct populations of midbrain dopamine neurons. Nevertheless, the underlying mechanism mediating the dissimilar response of VTA and SNpc neurons to Δ^9-THC in tolerant animals is unknown.

Previous electrophysiological studies using selective CB_1 agonists and an antagonist strongly point to the involvement of CB_1 receptors in mediating the excitatory response in both SNpc and VTA neurons (French, 1997; Gessa et al., 1998). Thus, it is possible that chronic cannabinoid treatment produces changes in receptor pharmacodynamics which differ between the VTA and SNpc. For example, a long-term regimen of Δ^9-THC produces a down-regulation of cannabinoid receptors in the striatum which is larger than that in the mesencephalon, but with no receptor changes in the limbic forebrain (Romero et al., 1995). The limbic area is innervated by VTA dopamine neurons while the striatum receives dopamine innervation from SNpc neurons. Since similar radioligand binding studies have not been conducted on circumscribed VTA or SNpc tissues, it still remains unknown whether cannabinoid receptors in these areas undergo changes similar to those in the terminal sites. Nonetheless, one study reported that chronic treatments with the CB_1 ligand, CP55,940, produced a down-regulation of cannabinoid receptor mRNA levels in the caudate-putamen, but not in the cerebellum (Rubino et al., 1994). Thus, not all brain areas appear to change in a uniform way under conditions producing behavioral tolerance. Furthermore, there may be regional brain differences in CB_1 activated G-protein coupled signal transduction processes which could be affected by chronic cannabinoid treatment (Sim et al., 1996; Rubino et al., 1997). Again, however, this finding has not been confirmed in midbrain dopamine neurons in either the SNpc or VTA. Lastly, VTA and SNpc neurons likely differ in the composition of excitatory and inhibitory afferents that control the activity of these two neuronal populations.

CB_1 receptors are considered to be presynaptically located, thereby modulating transmitter release (Herkenham et al., 1991; Miller and Walker, 1995; Shen et al., 1996). Thus, they are in a position to play an integral role in the control of the two major afferent transmitters, glutamate and GABA, that modulate the activity of midbrain dopamine neurons (Shen et al., 1996; Szabo et al., 1998). Therefore, any change in the receptor response to Δ^9-THC following chronic cannabinoid treatment could effectively alter the ratio of the excit-

atory and inhibitory drives governing the rate and patterns of dopamine cell firing. Such differences in afferent tone could also help explain why the magnitude of Δ^9-THC-induced excitation is greater in the VTA than in the SNpc of naive animals, and possibly how SNpc neurons become less responsive to Δ^9-THC after chronic treatment with THC. Nonetheless, the mechanism for the lack of tolerance in VTA neurons remains elusive.

Laboratory studies have provided compelling experimental data establishing a role for the VTA–mesolimbic dopamine system in animal models of addiction (Koob et al., 1998). While demonstrating that cannabinoids are reinforcing in animals has been problematic (Mansbach et al., 1994), recent evidence shows that animals will self-administer CB_1 receptor agonists (Harris et al., 1974; Martellotta et al., 1998). Earlier work and the present results that psychoactive cannabinoids increase dopamine neurotransmission in the VTA–limbic–cortical circuits may provide additional insight into possible mechanisms by which cannabinoids are positive reinforcers. However, it still remains to be established whether these same dopamine pathways also mediate the rewarding effects of marijuana in humans. Notwithstanding the lack of this evidence, imaging studies of the human brain have concluded that Δ^9-THC does alter neurotransmission in the prefrontal, frontal and orbitofrontal cortices and the basal ganglia, all dopamine innervated structures. Also, metabolism in the prefrontal cortex is significantly greater in marijuana abusers than in normal subjects (Volkow et al., 1996), while metabolism in the cerebellum is decreased. Since the prefrontal cortex receives dopamine innervation from the VTA, these results suggest that VTA dopamine neuronal activity, and the consequent increase in dopamine release, caused by Δ^9-THC is actually accentuated with chronic cannabinoid use. The electrophysiological data presented here would support this contention. Thus, based upon our findings, it is possibile that in humans the electrophysiological response of VTA dopamine neurons does not diminish during repeated exposure to cannabinoids, and that this may underlie the lack of tolerance to the eurphoric effects of marijuana even with chronic use.

Acknowledgements

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