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Δ⁹-Tetrahydrocannabinol Produced Positive Place Preference in Mice without Significant *Ex-Vivo* Effect on Hepatic Arylamine N-Acetyltransferase Activity: Implications for Its Addictive Liability and Absence of Effect on Xenobiotic Metabolism

Lauriann Young[1*], Karen Thaxter[1], Danielle Campbell[1], Sheena Francis[2], Nicola Laurieri[3], Rupika Delgoda[2]

[1]Physiology Section, Department of Basic Medical Sciences, The University of the West Indies (UWI), Mona Campus, Kingston, Jamaica
[2]Natural Products Institute, The University of the West Indies (UWI), Mona Campus, Kingston, Jamaica
[3]Department of Pharmacology, Oxford University, Oxford, England, UK
Email: *lauriann.young@uwimona.edu.jm

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Abstract

Aim: Δ⁹-Tetrahydrocannabinol (Δ⁹-THC) is a potentially addictive cannabinoid. Its impact on the activity of liver arylamine N-Acetyltransferase (NAT) has not been reported. This study investigated the rewarding effects of Δ⁹-THC in mice and whether Δ⁹-THC had any impact *ex-vivo* and *in-vitro* on NAT activity. **Methods:** Thirty-six Swiss albinomice randomly assigned to six groups (n = 6) completed a biased, 8-week Conditioned Place Preference (CPP) paradigm. Mice exhibiting ~80% preference for the black chamber at pre-conditioning were selected. Treatment groups were administered Δ⁹-THC (0.10, 0.50 or 2.0 mg/kg/mL, *ip*) or amphetamine (AMP, 5.0 mg/kg/mL, *ip*); while untreated groups (controls) received vehicle solutions (coconut oil or 0.9% saline). Entries and time spent in the white, drug-paired chamber during a 15-min post-conditioning exploration of the CPP apparatus were compared with the pre-conditioning exploratory scores. Livers from Δ⁹-THC treated and untreated mice were excised and NAT enzyme activity determined *ex-vivo* using a spectrophotometric assay with p-anisidine as substrate. The impact of varying concentrations of Δ⁹-THC (0.00 - 162 μM) on the activities of NAT from untreated mice livers were also investigated *in-vitro.* **Results:** Δ⁹-THC treated mice entered and spent significantly more time in the drug-paired CPP chamber

($p \leq 0.05$) at post-conditioning vs pre-conditioning ($F = 11.22$). Mice treated with 2.0 mg/kg Δ^9-THC made significantly more entries into the drug-paired chamber ($p \leq 0.05$) as compared with their vehicle controls. AMP-treated mice displayed significant ($p < 0.001$) increases in both entries and time spent in the drug-paired chamber at post-conditioning (positive place preference). *In-vitro* NAT evaluations revealed a dose-dependent inhibitory impact of Δ^9-THC on NAT activity with an IC_{50} value of 34.97 ±1.58 μM. *Ex-vivo* evaluations of livers from Δ^9-THC treated mice showed no significant impact on liver NAT enzyme activity. **Conclusion:** Δ^9-THC induced significant conditioned place preference (drug reward) and produced a moderate dose-dependent inhibition on NAT activity *in-vitro*, but not *ex-vivo*.

Keywords

Cannabis, Marijuana, Conditioned Place Preference, Addiction, Drug Reward

1. Introduction

Delta-9-tetrahydrocannabinol (Δ^9-THC) is the principal psychoactive component of the *Cannabis* plant, popularly known as marijuana. It is often introduced by smoking marijuana and the plant is being increasingly bred to avail higher Δ^9-THC to the user [1] [2]. Δ^9-THC is a partial agonist at endocannabinoid receptor subtypes CB1 and CB2 which are abundant in the brains of humans and rodents. These sites are implicated in functions that include regulation of mood, memory and reward processing [3]. Δ^9-THC's ability to induce euphoria in humans makes it potentially addictive. Han, *et al.* (2017) [4] attributed cannabis reward to activation of cannabinoid CB1 receptors on GABAergic neurons which then disinhibit dopaminergic neurons in the ventral tegmental area of the brain; and activation of CB1 receptors in glutamatergic neurons to aversion in rodents. They extrapolated that such neural interactions may explain the variety of reported hedonistic effects in humans [4]. However, rodents do not naturally consume Δ^9-THC or whole plant cannabis extracts. Thus, there is contradicting evidence to support and refute the addictive liability of Δ^9-THC from animal studies, due to reported aversive and rewarding drug effects [5] [6] [7].

The pharmacological effects of Δ^9-THC are largely dictated by the levels found in the plasma, which in turn is subject to metabolic processes primarily in the liver. Cytochromes P450 (CYP) superfamily of enzymes (specifically CYPs2C9, 2C19 and 3A4) and Uridine Diphosphate (UDP)-glucuronosyltransferases, have been identified as the main catalysts responsible for Δ^9-THC metabolism [8]. Inhibitors and inducers of these enzymes (as well as those of transporters) and genetic polymorphisms, may yield variability of Δ^9-THC plasma levels. Equally importantly, the presence of Δ^9-THC has been known to impact other key CYP enzymes (CYPs1A2, 2D6), in turn invoking undesirable drug-associated toxicities [9]. While there are increasing probes into the impact of Δ^9-THC on phase I en-

zymes, there is a dearth of information on its impact on several phase II metabolizing enzymes.

Arylamine N-Acetyltransferase (NAT) belongs to a set of cytosolic, polymorphic, phase II drug-metabolizing enzymes found expressed in many animal species including mouse and man, which acetylates a range of xenobiotics, including drugs, environmental pollutants and carcinogens [10]. They catalyze the conjugation of an acetyl group from acetyl coenzyme A to arylamines, hydrazines and N-hydroxyarylamines, using a ping-pong bi-bi mechanism where acetyl CoA and substrate bind to a Cys-His-Asp catalytic triad [11] [12] followed by the transfer of the acetyl moiety to the substrate. Selective inhibitors of prokaryotic and human NATs have been examined for use as therapeutic leads for tuberculosis [13] and breast cancer [14] although co-administration of such inhibitors in man may lead to drug adversities from pharmacokinetic drug interactions [15]. There is no reported data of Δ^9-THC on the activity of NAT enzymes in either a human or animal model.

The slow or rapid metabolism subject to the genetic variability of the polymorphic NAT2 enzyme, in various ethnicities, has been shown over the years to have important therapeutic and toxicity implications in pharmaceuticals such as isoniazid, hydralazine, sulfamethazine, amifamirpiridne, procainamide, sulfasalazine, amonafide and metamizole. Slow NAT2 acetylators seem to be more at risk from adversities arising from accumulated toxic intermediates, and thus a higher disposition to various cancers [14]. Albeit the scores of findings over the years, a recent review concludes that [16] phenotype/genotype remain somewhat ambiguous. While conclusions for genetic heterogeneity may require further evidence, there seems little doubt that pharmacokinetic alterations that impede the activity of NAT enzyme, can have clinical implications on drug efficacy and toxicity. Inhibition of enzyme activity can lead to elevated co-administered drugs or intermediates awaiting their metabolism by NAT. This is true also in the case of the recreational psychoactive designer drugs, "2C" derived from 2,5-dimethoxyphenethylamine, which have been shown to be reliant on NAT [17] for their N-acetylation-driven metabolism. In the space of psychoactive drug users, where co-use with multiple agents is likely, garnered data on potential interactions can be of tremendous use.

Therefore this investigation sought to elucidate the impact of Δ^9-THC on the activities of NAT enzyme *ex-vivo*, employing a Swiss albino mouse model, which was set up to also gauge the behavioral responses to three doses of Δ^9-THC, using a modified Conditioned Place Preference (CPP) paradigm.

2. Materials and Methods

2.1. Subjects

Forty (40) experimentally naïve Swiss albino mice weighing 22 - 24 g were initially obtained from the Animal House of The University of the West Indies (UWI), Mona Campus, housed in pairs and habituated in a Behavioral Laboratory main-

tained at 22˚C and 45% relative humidity with a 12:12 h light/dark cycle. The mice received food and water *adlibitum*. Institutional guidelines for the care and use of the mice as well as ethical approval for the animal study prior to the start of the investigation were obtained from the UWI Ethics Research Committee (ECP 01, 17/18).

2.2. The Conditioned Place Preference (CPP) Apparatus

A biased paradigm utilizing a three-chambered wooden box with visual cues was used in this study as previously described [18] [19]. Each chamber of the box (dimensions: 75 × 30 × 25 cm) was painted white, black or grey. The black and white chambers each measured 30 × 30 cm; while the grey chamber measured 15 cm × 30cm. The central grey chamber or corridor facilitated movement of the mice between the white and black chambers of the box. Perspex sliding doors (removable partitions) were used to isolate the grey chamber from the black and white chambers. A removable Perspex lid on the roof of the box enabled uninterrupted observation of the rodent's locomotory behavior. The apparatus was cleaned thoroughly between each mouse exploration.

2.3. Drugs

Δ^9-THC (100 mg/mL, Sigma-Aldrich) dissolved in ethanol was prepared for intraperitoneal injections (*ip*) by evaporating the ethanol from the THC:ethanol solution under a stream of nitrogen gas. A stock solution containing 50 mg Δ^9-THC was then dissolved in I mL of a local commercially manufactured cold-pressed virgin coconut oil vehicle from which dilutions of 0.10, 0.50 and 2.0 mg/kg/mL Δ^9-THC dissolved in coconut oil were prepared for *ip* administration. Amphetamine sulphate (AMP, Sigma-Aldrich) was dissolved in 0.9% normal saline and 5.0 mg/kg/mL prepared for *ip* administered. AMP was used as the positive control for the CPP experiments. Coconut oil and saline treated groups provided the respective negative controls for comparison with the Δ^9-THC and AMP drug treatment groups.

2.4. Experiment 1: The CPP Paradigm

The CPP paradigm demonstrates the rewarding or aversive effects of drugs in animal models by the expression of increased or decreased time and entries into the portion of the classic maze with which drug is paired. In a biased paradigm, the drug is deemed to be rewarding or potentially addictive if the animal seeks out a 'non-preferred' environment to which the drug has been paired (*i.e.* positive place preference or drug-seeking behavior). Alternatively, the animal avoids the chamber to which the drug is paired if the drug has no rewarding effect (*i.e.* place avoidance). The classic CPP paradigm as described elsewhere [20] was modified to employ a 15-minute duration conditioning period in this study.

2.4.1. Habituation

Mice were allowed free play and activity in the Behavioral Laboratory and han-

dled at least 15 min daily over 1 week prior to the start of the pre-conditioning phase of the study. This allowed the animals to become acclimatized to the experimenter and the lab in order to eliminate any undue anxiety at the start of the study.

2.4.2. Pre-Conditioning Phase & Group Selection

Prior to the conditioning phase of the CPP paradigm, each untreated mouse was coded and placed individually into the grey chamber with the sliding doors removed, and allowed to freely explore the apparatus for 15 min. The exploratory and locomotory behaviour of the mice were observed and a record of the number of entries and time spent upon entry into each chamber of the CPP box established the preference of each mouse for the white or black chamber. An entry was recorded when the mouse placed all four paws in the black or white chamber. Percent time spent in, and percent entries into these chambers by each mouse was determined as follows:

% Time spent in the white or black chamber = [Total time (min) spent in the chamber ÷ 15 min] × 100

% Entries into the white or black chamber = [Total no. of entries into the chamber ÷ Total entries] × 100

Mice showing approximately 80% preference for the black chamber with respect to the greater length of time spent in, and number of entries made, were selected for the study (Table 1). Thirty-six (36) mice showing preference for the black chamber were randomly assigned to one of six (6) treatment groups, consisting of 6 mice per group, as follows:

Group 1: Δ^9-THC (0.10 mg/kg/mL, *ip*)
Group 2: Δ^9-THC (0.50 mg/kg/mL, *ip*)
Group 3: Δ^9-THC (2.0 mg/kg/mL, *ip*)
Group 4: Amphetamine sulphate (5.0 mg/kg/mL, *ip*)
Group 5: Virgin coconut oil (1 mL/kg, *ip*)
Group 6: 0.9% Saline solution (1 mL/kg, *ip*)

Table 1. Summary of the means ± S.E.M. for time spent in, and entries into the white and black chambers for untreated mice at pre-conditioning to determine chamber preference during a 15-minute exploration of the CPP apparatus.

Animal Groups (Untreated) N = 6 per group	White Chamber		Black Chamber	
	Mean Percent Time Spent ± S.E.M.	Mean Percent Entries ± S.E.M.	Mean Percent Time Spent ± S.E.M.	Mean Percent Entries ± S.E.M.
Group 1 (THC 0.1)	11.49 ± 2.86	36.00 ± 3.16	89.51 ± 2.27	64.04 ± 3.16
Group 2 (THC 0.5)	20.05 ± 6.63	34.55 ± 3.97	81.74 ± 5.84	64.17 ± 3.96
Group 3 (THC 2.0)	11.04 ± 2.61	32.00 ± 3.55	89.16 ± 2.65	70.48 ± 3.98
Group 4 (AMP 5.0)	21.10 ± 4.36	21.11 ± 4.36	78.89 ± 4.36	78.89 ± 4.36
Group 5 (Coc. oil)	13.97 ± 2.66	37.97 ± 6.00	85.98 ± 2.48	62.03 ± 6.00
Group 6 (Saline)	21.11 ± 4.36	31.28 ± 5.18	75.56 ± 4.44	68.78 ± 5.14
Average	**16.46 ± 3.91**	**32.15 ± 4.37**	**83.47 ± 3.67**	**68.06 ± 4.43**

2.4.3. Conditioning Phase

An 8-day CPP schedule with alternating drug treatment days was employed. Each drug (Δ^9-THC or AMP) was administered for four (4) alternate days *i.e.* days 1, 3, 5 and 7, and the animal then confined to the non-preferred (white) chamber for 15 min. On days 2, 4, 6 and 8, Δ^9-THC treated mice were administered coconut oil vehicle and subsequently confined individually for 15 min to the preferred (black) chamber. AMP treated mice were administered saline on the days between drug treatment, *i.e.* days 2, 4, 6 and 8. Untreated mice were administered either coconut oil or normal saline on all 8 days of the CPP schedule and individually confined for 15 min to the white chamber on days 1, 3, 5 and 7 and the black chamber on days 2, 4, 6, and 8. During conditioning, the sliding doors were used to confine the animal to the non-preferred, white chamber or the preferred, black chamber.

2.4.4. Post-Conditioning Phase to Assess CPP Performance

On Day 9, each mouse was individually placed in the grey chamber and the sliding doors removed to facilitate a 15-min free exploration of the CPP apparatus. The time spent in each chamber and the number of entries made with all four paws of the mouse placed in a chamber, were again recorded. The means of the percent time spent in, and entries into the white chamber, were determined for each group as previously described.

2.5. Experiment 2: Arylamine N-Acetyltransferase (NAT) Activity

2.5.1. Liver Homogenate Preparation for Both *In-Vitro* and *Ex-Vivo* Studies

Livers from the Δ^9-THC treated mice were obtained immediately after the final CPP exploration. Initial cervical dislocation was performed for drug-treated and drug-naïve (untreated) mice, following which livers were promptly excised and washed in phosphate buffered saline solution. The liver tissue was homogenized at 440 rpm in 25% w/v of chilled buffer (10 mM KPB, pH 7.4) containing 0.15 M KCl and 0.1 mM PMFS protease inhibitors (Roche, NJ, USA). The suspensions were spun in a refrigerated centrifuge (4˚C) at 9000 g for 20 min and the supernatants from each tube were labelled as S9 fractions and stored in −80˚C until further analysis. S9 fractions from each of the 6 mice per treatment group were prepared individually for *ex-vivo* studies.

2.5.2. Protein Quantification

The total protein content in the S9 fractions was determined in triplicates, using a Bradford Assay (BioRad, CA, USA) as described elsewhere [21].

2.5.3. Determination of Arylamine N-Acetyltransferase Aactivity

NAT activity was determined in both *in-vitro* and *ex-vivo* studies using a modified method with *p*-Anisidine (*p*ANS) as substrate [22]. Routinely, reactions were conducted in triplicate at 37˚C in a final volume of 250 µL containing 100 µL of S9 fraction such that NAT activity was within a linear range, with 160 µM *p*ANS

in Tris-EDTA buffer (20 mM Tris-HCl, 10 mM NaCl, 1 mM EDTA and pH 7.5) and 160 μM acetyl coenzyme A (AcCoA) added to start the reaction. After 50 minutes, the reaction was quenched by adding 100 μL of 30% (w/v) cold trichloroacetic acid. The assay mixture was centrifuged to remove precipitated protein, followed by the addition of 200 μL of 5% (w/v) dimethylaminobenzaldehyde (DMAB). Unacetylatedarylamine substrate was detected at 450 nm using a UV-spectrophotometer (μQuant universal microplate spectrophotometer, Bio-Tek Instruments, Winooski, VT, USA). Enzymatic NAT activity values are expressed as specific NAT activity (μmol *p*ANS/min/μg of liver protein), using S9 fractions (10 - 11 μg/μL protein) of treated or untreated mice. Mouse S9 activity (6 per group) was determined individually. S9 fractions from 3 untreated mice livers were pooled and employed to assess the impact of varying concentrations of Δ^9-THC (0.00 - 162 μM) dissolved in coconut oil. Control experiments for this vehicle were also conducted.

3. Data Analysis

Comparisons of the group means of the percent time (from a total of 15 minutes) and percent entries (from the total entries in all chambers of the CPP box during the 15-minute exploration) between the pre- and post-conditioning phases of the study were analyzed using ANOVA with Tukey's post hoc test (SPSS-19 software). Mean scores for the white chamber between groups were also analyzed using Student's t-test. For the *in-vitro* and *ex-vivo* analyses, the IC_{50} value was determined by plotting NAT activity against log ([Δ^9-THC]) and fitting the data in Sigma Plot 10.0 from Systat Software Inc., San Jose California USA, https://systatsoftware.com/. Values are presented as the mean ± S.E.M. and statistical significance was taken at $p \leq 0.05$.

4. Results

Exploratory performance of MICE in the CPP apparatus during preconditioning

The performance of mice that were selected based on their exploration of the CPP apparatus is summarized in Table 1. A comparison of the means scores obtained for the black and white chambers revealed a significant increases ($p < 0.001$) in the mean percent time spent and entries into the black chamber of the CPP apparatus ($F = 78.358$). All 36 mice selected for the study and assigned to the 6 treatment groups therefore demonstrated strong preference for the black chamber. Animals also exhibited normal locomotory behavior during their exploration of the CPP box.

Effect of Δ^9-THC on the CPP performance of mice

ANOVA revealed significant increases ($p < 0.001$) in the mean scores in percent time and entries to the white, non-preferred chamber ($F = 11.220$) between the different treatment groups. Significant increases ($p < 0.05$) in mean percent time in the drug-paired, white chamber at post-conditioning compared to pre-

conditioning was obtained for the 0.1 and 0.5 mg/kg Δ^9-THC treated mice, and a strong tendency towards significance ($p = 0.059$) for the 2.0 mg/kg Δ^9-THC treated mice. Additionally, the three Δ^9-THC treated rodent groups (0.1, 0.5 and 2.0 mg/kg) spent significantly ($p < 0.01$) more time in the white chamber at post-conditioning as compared with their coconut oil treated controls. Data illustrated in Figure 1 show that the 2.0 mg/kg Δ^9-THC treated group made significantly ($p < 0.05$) more entries into the white chamber at post-conditioning compared to pre-conditioning, unlike the 0.1 and 0.5 mg/kg Δ^9-THC-treated mice. Additional comparisons revealed that the 2.0 mg/kg Δ^9-THC treated mice also made significantly ($p < 0.01$) more entries into the white chamber post-conditioning as compared with their oil vehicle treated controls.

Effect of amphetamine on the CPP performance of mice

A comparison of post- and pre-conditioning mean scores for AMP treated mice revealed significant increases ($p < 0.001$) in both post-conditioned mean time spent in (Figure 2) and entries into (Figure 1) the white chamber ($F = 11.220$). Additional comparison of the mean scores for the AMP treated mice also revealed significant increases in time spent in, and entries into the white, non-preferred chamber at post-conditioning as compared with their saline treated controls ($p < 0.05$). The significant positive place preference exhibited by the AMP treated mice validates the CPP paradigm.

Effect of Δ^9-THC on *in-vitro* mouse liver NAT activity

Preliminary *in-vitro* investigation of the effect of Δ^9-THC on the activity of NAT in livers obtained from untreated mice is illustrated in Figure 3(a). A moderate dose dependent inhibition on NAT activity was observed, with an $IC_{50}$of 34.97 ± 1.58 μM.

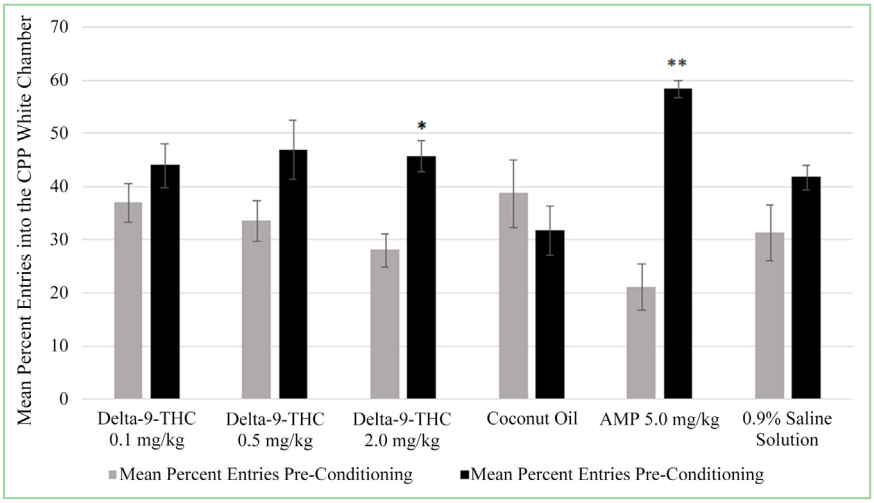


Figure 1. A comparison of the pre-conditioning (grey bars) and post-conditioning (black bars) mean percent entries into the white, drug-paired chamber by different treatment groups of mice [Δ^9-THC (0.1-2.0 mg/kg), amphetamine (5 mg/kg) and vehicle (coconut oil or normal saline)]. Values for groups are expressed as the mean percent entries ± S.E.M. (n = 6 per group). *$p < 0.05$, Δ^9-THC (2 mg/kg); post-conditioning vs pre-conditioning.**$p < 0.001$, AMP (5 mg/kg); post-conditioning vs pre-conditioning.

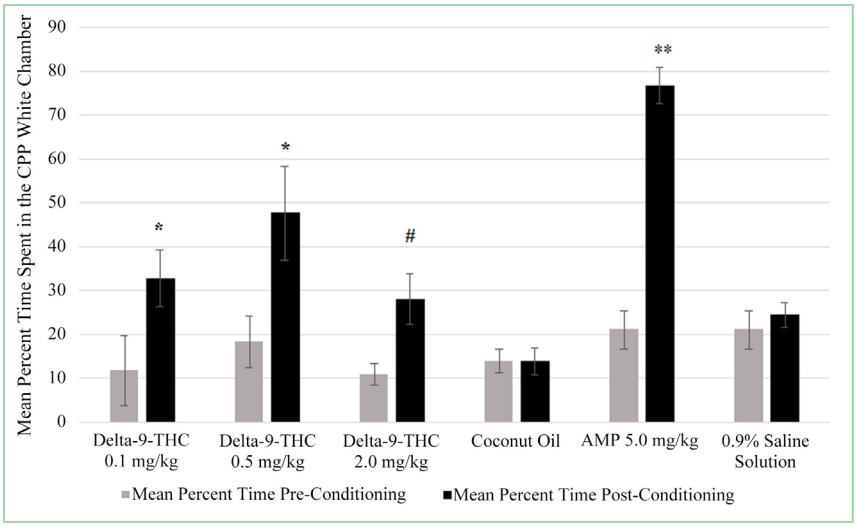
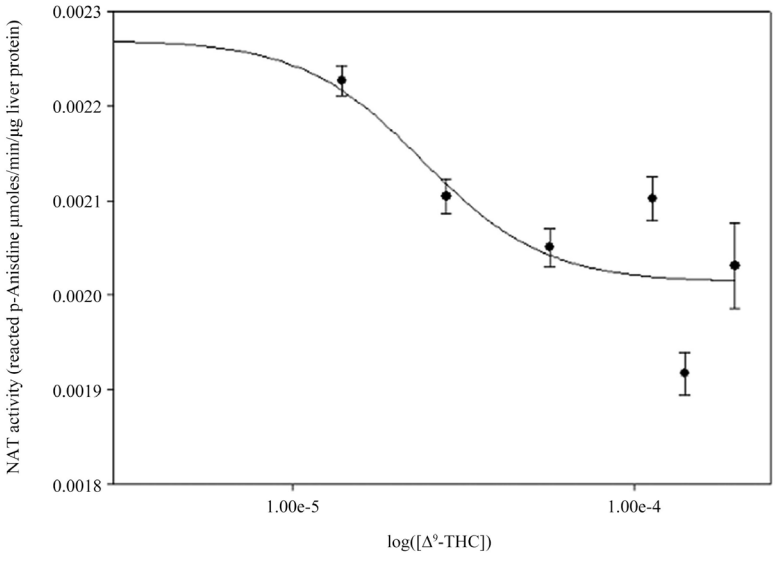


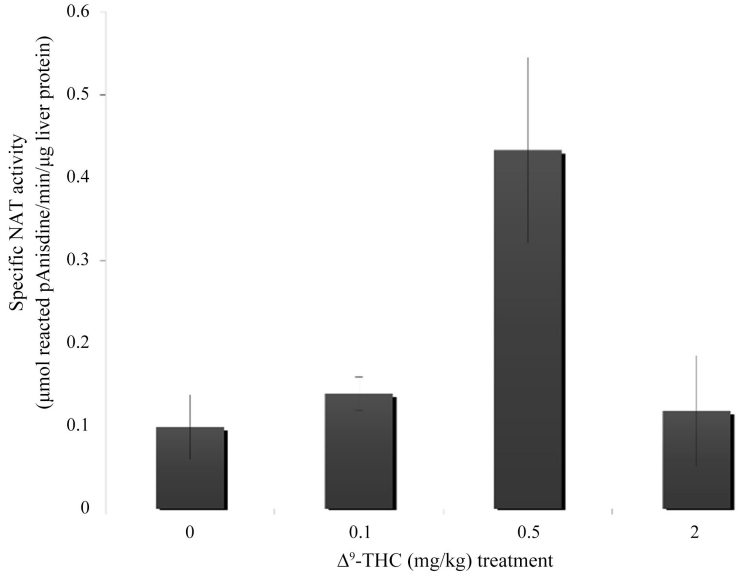
Figure 2. A comparison of the pre-conditioning (grey bars) and post-conditioning (black bars) mean percent time spent by different treatment groups of mice in the white, drug-paired chamber [Δ^9-THC (0.1-2.0 mg/kg), amphetamine (5 mg/kg) and vehicle (coconut oil or normal saline)]. Values for groups are expressed as the mean percent time ± S.E.M. (n = 6 per group). *p < 0.05, Δ^9-THC (0.1 & 0.5 mg/kg); post-conditioning vs pre-conditioning. #p = 0.059, Δ^9-THC (2.0 mg/kg); post-conditioning vs pre-conditioning time. **p < 0.001, AMP (5 mg/kg); post-conditioning vs pre-conditioning time.

Effect of Δ9-THC on *ex-vivo* mouse liver NAT activity

Livers from mice that had previously shown positive place preference using Δ^9-THC (0.1 - 2.0 mg/kg) were tested for NAT activity. This yielded variable non-significant results. After Δ^9-THC exposure, there was a non-significant, elevated level of liver NAT activity between 0.1 and 0.5 mg/kg Δ^9-THC. As shown in **Figure 3(b)**, there was a statistically non-significant increase in NAT activity *ex-vivo* for the 0.5 mg/kg Δ^9-THC exposed livers, but the lower (Δ^9-THC 0.1 mg/kg) and higher (Δ^9-THC 2.0 mg/kg) doses did not display this effect (**Figure 3(b)**).



(a)



(b)

Figure 3. (a) The impact of varying concentrations of Δ^9-THC (0.00 - 162 µM) on the activities of NAT from S9 fractions (10.17 µg protein) of untreated mice livers, were investigated *in-vitro*, as described in methods. Assays were conducted in triplicate and all values are expressed as mean ± S.E.M. (b) The NAT activity of the liver S9 fractions (10 - 11 µg/µl protein) obtained from coconut oil controls (untreated mice) and mice treated with low doses of Δ^9-THC (treated mice) was determined *ex-vivo* using *p*ANS and AcCoA, as described in methods. The average value of NAT activity for the untreated group was compared with each NAT activity value for the treated groups. Assays were conducted in triplicate and all values are expressed as mean ± S.E.M. There were no significant differences between the groups [F = 2.11].

5. Discussion

Data revealed that Δ^9-THC and amphetamine treated mice exhibited positive place preference, supporting the rewarding effects of both drugs. At all three relatively low doses of Δ^9-THC (0.1-2.0 mg/kg), mice spent significantly more time in the drug-paired chamber post-conditioning; and additionally at the highest dose investigated in this study, (2 mg/kg Δ^9-THC), mice made significantly more frequent entries (**Figure 1**, **Figure 2**). Both behavioral indices demonstrate positive place preference, indicating the motivational or rewarding effect of the Δ^9-THC which drives the animal to seek the drug in an undesirable or non-preferred environment. The data derived from the animal model used in this study provide support for the potential addictive liability of Δ^9-THC, albeit a low liability when compared to the addictiveness of amphetamine. The differential dose-dependent rewarding effect obtained also supports the observation that Δ^9-THC produces a mixture of depressant and stimulatory effects at low doses and, at higher doses, predominantly CNS depression [23]. It is important to note that the reduced conditioning period employed in this study enabled us to allow the rodents to associate the rewarding effect of Δ^9-THC in a timely manner before any dysphoric effects were induced. Amphetamine, a known CNS stimulant and highly addictive

drug, produced significant positive place preference in the animal model (**Figure 1**, **Figure 2**), thus validating the modified CPP paradigm that was used.

Δ^9-THC place preference, place aversion or no effect have all been reported, and are considered to be dependent on dose, timing within the apparatus and animal strain. Failure to induce place preference at 1 mg/kg Δ^9-THC was attributed to the impact of Δ^9-THC's simultaneous aversive effects [24]. Other reports now suggest that Δ^9-THC place preference can be induced at very low doses, but as the dose concentration increase so do the aversive effects, thus decreasing the ability of Δ^9-THC to induce place preference [5] [25] [26]. At high doses, enough place aversion is induced, such that aversion overshadows Δ^9-THC's rewarding effects [6] [7] [25]. Braida, *et al.* (2004) [26] utilizing a conditioning time of 30 minutes reported positive place preference in Wistar rats at the low dose range of 0.075 mg/kg to 0.75 mg/kg Δ^9-THC. We were able to report that an 18-minute conditioning time period was equally effective in producing positive place preference in adult Sprague-Dawley rats treated with a very low dose of 0.05 mg/kg Δ^9-THC [27].

The observed *in-vitro* direct inhibition of NAT activity by Δ^9-THC is not entirely surprising, given the structural motif of Δ^9-THC; as prior observations of compounds containing triazole and pyridine moieties have shown particular potency against NAT catalysis of *p*-anisidine [13]. However, most importantly, such inhibition did not manifest *ex-vivo*. Thus we report here, the absence of significant inhibition *ex-vivo* by Δ^9-THC on the activities of mouse NAT. The likely first pass elimination of the doses of Δ^9-THC used in this study can explain the discrepancy between *in-vitro* and *ex-vivo* observations.

The non-significant impact *ex-vivo* on NAT activity suggest that Δ^9-THC is unlikely to impart metabolism-based interactions for drugs reliant on hepatic NAT enzyme for their bio-transformations. This conclusion can have major implications for human drug use, although extrapolating data from mouse to human ought to be exercised with care, particularly given species variability in drug metabolism. Variability in NAT activity has been shown for leading pharmaceutical interventions in major diseases that include hypertension, arrhythmia, rheumatoid arthritis, tuberculosis, cancers, as well as those used for alleviating symptoms of infectious diseases [28]. Our findings imply that co-use of Δ^9-THC is unlikely to impart an impact on these therapeutic drugs, as well as others in the recreational space (e.g. 2C), although determinations of impact of Δ^9-THC on human NAT1 and NAT2 enzymes are highly recommended in future studies, to distinguish any disparity in impact between these isozymes. In our study, *p*-Anisidine may have been metabolized by both mouse NAT isoforms (NAT1 and NAT2), although it may be preferentially acetylated by mouse NAT2 [10]. In fact, NAT2 is a polymorphic enzyme, and its genotypic variability within the groups may explain the apparent discrepancy of NAT activities in liver fractions from differently treated mice, as observed in **Figure 3**; nevertheless, this difference was statistically insignificant. Also, a limitation of the study is that mice were not genotyped.

This study reports for the first time, that although a moderate inhibition of NAT activities was observed *in-vitro,* Δ^9-THC has a statistically non-significant impact on the activities of mice liver NAT enzymes *ex-vivo* at the doses adopted from the behavioral study. This supports and underscores the need for further investigations on the exposures of marijuana or cannabis smoke (which contains multitudes of other polyaromatic hydrocarbons and xenobiotics) in relation to genotype variants of NAT enzymes in human subjects.

6. Conclusion

Δ^9-THC induced significant conditioned place preference (drug reward) at all three doses employed and produced a moderate dose-dependent inhibition on NAT activity *in-vitro,* but had no significant impact on the activity of NAT enzymes *ex-vivo*. The results of this mouse model provide evidence to support Δ^9-THC's addictive potential and the absence of significant effect on xenobiotic metabolism of hepatic NAT enzymes.

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Author Disclosure Statement

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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