ORIGINAL ARTICLE

Dosage and Conditioning Period Determine Reward or Aversion to Cannabis-induced Conditioned Place Preference in Sprague-Dawley Rats

LE Young, KP Chin-Quee

ABSTRACT

Objective: *To assess the addictive potential of cannabis by investigating the motivational responses to low doses of delta-9-tetrahydrocannabinol (Δ^9-THC) and a marijuana tea extract (MTE), and to determine if the length of the conditioning period in the conditioned place preference (CPP) paradigm influences reward or aversion to these cannabinoid preparations.*
Methods: *Thirty-eight Sprague-Dawley rats were taken through a biased CPP paradigm utilizing an eight-day schedule. The pre-conditioning phase consisted of three trials of 15 minutes each, and the conditioning phase lasted either 40 or 18 minutes in the drug-paired, 'non-preferred' white chamber or the vehicle-paired, 'preferred' black chamber. Spontaneous motor activity (SMA) was used to determine the 18-minute conditioning period, dosage of a crude MTE and the oil-based vehicle for Δ^9-THC (coconut oil), which did not alter the SMA of the rats. Differences in the mean times spent in the 'non-preferred' white chamber during the pre-conditioning and post-conditioning periods were compared using paired* t-test*.*
Results: *Significant place aversion (*$p < 0.0001$*) to the MTE occurred at the 40-minute conditioning period, but not at the 18-minute period. Also, significant CPP reward (*$p < 0.01$*) to 0.05 mg/kg Δ^9-THC occurred with the reduced 18-minute conditioning period, while a non-significant increase in post-conditioning time at the higher dose of 2.0 mg/kg Δ^9-THC was obtained.*
Conclusion: *Drug-seeking, motivational reward to 0.05 mg/kg Δ^9-THC confirmed the addictive potential of Δ^9-THC. However, the duration of the conditioning period in the CPP design was a determinant of the outcome to CPP-reward or -aversion to marijuana.*

Keywords: Addiction, aversion, cannabis, conditioned place preference, delta-9-tetrahydrocannabinol, marijuana, motivation, reward

La Dosificación y el Período de Condicionamiento Determinan la Recompensa o la Aversión a la Preferencia del Lugar Condicionada por Inducción con Cannabis en Ratas Sprague-Dawley

LE Young, KP Chin-Quee

RESUMEN

Objetivo: *Evaluar el potencial adictivo del cannabis investigando las respuestas motivacionales a dosis bajas de delta-9-tetrahidrocannabinol (Δ^9-THC) y un extracto de té de marihuana*

From: Department of Basic Medical Sciences (Physiology Section), The University of the West Indies, Mona, Jamaica, West Indies.

Correspondence: Dr L Young, Department of Basic Medical Sciences (Physiology Section), The University of the West Indies, Mona, Kingston 7, Jamaica, West Indies. Email: lauriann.young@uwimona.edu.jm

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(ETM), y determinar si la longitud del período de condicionamiento en el paradigma de preferencia al lugar condicionada (PLC), influye sobre la recompensa o la aversión a estas preparaciones del cannabinoide.

Métodos: *Treinta y ocho ratas Sprague-Dawley fueron llevadas a través de un paradigma sesgado PLC utilizando un programa de ocho días. La fase de acondicionamiento previo consistió en tres ensayos de 15 minutos cada uno, y la fase de acondicionamiento duró ya sea 40 o 18 minutos en la cámara blanca 'no preferida' pareada con la droga, o la cámara negra 'preferida' pareada con el vehículo. La actividad motora espontánea (AME) fue utilizada para determinar el período de condicionamiento de 18 minutos, la dosificación de un ETM crudo y el vehículo a base de aceite para el* Δ^9*-THC (aceite de coco), que no alteró la AME de las ratas. Se compararon las diferencias en los tiempos promedios en la cámara blanca 'no preferida' durante los períodos de pre-condicionamiento y de post-condicionamiento mediante el uso de* t-tests *pareados.*

Resultados: *Aversión del lugar significativa (*$p < 0.0001$*) frente al ETM ocurrió en el período de condicionamiento de 40 minutos, pero no en el período de 18 minutos. Por otro lado, la recompensa significativa de PLC (*$p < 0.01$*) para 0.05 mg/kg* Δ^9*-THC se produjo con el período de acondicionamiento reducido de 18 minutos, en tanto que se obtuvo un aumento no significativo en el tiempo de post-acondicionamiento con una dosis más alta aumentada a 2.0 mg/kg* Δ^9*-THC.*

Conclusión: *La recompensa motivacional por busca de drogas en relación con 0.05 mg/kg* Δ^9*-THC confirmó el potencial adictivo de* Δ^9*-THC. Sin embargo, la duración del período de acondicionamiento en el diseño PLC fue un factor determinante en el resultado de recompensa o la aversión del PLC frente a la marihuana.*

Palabras clave: Adicción, aversión, cannabis, preferencia del lugar condicionada, delta-9-tetrahidrocannabinol, marihuana, motivación, recompensa

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INTRODUCTION

Marijuana, also known as cannabis or ganja, is the most frequently abused illicit drug in the United States of America (USA) and other countries (1). Jamaica has had a long history and association with the cultivation and use of marijuana, where it is popularly smoked, drunk or eaten for recreational, religious and medicinal purposes. This widespread use of marijuana, despite its illegal status, strongly suggests that it has addictive or reinforcing properties. However, the *Cannabis sativa* plant contains not only the psychoactive compound, delta-9-tetrahydrocannabinol (Δ^9-THC), but also a myriad of other non-psychoactive cannabinoid compounds, such as cannabidiol. Marijuana is often used as a pharmacopeia for many disorders. As a result, marijuana compounds and preparations are under active investigation for their medicinal properties. The popularity of marijuana as both a recreational drug and a medicinal herb (2) is likely to increase its abuse potential.

Behavioural demonstration of the reinforcing or rewarding property of a drug helps to establish its addictiveness. Unlike humans, animals do not voluntarily consume or self-administer marijuana, and the rewarding properties of Δ^9-THC are difficult to demonstrate in rodents using standard procedures (3). In fact, experimental data obtained from the classical conditioned place preference (CPP) animal model of addiction show that rodents often exhibit conditioned place aversion due to the dysphoric properties associated with the psychoactive Δ^9-THC. Humans have also reported dysphoria associated with the use of marijuana containing high levels of Δ^9-THC. Thus, the inconsistencies regarding the addictiveness of marijuana may be partly due to the biphasic euphoric and dysphoric effects associated with the drug. Depending on dosage and time phase, drugs such as alcohol and marijuana can act as either stimulants or depressants of the central nervous system. Thus, modifications of the classical experiments using

animal addiction models of reward (including a period of sensitization, attention to drug dosages that produce stimulation, and time of recording after drug administration) have been demonstrated for drugs that can produce addiction in humans, but do not generally elicit a reward or motivational response in animals. Additionally, reports on the effective dose of Δ^9-THC in rodents that produces drug reward have been inconsistent. We therefore hypothesised that motivation to drug-seeking behaviour at low doses of marijuana or Δ^9-THC administration could be achieved by modification of the confinement phase of the CPP paradigm to minimize the dysphoric cue-associated effects of Δ^9-THC in order to increase CPP-induced drug reward.

SUBJECTS AND METHODS

Subjects

A total of 40 Sprague-Dawley rats weighing 300–350 g were initially selected for the study; 38 rats completed the study. They were housed two to three per cage under normal laboratory conditions and were provided with food and water *ad libitum*. Rats were also habituated to the test room of the behavioural laboratory and handled three to four minutes daily over three days prior to the start of the CPP paradigm. Spontaneous motor activity (SMA) of the rats was quantitatively assessed using a computerized jiggle platform (model: BRS/LEV JPA-001, USA) in order to select an inert vehicle for dissolving the Δ^9-THC that was without stimulant effect and determine the approximate onset at which drug-induced hypomotility in rats occurred. The observed start of hypomotility was equated to the approximate onset of the dysphoria-induced effect of the marijuana tea or Δ^9-THC.

Preparation of the marijuana tea extract

Dried leaves and stems of a marijuana plant (*Cannabis sativa*, 100 g) used for recreational purposes were boiled under reflux for six hours, and the resulting liquor was vacuum-filtered. The filtrate was subsequently distilled, and the distillate freeze-dried to a powder at the International Centre for Environmental and Nuclear Sciences, The University of the West Indies, Mona, Jamaica. The distillate (1 L) yielded 127 g of the freeze-dried powder. A stock solution of the marijuana tea extract (MTE) was first made by dissolving 7.5 g of the freeze-dried powder in 25 ml of saline. This was then diluted to produce half its concentration and used as the diluted MTE in the experiments.

Drug administration

The THC-ethanol solution (Sigma, USA) was evaporated under a stream of nitrogen gas to separate the pure Δ^9-THC. Coconut oil, which was found to have the least effect on the SMA of rats, was used as the inert vehicle to dissolve the Δ^9-THC. The MTE was dissolved in saline as previously described. Drugs and vehicle were administered by intraperitoneal injection (*ip*) in a volume of 1.0 ml/kg body weight.

Conditioned place preference paradigm

The CPP paradigm utilized a three-compartment wooden shuttle box with a black and a white compartment of equal dimensions (30 x 30 x 15 cm) separated by a neutral grey corridor (15 x 30 x 15 cm), that was fitted with removable partitions to allow easy access to the other chambers. The floor of the white compartment was covered with wood shavings, while the floor of the black chamber was covered with wire mesh, to provide tactile and visual cues that biased the CPP paradigm. Following habituation, rats were allowed daily access to all three compartments of the CPP box for 15 minutes per day for three consecutive days; the last day served as an indication of the animal's initial place preference. Time spent in each chamber was recorded as the pre-conditioned time. Two drug-naïve rats that did not show at least 80% preference for the black ('preferred') chamber were removed from the study. Rats were randomly assigned to three treatment groups consisting of equal numbers of male and female rats. For the conditioning trials, rats were administered either the dilute MTE (1.0 ml/kg, *ip*) or Δ^9-THC (0.05 and 2 mg/kg, *ip*) and confined to the 'non-preferred' white chamber for either 18 or 40 minutes on days 1, 3, 5 and 7. On alternate non-drug days of an eight-day schedule, rats were injected with vehicle (*ie* coconut oil or saline, 1.0 ml/kg) and confined to the 'preferred' black chamber. On day 9, the test day, each rat was given free access to all three chambers for 15 minutes and the time spent in each chamber again recorded.

Statistical analyses

The effect of the two doses of Δ^9-THC and MTE on the mean time spent in the drug-paired, 'non-preferred' white chamber on the test day was compared with the pre-conditioned mean times. Differences in the means for the 40-minute and 18-minute confinement periods of the conditioning phase of the CPP were also compared. All data were analysed using Students' paired *t-test*. Significance level was taken at $p < 0.05$.

RESULTS

Animals that were administered the MTE and confined to the 'non-preferred' white chamber of the CPP box using a 40-minute conditioning period exhibited a significant reduction ($p < 0.0001$) in post-conditioning time on the test day as compared with their pre-conditioned time (Fig. 1). In contrast, both male (Fig. 2) and female (Fig. 3) MTE-treated rats exhibited a non-significant increase in the post-conditioning mean time spent in the 'non-preferred' white chamber when an 18-minute conditioning period was used in the experimental design. Thus, the shortened conditioned period in the MTE-paired chamber resulted in reduced place aversion when compared to the longer confinement of 40 minutes. Rats administered 0.05 mg/kg of Δ^9-THC and conditioned for 18 minutes in the drug-paired 'non-preferred' chamber of the CPP exhibited a significant increase ($p < 0.01$) in time spent on the test day as compared with their pre-conditioned mean time (Fig. 4). However, for a similar 18-minute conditioning period, rats treated with 2.0 mg/kg of Δ^9-THC exhibited a non-significant ($p > 0.02$) increase in post-conditioned mean time in the white chamber. There were no observed hypomotility effects with the low dose of Δ^9-THC, but the effects were seen with the MTE.

DISCUSSION

This is the first reported study of significant CPP-induced reward with 0.05 mg/kg of Δ^9-THC and supports evidence of an addictive potential of marijuana at a very low dose in an animal model of addiction using Sprague-Dawley rats. Marijuana, Δ^9-THC and other synthetic cannabinoid agonists, such as CP 55 940 (4), WIN 55 212-2 (5) and HU 210 (6), generally induce conditioned place avoidance rather than place preference in rats and mice. Data also suggest that higher doses of Δ^9-THC are more likely to produce aversion than lower doses (7). In this study, the hypomotility effects of the marijuana tea may also suggest that it contained higher levels of Δ^9-THC, especially given that the extract was prepared from plants grown for recreational use. Another interesting finding of the study is that the female rats exhibited a stronger motivational behaviour to the drugs, suggesting an oestrogenic effect on CPP-induced reward to Δ^9-THC. This observed CPP to cannabinoids by female rats has been shown to be reversed when they are ovariectomised (8). Studies on THC-induced CPP have been reported at limited dose-ranges and under restricted experimental conditions in mice, Long Evans and Wistar rats (3, 9, 10).

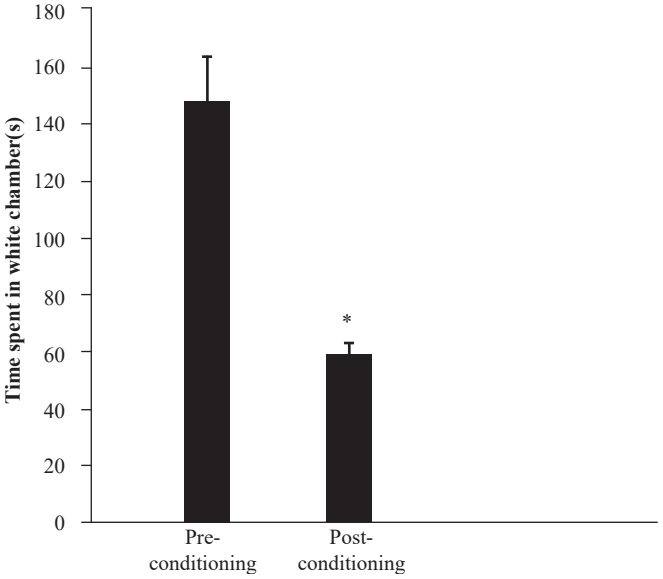

Fig. 1: Graph showing pre-conditioned and post-conditioned mean times spent by male Sprague-Dawley rats (n = 14) that were treated with the marijuana tea extract (MTE) and confined to the non-preferred, white chamber using a 40-minute conditioning period.

* Post-conditioning time for MTE-treated rats was significantly reduced ($p < 0.0001$).

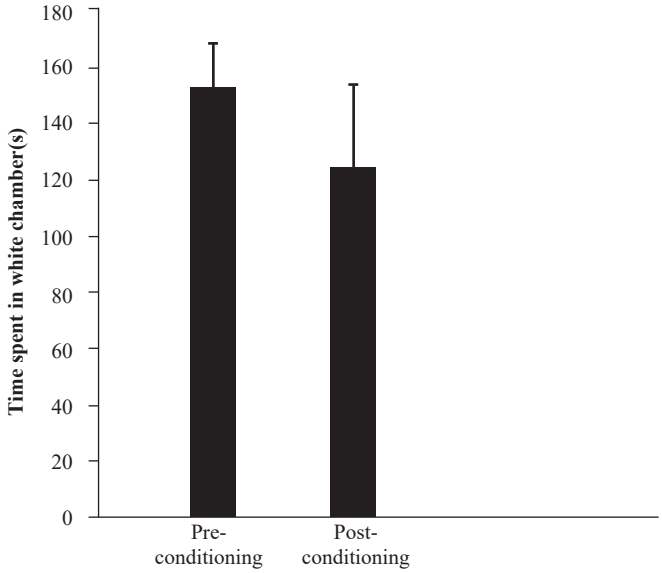

Fig. 2: Graph showing pre-conditioned and post-conditioned mean times spent by male Sprague-Dawley rats (n = 6) that were treated with the marijuana tea extract and confined to the non-preferred, white chamber using an 18-minute conditioning period. Post-conditioned mean time was not significantly different from pre-conditioned mean time ($p > 0.05$).

While the literature is replete with evidence for self-administration behaviour in animal studies of drugs abused by humans (including psychostimulants, opiates, ethanol and nicotine), there has been a paucity of similar studies for marijuana (11). However, other evidence points to the addictive potential of marijuana and includes the lowering of electrical brain-stimulation reward thresholds and the increased firing rate of ventral tegmental dopaminergic neurons that project to the nucleus accumbens and result in an increased release of dopamine (12–14).

The subjective 'high' that follows marijuana use in humans seems to peak in about 15 minutes and may correspond to an increased heart rate (15). Like alcohol (16), marijuana appears to exhibit an early stimulant or euphoric phase, followed by a dysphoric or depressive phase and explains the requirement for modifications to the classical experiments to demonstrate reinforcing or motivational behaviour to the drug. Thus, the often-used conditioning period of 30 to 40 minutes could cause animals to cue-associate the drug-paired environment of the CPP with the dysphoria-induced effects of Δ^9-THC. Interestingly, a similar divergence of CPP-induced reward with conditioning time was observed for cocaine where it was shown that administration 15 minutes before CPP induced cocaine aversion (17, 18). This temporal dependence of drug-induced reward further explains our data that showed marijuana aversion with a long conditioning period and a reversal when the period was reduced.

Limitations of the study

The limited supply of animals restricted the number of dosages of the drug that could be investigated. There may have been a possible hormonal effect on cannabis-induced reward which could have influenced the results of this study. For comparison, the drugs were administered intraperitoneally. Normally, teas are ingested and subject to digestive processes that may alter the cannabinoids and, consequently, the addictive potential of the tea.

CONCLUSION

Conditioned place preference reward to a 0.05 mg/kg dose of Δ^9-THC confirmed its addictive potential. Marijuana has both euphoric and dysphoric effects mainly due to Δ^9-THC. Thus, dosage of the drug and duration of the conditioning period in the CPP paradigm are factors that will influence the outcome of the experimental design with respect to conclusions regarding the addictive potential of marijuana or the psychoactive component Δ^9-THC.

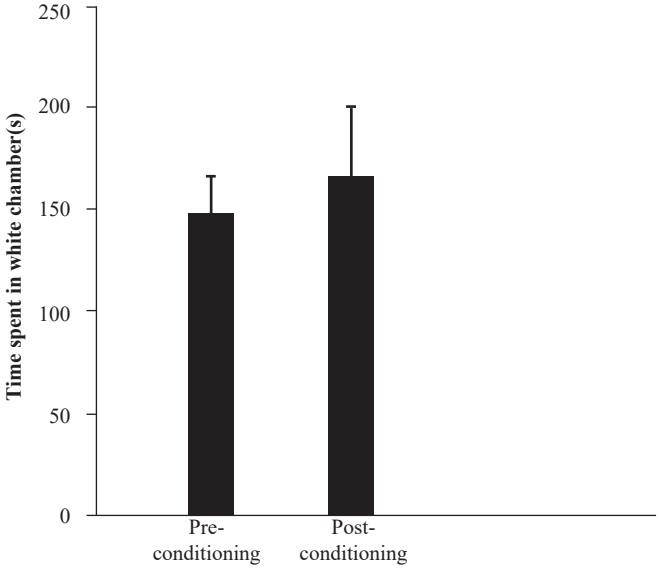

Fig. 3: Graph showing pre-conditioned and post-conditioned mean times spent by female Sprague-Dawley rats (n = 6) that were treated with the marijuana tea extract and confined to the non-preferred, white chamber using an 18-minute conditioning period. Post-conditioned mean time was not significantly different from pre-conditioned mean time ($p > 0.05$).

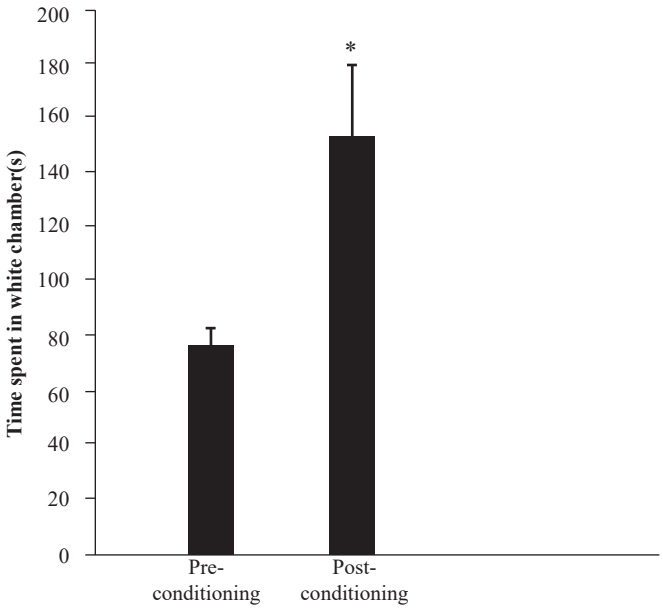

Fig. 4: Graph showing pre-conditioned and post-conditioned mean times spent by female Sprague-Dawley rats (n = 6) that were treated with 0.05 mg/kg of delta-9-tetrahydrocannabinol and confined to the non-preferred, white chamber using an 18-minute conditioning period.

* Post-conditioned mean time was significantly different from pre-conditioned mean time ($p < 0.01$).

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AUTHORS' NOTE

The authors declare that they have no conflicts of interest.

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