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Functional consequences of the repeated administration of Δ^9-tetrahydrocannabinol in the rat

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Abstract

The repeated administration of Δ^9-tetrahydrocannabinol (THC) results in tolerance to many of its behavioral and physiological effects. It also produces changes in the functionality of cannabinoid receptors. What is not completely understood is how these cellular events translate into the behavioral and physiological changes that are associated with repeated cannabinoid agonist treatment. The purpose of these studies was to determine the development of changes in the patterns of functional activity, as measured by the 2-[^{14}C]deoxyglucose method (2DG), associated with repeated THC exposure. Male Sprague–Dawley rats ($n = 4$–5) were administered THC (vehicle or 10 mg/kg, intraperitoneally), daily for 7 or 21 days. Fifteen minutes following the final THC treatment the 2DG procedure was initiated. In separate sets of rats similarly treated with THC, locomotor activity and core body temperature were measured at corresponding time points in order to establish the behavioral profile of repeated THC administration. The acute administration of THC following 7 or 21 days of drug exposure resulted in a significant attenuation of changes in rates of glucose utilization throughout the majority of brain regions analyzed when compared to the large global decreases observed following a single administration of THC. After 7 and 21 days of treatment, cerebral metabolic rates were no longer different from vehicle-treated controls in most cortical, thalamic and basal ganglia regions. This attenuation closely paralleled the development of tolerance to the effects of THC on locomotor activity and core body temperature. However, glucose utilization remained altered in the nucleus accumbens, mediodorsal thalamus, basolateral amygdala, portions of the hippocampus and median raphe. These data suggest that the development of tolerance to the cerebral metabolic effects of THC is regionally specific and temporally distinct. The persistence of effects in limbic areas as well as portions of the hippocampal complex, however, suggests that processes such as stress, reward, and aspects of memory mediated by these brain regions may continue to be affected by THC even after prolonged THC exposure.

Keywords: Marijuana; Tolerance; Deoxyglucose; Cannabinoid receptors; Rats

1. Introduction

Chronic exposure to a drug often results in the development of tolerance, such that the physiological and/or behavioral effects produced by the drug diminish with repeated exposure. This phenomenon has been described for substances of abuse such as opiates and alcohol. Tolerance to the physiological and behavioral effects of Δ^9-tetrahydrocannabinol (THC), the primary psychoactive constituent in marijuana, has also been well-characterized (for review see Adams and Martin, 1996). Following chronic THC administration, tolerance has been shown to develop to THC-induced hypothermia, locomotor suppression, as well as disruption of complex integrative functions like short-term memory (Carlini, 1968; Perron et al., 2001). Ratings of 'high' in human subjects, however, have been reported to remain relatively stable in magnitude despite repeated marijuana administration, suggesting that not all effects of cannabinoids show complete tolerance nor develop tolerance at the same rate (Perez-Reyes et al., 1991).

On the cellular level, the repeated administration of cannabinoid agonists including THC is accompanied by

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changes in the functionality of central nervous system cannabinoid receptors (CB_1). Studies of CB_1 receptor and cannabinoid agonist-stimulated [^{35}S]GTPγS binding, as well as cannabinoid-related gene regulation in rodents have demonstrated both CB_1 receptor down-regulation and desensitization in many brain structures after chronic THC administration (Oviedo et al., 1993; Rodriguez de Fonseca et al., 1994; Romero et al., 1995; Sim et al., 1996; Romero et al., 1997, 1998; Zhuang et al., 1998; Breivogel et al., 1999). These changes can follow distinct time courses in different regions suggesting that their regulation is quite complex and regionally heterogenous. In several of these studies, changes in CB_1 receptor properties in some brain areas were shown to parallel the development of tolerance to the acute locomotor depressing effects produced by THC administration, but the correspondence was not as evident in other areas (Oviedo et al., 1993; Rodriguez de Fonseca et al., 1994).

What is not completely understood is how these cellular events translate into the behavioral and physiological changes that are associated with repeated cannabinoid agonist treatment. Whole brain neuroimaging methods, such as metabolic mapping, may be particularly well-suited for assessing changes in CNS function associated with chronic THC exposure. The 2-[^{14}C]deoxyglucose method, which measures rates of local cerebral glucose utilization (LCGU) has been useful in characterizing the effects of the repeated administration of other pharmacological agents like alcohol, psychomotor stimulants, and opioids (Cohen et al., 1991; Porrino, 1992; Stein and Fuller, 1992, 1993; Pontieri et al., 1995; Kraus et al., 1997; Porrino et al., 1998). Such studies have demonstrated that the acute cerebral metabolic effects produced by the single administration of a drug differ from the acute effects following repeated drug administration. Although there have been a number of neuroimaging studies investigating the effects of both endogenous and exogenous cannabinoids (Goldman et al., 1975; Margulies and Hammer, 1991; Bloom et al., 1997; Stein et al., 1998; Pontieri et al., 1999; Freedland et al., 2002; Whitlow et al., 2002), these studies have focused mainly on the effects of a single acute drug administration. To date, few studies have employed neuroimaging methods to examine the consequences of cannabinoid administration on functional activity throughout the brain following repeated cannabinoid exposure.

Previous studies from this laboratory have demonstrated large and widespread decreases in rates of LCGU in the majority of structures analyzed after a single dose (10 mg/kg) of THC (Whitlow et al., 2002). The purpose of the present study was to determine if this pattern of acute cerebral metabolic decreases would be altered by repeated THC exposure, as measured by the quantitative autoradiographic 2-[^{14}C]deoxyglucose (2DG) method (Sokoloff et al., 1977). This investigation assessed whether the acute changes in rates of LCGU produced after THC administration diminish with repeated THC exposure, suggesting CNS adaptation to the effects of THC, or continue to be present despite repeated administration. Parallel studies measured the effects of repeated THC administration on spontaneous locomotor activity and core body temperature.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 300–375 g at the time of testing were single-housed in standard plastic cages and maintained in a temperature (20 ± 2 °C) and humidity ($50 \pm 10\%$) controlled vivarium on a 12/12 light/dark cycle (lights on at 7:00 AM). Food and water were available ad libitum. Rates of glucose utilization, locomotor activity, and core body temperature were measured in separate groups of animals. All procedures were performed in accordance with established practices as described in the Guide for the Care and Use of Laboratory Animals (National Research Council 1996). In addition, all protocols were reviewed and approved by the Animal Care and Use Committee of Wake Forest University School of Medicine.

For all animals included in the 2DG experiments, surgical procedures were performed a minimum of 24 h before the 2DG procedure to allow for anesthetic clearance and recovery. Rats were lightly anesthetized with a mixture of nitrous oxide and halothane; polyethylene catheters were subsequently inserted into a femoral artery and vein, then run subcutaneously to exit at the nape of the neck. Catheters were filled with heparinized saline (0.1 units/ml), coiled, and secured at the neck. Following surgery, animals were returned to their home cages and given access to food and water. To reduce variability in plasma glucose levels, animals were food-deprived for 8 h before the initiation of the 2DG procedure.

2.2. Drugs

THC was obtained from the National Institute on Drug Abuse (Rockville, MD) as a resin dissolved in 100% ethanol at a 50 mg/ml concentration. The ethanol/THC solution was suspended in a 1:4:1 ratio with Pluronic F68 detergent in ethanol and saline, as previously described (Heyser et al., 1993). Ethanol was evaporated under a stream of nitrogen, and the drugs were diluted to 10 mg/ml in saline for injection. Animals in the control groups received an equal volume of vehicle.

2.3. Behavioral and physiologic measurements

All behavioral and physiological measurements were made in separate groups of similarly treated animals from those used for the measurement of rates of LCGU.

2.3.1. Locomotor activity

Locomotor activity was measured in Plexiglas® test chambers (42 × 42 × 30 cm) by electronic counters that detected interruptions of 8 independent infrared photocell beams (Omnitech, Columbus, OH). Beam breaks were recorded and stored in 5 min bins. Rats were habituated to all experimental procedures, including injections, for 2 days prior to testing. On these days, rats were injected with saline and placed in the locomotor chambers for 30 min. Rats were randomly divided into two groups ($n = 4$): (1) vehicle or (2) THC 10 mg/kg. On the first day of testing, all rats received saline injections and were placed into experimental chambers 15-min post injection. Locomotor activity was recorded for 30 min, after which rats were removed and returned to their home cages. Rats then received single daily injections of THC (vehicle or 10 mg/kg/day) for 21 days. Locomotor activity was measured 15 min after injection of THC (vehicle or 10 mg/kg/day) on day 1, 7 and 21.

2.3.2. Core body temperature

Core body temperature was measured with a digital rectal thermocouple thermometer (Barnant Co, Barrington, IL). Rats were habituated to light hand restraint and rectal probe insertion for 2 days prior to testing. Rats were randomly divided into two groups ($n = 4$): (1) vehicle or (2) THC 10 mg/kg. On the first day of testing, baseline temperature was measured in all animals 15 min after the injection of saline. Rats then received single daily injections of THC (vehicle or 10 mg/kg/day) for 21 days. Core body temperature was measured 15 min after injection of THC (vehicle or 10 mg/kg/day) on day 1, 7 and 21.

2.4. Measurement of local cerebral glucose utilization

Rates of LCGU were measured according to the method (Sokoloff et al., 1977) as adapted for use in freely moving animals (Crane and Porrino, 1989). Separate groups of rats were habituated to all experimental procedures, including injections, for 5 days prior to testing. All procedures were carried out in the home cage. Groups of rats ($n = 5$–6) received single daily intraperitoneal (i.p.) injections of THC (vehicle or 10 mg/kg/day) for 7 or 21 days. The 2DG procedure was initiated 15 min after injection on day 7 or 21 via an intravenous infusion of a pulse of 2-[^{14}C]DG (100 μCi/kg; specific activity 55 mCi/mmol; New England Nuclear, New Boston, MA). Timed arterial blood samples were collected at approximately 5, 10, 15, 30 and 45 s, and 1, 2, 3, 5, 7.5, 15, 25, 35 and 45 min. Samples were then centrifuged immediately. Plasma concentrations of 2-[^{14}C]DG were determined by liquid scintillation counting (Beckman Instruments, Fullerton, CA) and glucose levels determined by a Beckman Glucose Analyzer II (Beckman Instruments). Immediately after the 45-min sample, animals were killed by intravenous administration of sodium pentobarbital. Brains were rapidly removed, cooled in isopentane at −45 °C, and stored at −80 °C until sectioning. Brains were sectioned coronally (20 μm) in a cryostat maintained at −20 °C, collected on glass coverslips and immediately transferred to a hot plate at 60 °C to dry. Coverslips were apposed to Kodak EMC film for 13–15 days along with a set of calibrated [^{14}C]methylmethacrylate standards (Amersham, Arlington Heights, IL) previously calibrated for their equivalent wet weight ^{14}C concentration. Films were developed in GBX developer (Kodak, Rochester, NY).

Autoradiograms were analyzed by quantitative densitometry with a computerized image analysis system (MCID, Imaging Research, St. Catharines, Ontario). Tissue ^{14}C concentrations were determined from densitometric analysis of autoradiograms of the calibrated standards. Rates of glucose utilization were then calculated using the optical densities and a calibration curve obtained from local ^{14}C tissue concentrations, time-courses of the plasma glucose and ^{14}C concentrations, and the constants according to the operational equation of the method (Sokoloff et al., 1977). Glucose utilization measurements were determined for 40 discrete brain regions according to the rat brain atlas of Paxinos and Watson (Paxinos and Watson, 1997). Each brain region was analyzed bilaterally in a minimum of five brain sections per animal.

2.5. Statistical analysis

Locomotor activity and core body temperature were analyzed by means of a two-way analysis of variance for repeated measures (dose × duration as the repeated measure), followed by appropriate post-hoc analysis. Statistical analysis of rates of LCGU was carried out for each brain region individually by means of a one-way analysis of variance followed by Bonferroni tests for multiple comparisons.

3. Results

3.1. Effects of the administration of THC on behavioral and physiologic measures

3.1.1. Locomotor activity

A statistically significant interaction of treatment × duration of drug administration, $F\ (2,18) = 9.42$, $P <$

0.001, was found such that the depressive effects of THC on spontaneous locomotor activity as compared to vehicle treatment differed across treatment duration (Table 1). Post hoc analyses revealed that the administration of THC significantly reduced locomotor activity following 1 and 7 days of treatment, but not after 21 days of exposure. Locomotor activity was significantly reduced on day 1 of THC treatment as compared to locomotor activity measured after 7 or 21 days of exposure. In addition, spontaneous locomotor activity was depressed on day 7 as compared to day 21.

3.1.2. Core body temperature

A statistically significant interaction of treatment × duration, F (2,18) = 57.38, $P < 0.001$, was found such that the effects of THC on core body temperature when compared to vehicle differed across duration of THC administration (Table 1). Post hoc analyses revealed that THC exposure significantly depressed core body temperature on day 1 of treatment, but not after 7 or 21 days of repeated treatment, when compared to controls. Core body temperature was significantly lower in those rats treated with THC for one day, as compared to those treated for 7 or 21 days.

3.2. Effects of the administration of THC on cerebral metabolism

After 7 days of repeated daily doses of THC rates of glucose utilization did not differ from values of glucose utilization of vehicle-treated controls in 28 of 40 regions analyzed. Conversely, significant differences were evident in the other 12 brain areas (Fig. 1). After 21 days of treatment rates of glucose utilization differed from controls in 10 brain regions (Fig. 1). These data are shown in Table 2. This pattern of changes in cerebral metabolism is in marked contrast to that observed following a single injection of THC where significant differences of up to 40–50% were seen in of 31 of 35 regions examined (Whitlow et al., 2002) assessed 15 min after THC exposure (Fig. 1). Differences expressed as percent changes between rates of glucose utilization of rats following a single acute administration of THC and vehicle-treated controls from this previous study (Whitlow et al., 2002) are shown in Table 2 for purposes of comparison with the present data.

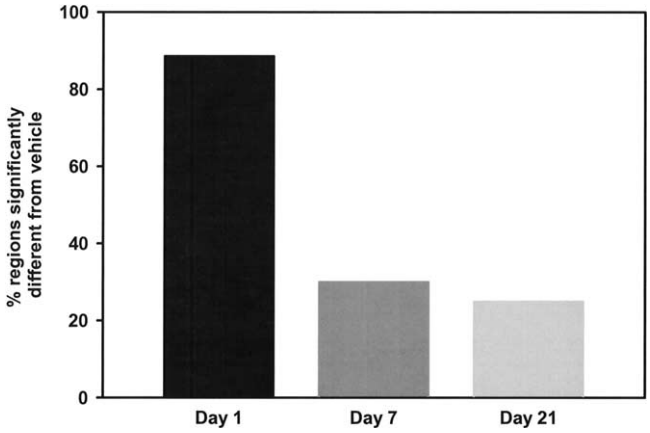

Fig. 1. Comparison of the effects of the administration of Δ^9- THC (10 mg/kg) on rates of LCGU measured in animals treated for 1, 7 and 21 days. Shown are the number of regions significantly decreased from vehicle treated baseline groups in 1, 7 and 21-day groups. Data for the 1-day group are taken from a previous study (Whitlow et al., 2002). Note that the development of tolerance to the effects of repeated THC administration on cerebral metabolism is rapid, occurring in 28 of 40 brain regions after 7 days, slowing thereafter, with only 2 more regions included at the 21-day time point (30 of 40 regions).

3.2.1. Seven-day THC treatment duration

The majority of significant decreases in rates of glucose utilization were in limbic areas and included the anterior and core portions of the nucleus accumbens, dorsomedial and ventral caudate, mediodorsal thalamus, and basolateral amygdala, as well as in the CA3 and CA1 fields of the hippocampus (Table 2). Cerebral metabolism was also significantly decreased in portions of the motor system including the dorsolateral caudate and globus pallidus. In addition, differences were

Table 1
Effects of repeated THC administration on locomotor activity and core body temperature

	Group	Baseline	1-day	7-day	21-day
Horizontal locomotor activity	Control ($n = 4$)	5379.5 ± 933	5476.5 ± 316	5596.0 ± 549	5142.3 ± 579
	THC ($n = 4$)	5810.5 ± 288	488.5 ± 110*,**,***	2698.1 ± 421*,***	5542.5 ± 326
Core body temperature	Control ($n = 4$)	100.6 ± 0.3	100.6 ± 0.3	100.2 ± 0.1	100.5 ± 0.4
	THC ($n = 4$)	100.8 ± 0.2	95.1 ± 0.5*,**,***	100.1 ± 0.2	100.6 ± 0.3

Shown are the effects of the administration of Δ^9-THC (10 mg/kg/day) on horizontal locomotor activity and core body temperature on day 1, 7 and 21 of treatment measured 15 min after injection. Data are expressed as means ± SEM of total beam breaks and degrees F, respectively. Two-way analysis of variance followed by multiple comparisons using Bonferroni adjustment.

* $P < 0.01$, different from vehicle-treated control values.

** $P < 0.01$, different from 7-day treatment duration values.

*** $P < 0.01$, different from 21-day treatment values.

Table 2
Effects of the administration of Δ^9-THC following repeated THC exposure

Structures	Vehicle ($n=6$)	7-day ($n=4$)	21-day ($n=5$)	1-day* % diff.	7-day % diff.	21-day % diff.
Infralimbic cortex	72.0±2	68.1±3	65.8±2	−39.5	−5.4	−8.6
Agranular insular cortex	75.8±1	77.4±5	74.2±3	−38.4	2.1	−2.2
Olfactory tubercle	85.2±2	78.6±3	77.5±1	−36.8	−7.7	−9.0
Anterior cingulate cortex	106.9±3	97.4±5	94.3±3	−41.6	−8.6	−11.8
Motor cortex	93.9±4	91.9±3	89.2±2	−39.2	−2.1	−5.1
Nucleus accumbens						
Anterior	92.9±3	72.4±6**	80.3±2**	−39.0	−22.1	−13.5
Core	80.0±2	72.7±3**	73.1±2	−38.2	−18.2	−14.9
Shell	74.6±2	66.2±2	68.7±2	−42.1	−11.2	−7.9
Corpus callosum	27.0±1	29.3±3	28.4±1	−19.0	8.4	5.1
Caudate						
Dorsomedial caudate	103.1±3	80.2±5**	89.8±1**	−39.4	−22.2	−12.9
Dorsolateral caudate	112.7±3	87.2±6**	96.7±1**	−38.7	−22.6	−14.2
Ventral caudate	97.2±2	77.6±4**	86.4±2**	−37.7	−20.2	−11.2
Lateral septum	54.1±2	47.3±5	44.2±1**	−40.0	−12.6	−18.3
Medial septum	79.2±2	73.3±5	76.1±2	−32.8	−7.4	−3.9
Bed nucleus of stria	44.2±2	40.2±3	45.6±1	−33.0	−9.1	3.1
Ventral pallidum	53.6±1	53.3±4	56.0±1	−31.9	0.5	4.5
Globus pallidus	52.2±1	43.7±4**	49.3±1	−40.6	−16.3	−5.5
Mediodorsal thalamus	110.9±3	89.0±7**	94.3±3**	−52.8	−19.8	−14.9
Amygdala						
Basolateral	83.9±2	65.9±5**	72.9±2**	−56.0	−21.4	−13.1
Central	42.6±2	43.6±3	43.6±2	−32.5	2.3	2.4
Entopeduncular nucleus	53.9±1	53.6±6	49.6±2	−40.4	−0.5	−8.0
Hypothalamus						
Medial preoptic	46.1±1	49.0±4	46.7±2	−28.7	6.3	−0.9
Lateral preoptic	77.5±2	73.4±3	73.3±2	−34.7	−5.3	−8.0
Anterior	54.6±4	50.3±2	49.5±1	−41.4	−7.9	−13.2
Lateral	62.6±2	56.3±5	58.1±2	−42.5	−10.1	−7.2
Hippocampus						
CA1	69.6±3	53.0±4**	58.8±2**	−44.8	−23.8	−15.5
CA3	78.6±3	55.5±4**	64.7±4**	−41.5	−29.4	−17.7
DG	58.0±2	54.1±4	54.8±2	−31.4	−6.7	−5.5
Subthalamic nucleus	96.2±3	91.2±6	93.8±3	−31.2	−5.7	−2.5
Auditory cortex	135.4±4	120.6±6	132.0±6	−39.3	−10.9	−2.5
Medial geniculate	132.8±6	114.5±7	123.0±3	−44.7	−13.4	−2.4
Substantia nigra						
Pars compacta	69.6±2	65.2±4	68.8±2	−29.9	−6.3	−1.2
Pars reticulata	56.5±1	56.7±4	56.7±2	−24.7	0.4	0.4
Ventral tegmental area	64.8±2	53.7±6**	60.1±2	−32.5	−17.1	−7.2
Superior colliculus	94.5±2	87.7±8	89.9±2	−39.3	−7.2	−4.9
Periaqueductal gray	72.0±3	65.9±2	65.8±1	−38.5	−8.5	−9.4
Dorsal raphe	83.8±2	75.7±3	82.2±3	−37.5	−9.7	−1.9
Median raphe	98.5±3	81.4±3**	88.3±1**	−44.5	−17.3	−10.4
Locus coeruleus	62.9±2	62.9±2	63.8±1	−32.9	−0.1	1.4
Cerebellum	70.6±3	61.4±4	61.2±2	−41.6	−13.0	−13.3

Shown are the rates of glucose utilization (μmol/100 g/min) of rats measured after 7 or 21 days of administration of Δ^9-THC (10 mg/kg) compared to rates of glucose utilization of vehicle-treated rats. Shown at right are percent differences between rates of glucose utilization measured after 1, 7 or 21 days of administration of Δ^9-THC and vehicle treatment. Data are expressed as means ±SEM.

* Data taken from Whitlow et al. (2002).

** $P < 0.05$, different from vehicle-treated control values, one-way analysis of variance followed by Bonferroni t-tests for multiple comparisons.

observed in the ventral tegmental area and median raphe. In all other brain areas examined, however, glucose utilization did not differ significantly from vehicle levels.

3.2.2. Twenty-one-day THC treatment duration

After 21 days of daily THC exposure glucose utilization was still depressed in many of the same regions altered after administration of 10 mg/kg/day THC for 7

days. The majority of decreases in rates of LCGU were again located in limbic regions, including, anterior accumbens, lateral septum, dorsomedial and ventral caudate, mediodorsal thalamus, basolateral amygdala, as well as in the CA1 and CA3 subfields of the hippocampus (Table 2). Rates of LCGU were also significantly decreased in the dorsolateral caudate and median raphe. Significant changes in glucose utilization were not evident in any other brain regions analyzed at this time point, with rates of glucose utilization equivalent to or approaching those of vehicle-treated controls. Furthermore, there were no significant differences between rates of glucose utilization measured on day 7 of THC treatment and those measured on day 21 of THC exposure (Table 2).

4. Discussion

The chronic administration of THC produces tolerance to many of its behavioral and physiological effects including antinociception, depression of spontaneous locomotor activity, hypothermia, catalepsy, and suppression of operant behavior. Tolerance to many of these effects develops rapidly and can in some cases be relatively complete after only a few exposures. The results of the present study in which the effects of THC were assessed after 7 or 21 days of drug treatment demonstrate a progressive decrease in the magnitude of changes in functional activity with repeated exposure to THC (Fig. 1). This is in sharp contrast to the large reductions in functional activity throughout virtually the entire central nervous system that have been shown to accompany a single acute injection of a similar dose of THC (Whitlow et al., 2002). It should be noted that in most areas tolerance to the effects of THC administration on cerebral metabolism appears to have developed almost completely by 7 days. The rate of tolerance development appears to have slowed appreciably after the first 7 days of drug exposure with tolerance manifest in only a few additional areas after 21 days. Although the magnitude of acute cerebral metabolic alterations produced by THC in the present study diminished throughout most brain regions following chronic THC exposure, there was a subset of structures localized primarily within the mesocorticolimbic system in which THC significantly altered functional activity even after 21 days of drug treatment.

In the present study, the administration of THC produced acute decreases in spontaneous locomotor activity and core body temperature that also were completely attenuated following repeated THC administration. The acute THC-induced effects on core body temperature, however, returned to control levels following a shorter duration of THC exposure than was required for the complete attenuation of the acute locomotor effects. That this attenuation of behavioral and physiological effects occurred at different rates in the present study is consistent with the variable time-course of the development of tolerance to the behavioral and physiological effects of THC in other studies. Acute decreases in core body temperature produced after the initial injection of THC were no longer apparent following 4 days of administration (Perron et al., 2001). In contrast, locomotor activity was significantly decreased in rats that received a single injection of THC, but no different than vehicle treated controls after THC administration for 7 days (Oviedo et al., 1993). Delayed match-to-sample performance, a measure of short term memory, was profoundly impaired following initial administration of THC, but recovered by 75–80% after 21 days of exposure, and completely recovered after 30–35 days of THC administration (Deadwyler et al., 1995), again demonstrating that tolerance to repeated THC administration develops at different rates depending on the behavioral effects assessed.

The differential temporal course of the development of tolerance to acute THC-induced effects on locomotor activity, core body temperature, and short term memory parallels the cerebral metabolic adaptations in CNS structures. In the present study, the lack of any significant core body temperature changes following 7 or 21 days of THC administration is consistent with a similar absence of cerebral metabolic alterations in hypothalamic regions, which mediate many of these actions, at these same time points. Similarly, rates of LCGU in globus pallidus, for example, a motor-related structure, were depressed after 7 days, but not 21 days, of THC administration, which parallels the THC-induced changes in locomotor activity. It is interesting to note that decreases in cerebral metabolism were still evident in CA1 and CA3 portions of the hippocampus following THC for 21 days, paralleling the disruptions of delayed match-to-sample performance that endure even after the administration of THC for this duration (Deadwyler et al., 1995). These data suggest that adaptations to the acute cerebral metabolic effects of THC occur with distinct temporal courses in different neuroanatomical circuits paralleling the variable development of tolerance to some of the physiological and behavioral effects produced by THC.

In contrast to the majority of brain structures analyzed in which rates of LCGU were no longer different than controls following THC for 7 or 21 days, acute THC-induced cerebral metabolic decreases were still evident in some brain areas. These structures included the nucleus accumbens, caudate, basolateral amygdala, mediodorsal thalamus, portions of the hippocampus, and the median raphe. Although some adaptation to the acute effects of THC following chronic THC administration was evident, adaptation to the acute cerebral metabolic effects of THC was not

complete in some regions, mainly within the limbic system, even after 21 days of drug exposure.

That significant cerebral metabolic effects are still evident in these regions following prolonged THC administration is somewhat surprising given the relatively rapid and robust CB_1 receptor downregulation and desensitization that has been reported to occur in many of these same structures. For example, CB_1 receptor downregulation occurred most rapidly in hippocampus, as significant decreases were evident 24 h after a single dose of THC (Romero et al., 1998), although the presence of residual drug may have been responsible for these effects. Furthermore, in comparison to other brain regions, the CA1, CA3, and dentate gyrus subregions of the hippocampus exhibited the largest CB_1 receptor binding decreases following the administration of THC for 14 days (Romero et al., 1998). The hippocampus also exhibited the most rapid and robust CB_1 receptor desensitization, as WIN 55212-2-stimulated [^{35}S]GTPγS binding was significantly decreased after 3 days, and exhibited a relatively large and stable 35% decrease following THC administration for 7 and 21 days (Breivogel et al., 1999). In the present study, significant cerebral metabolic decreases in the hippocampus were observed even after THC administration for 7 or 21 days. Acute cerebral metabolic decreases after 7 or 21 days of THC administration were also evident in other areas that have been shown to exhibit large magnitude CB_1 receptor downregulation and desensitization following chronic THC exposure (e.g. nucleus accumbens, caudate, basolateral amygdala, mediodorsal thalamus, and median raphe) (Oviedo et al., 1993; Sim et al., 1996; Romero et al., 1998; Breivogel et al., 1999). Even very large magnitude CB_1 receptor changes, in the context of chronic THC exposure, therefore, may not result in complete adaptation of CNS functional activity.

There are several potential explanations for the presence of persistent functional activity changes observed in the present study. It is possible that 21 days of treatment was simply not of sufficient duration to produce maximal effects on CB_1 receptor regulation, leading to incomplete adaptation. Longer periods of exposure to THC may be required to produce tolerance in these brain regions. Another factor that may have contributed to the continued alterations in functional activity is the involvement of other transmitter systems. The activation of CB1 receptors has been shown to produce interactions with dopamine and GABA systems, among others (see Maldonado and Rodriguez de Fonseca, 2002). Many of the regions in which continued alterations were observed after chronic THC exposure are components of the nigrostriatal and mesolimbic dopamine systems. Persistent changes in rates of LCGU, therefore, might result from compensatory changes in the regulation of dopamine or other non-cannabinoid neurotransmitter-receptor systems following repeated THC administration. Along these lines, THC has been shown to produce behavioral and physiological effects in CB_1 receptor knockout mice, suggesting that cannabinoids may also directly affect the activity of non-CB1 receptors in the CNS (Di Marzo et al., 2000; Breivogel et al., 2001). Whether or not these non-CB_1 mediated effects would diminish or remain evident following repeated THC exposure is not know. Finally, recent reports have shown that different brain regions have unique distributions of G-protein subunits with different potencies and/or efficacies (Prather et al., 2000). It is possible that some combination of G-protein subunits may be more resistant to the development of tolerance.

It is interesting to note that many of persistent metabolic decreases were localized largely within the mesocorticolimbic system. That functional activity remains depressed primarily in limbic structures, known to be involved in the processing of motivational and emotional information, suggests that Δ^9-THC may still affect these behaviors even after prolonged exposure to the drug. THC-induced alterations in limbic structures despite chronic THC administration may reflect the fact that, in humans, the acute subjective effects produced by marijuana use (e.g. ratings of 'high') remain evident after repeated marijuana exposure (Perez-Reyes et al., 1991). In addition to potentially mediating subjective cannabinoid effects, changes in limbic-related regions, such as the basolateral amygdala, may have other important behavioral implications for habitual marijuana users. Recent new data, for example, has shown that cannabinoids may play a role in the regulation of adverse memories via actions in the basolateral amygdala (Marsicano et al., 2002). In addition, the continued depression of functional activity within portions of the hippocampus may be related to the deficits in cognitive function that have been associated with long term marijuana use (cf. Pope and Yurgelun-Todd, 1996; Pope et al., 2001; Solowij et al., 2002).

In summary, the present report describes the acute effects of THC on rates of LCGU, following chronic THC exposure. In the majority of structures, rates of LCGU are no different than control levels following the administration of THC for 7 or 21 days. In a subset of regions concentrated within the extended amygdala and mesolimbic system, however, acute decreases in metabolic activity are still evident after 7 and 21 days of THC exposure. That THC produces acute alterations in functional activity primarily in mesolimbic and amygdalar regions, despite repeated THC administration, suggests that behaviors subserved by these structures (e.g. anxiety, stress, reward and memory) may continue to be affected by THC, even after 21 days of exposure.

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