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Evaluation of Reinforcing and Aversive Effects of Voluntary Δ^9 -Tetrahydrocannabinol Ingestion in Rats

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Abstract

Edible cannabis-infused products are an increasingly popular method of using cannabis in the United States. Yet, preclinical research to determine mechanisms underlying abuse of Δ^9 -tetrahydrocannabinol (THC), the primary psychoactive constituent of cannabis, has focused primarily on the effects of parenteral administration. The purpose of this study was to examine the rewarding and aversive effects of oral THC in a novel rodent voluntary ingestion model. Adult male and female Sprague Dawley rats were given access to sucrose-sweetened solutions during daily sessions. A range of THC concentrations, each paired with a unique flavor previously tested alone, was introduced into these solutions for four-session exposure periods and drinking volumes were measured. Injected (i.p.) THC doses were also paired with unique flavors to compare the effects of route of THC administration on drinking. Introduction of THC into sucrose solutions dose-dependently decreased drinking upon initial exposure, though drinking generally increased in subsequent sessions. By contrast, i.p. THC produced sustained dose-dependent decreases in drinking in rats of both sexes. Subsequent exposure to paired flavors in the absence of THC resulted in further decreases in drinking, suggesting route-specific aversion. Additional testing using saccharin-sweetened solutions in a two-bottle choice paradigm was also conducted, with THC producing sustained dose-dependent decreases in drinking after initial exposure in rats of both sexes. Though self-administration of ingested THC was not demonstrated, evidence of route-specific THC aversion was observed, which suggests that certain routes and/or rates of THC administration may mitigate some of its aversive effects.

Keywords

cannabinoid; Δ^9 -tetrahydrocannabinol; oral; self-administration; taste preference

1.0 Introduction

The increased acceptance and legality of cannabis use in the United States (U.S.) has been accompanied by increased availability of cannabis in myriad forms. Most notably, edibles, or food products containing cannabis extract (reviewed in Barrus et al., 2016), have become a major method of cannabis consumption. For example, in the first year after the state of Colorado legalized recreational cannabis use, 53.6% of respondents to an online survey

indicated use of an edible product (Allen et al., 2017). Because ingested cannabinoids enter circulation through the digestive system rather than the pulmonary system, there are notable differences in the effects of edibles, as compared to inhaled cannabinoids. Most prominently, while the effects of inhaled cannabinoids are typically experienced within minutes of inhalation and dissipate within two to three hours, the effects of edibles may not be felt until 30 to 90 minutes after ingestion and generally persist for several hours (Grotenhermen, 2003). In addition, edibles undergo first-pass metabolism in the liver, where ⁹-tetrahydrocannabinol (THC; the major psychoactive constituent of the cannabis plant) is metabolized to 11-hydroxy-tetrahydrocannabinol, 11-nor-carboxy-THC, and other minor metabolites (Huestis, 2007). Because 11-hydroxy-tetrahydrocannabinol is also psychoactive, it may contribute to the differences in strength and duration of intoxication observed between ingested and inhaled cannabinoids (Favrat et al., 2005). The slow onset of intoxication with edibles and a documented lack of consistency in their formulation and regulation have been implicated in a number of cannabis-related health incidents in states within the U.S. in which edible cannabis products are commercially available (Barrus et al., 2016).

Despite self-administration of cannabis in humans primarily via inhalation and oral routes, the majority of extant preclinical research on the abuse-related behavioral properties of cannabinoids has utilized parenteral routes of administration, including intravenous (i.v.), intraperitoneal (i.p.), and subcutaneous (s.c.) injections. In particular, preclinical investigation of the reinforcing effects of cannabinoids has relied on i.v. self-administration. This method failed to engender self-administration of cannabinoids until success was demonstrated in squirrel monkeys in the early 2000s (Justinova et al., 2003). In rodents, reliable robust i.v. self-administration of THC has still not been observed (Lefever et al., 2014; Wakeford et al., 2017), although intracerebroventricular self-administration THC has been reported (Braida et al., 2001; Zangen et al., 2006). In addition, i.v. self-administration of the synthetic cannabinoid WIN 55,212-2 has been reported (Fattore et al., 2001; Mendizábal et al., 2006). Results from studies using other behavioral measures to assess cannabinoid rewarding effects in rodents, such as conditioned place preference and intracranial self-stimulation (ICSS), have been inconsistent, with some studies reporting rewarding effects of cannabinoids (Braida et al., 2004; Valjent and Maldonado, 2000) and others finding little evidence that cannabinoid agonists induce these effects (Hempel et al., 2016; Vlachou et al., 2005). Katsidoni et al. (2013) suggested that both rewarding and aversive effects of THC may be observed in the ICSS model, but occur at different doses. While some researchers have suggested that aversive properties of THC that occur in conjunction with its rewarding effects may cause initial suppression of THC's rewarding properties (Valjent and Maldonado, 2000), others have shown that the rewarding and aversive effects of THC may occur independently and may be separately measured (reviewed in Verendeev and Riley, 2012). For example, in a novel experimental design that examines reward (conditioned place preference) and aversion (conditioned taste avoidance) in the same animals, THC induced conditioned taste avoidance, but did not produce conditioned place preference (Hempel et al., 2017; Wakeford et al., 2016). Hence, numerous attempts to demonstrate robust reinforcing or rewarding effects of injected THC in rodents have met with limited success.

The goal of the present study was to determine the extent to which rats would self-administer oral THC. In addition to being a potentially more palatable method of THC self-administration for rodents than parenteral routes, a voluntary ingestion model of THC administration would also be more translational to human cannabis use. To this end, we conducted a series of experiments in which adult male and female rats were given access to sweetened solutions containing THC and assessed the extent to which various concentrations of THC altered drinking.

2.0 Materials and methods

2.1 Subjects

To facilitate comparisons with prior attempts to train i.v. self-administration in rats, the present study also employed rats as subjects. Forty experimentally naïve adult male and female adult Sprague Dawley rats (125–174g, PND 41–43 on arrival) were used (Harlan, Frederick, MD). Rats were randomly assigned to conditions upon arrival. Within subject designs were used for each experiment and number per condition (n=6/sex) was based upon our previous preclinical work with THC (Wiley et al., 2007). Upon arrival, rats were housed in clear polycarbonate cages in a colony room with a 12 h light-dark cycle (lights on at 6 a.m.). Because food restriction has been shown to facilitate acquisition of self-administration (Carroll et al., 1979), rats were food restricted to ~90% of free-feeding weight over the course of each experiment and, consequently, were housed individually to allow tracking of food consumption. Rats had *ad libitum* water access in their home cages. All studies were carried out in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). All experiments were approved by our Institutional Animal Care and Use Committee.

2.2 Apparatus

Rats were trained and tested in standard operant conditioning chambers (ENV-007CT, Med Associates, St. Albans, VT) housed in sound-attenuating cubicles (ENV-018V, Med Associates). For the one bottle procedures (Experiments 1–3), each chamber was outfitted with a holster for a 100 ml rubber-stopped glass vial with a two-inch vacuum-style sipper tube (Lab Products Inc, Seaford, DE). A 50 ml plastic conical centrifuge tube was attached to the base of each holster in order to collect any drips or leakage from the glass vial. To reduce the risk of lingering flavor smells in the chambers, drop pans in the chambers were used without bedding. For the two bottle choice tests (Experiment 4), each chamber was outfitted with two lickometers (Med Associates). Each lickometer consisted of a plastic water bottle with a rubber stopper and ball-style sipper tube attached to a photobeam controller that was connected to the grid floor. Lickometers allowed measurement of number of head entries into the recess containing each sipper tube as well as the number of licks for each bottle during each session. All chambers and drop pans were wiped down with Virkon (Pharmaceutical Research Laboratories, Inc, Naugatuck, CT) after each session.

2.3 Chemicals

⁹-Tetrahydrocannabinol (National Institute on Drug Abuse, NIDA, Rockville, MD) was suspended in a 7.8% polysorbate 80 (Fisher Scientific, Hampton, NH) and saline (Patterson

Vet Supply, Charlotte, NC) mixture. Experiments 1–3 used a 10% sucrose (Fisher Scientific) mixture combined with the THC solution or comparable vehicle. Experiment 4 used a 0.1% saccharin (Fisher Scientific) solution. Flavorants (LorAnn Oils, Lansing, MI) were purchased commercially (see supplemental information for available details regarding chemical composition). Injected THC or vehicle was given i.p. at a volume of 1 ml/kg.

2.4 Procedure

This study is comprised of four inter-related experiments designed to examine the rewarding and aversive effects of oral THC. Because oral THC effects do not have immediate onset, a specific flavor was paired with each concentration of THC (or vehicle) to serve as a discriminative stimulus to signal availability of the associated THC concentration during subsequent exposures. The purposes of Experiment 1 were (1) to choose the flavors to use in subsequent experiments and (2) to provide comparison data for rats exposed to the flavors (plus vehicle), but not to THC. In Experiment 1, flavors were evaluated for their effects on drinking of THC-free sucrose and vehicle solution in a one-bottle procedure. Flavors that were associated with significant differences in volume consumed compared to unflavored sucrose solution were eliminated from further consideration. Experiment 2 also consisted of a one-bottle sucrose solution drinking procedure; however, during some sessions, the solution also contained THC or its vehicle. A specific flavor (chosen from those tested in different rats in Experiment 1) was paired with each concentration of THC (or vehicle) to serve as a discriminative stimulus to signal availability of the associated THC concentration during subsequent exposures. In Experiment 3, the effects of i.p. injection of THC on sucrose drinking were determined in the same group of rats. To facilitate comparisons with the results of Experiment 2, each injection dose was paired with a specific flavor added to the sucrose solution. Experiment 1 data were also used for comparison to a “flavor-only, no injection” condition. Experiment 4 employed a choice procedure, in which rats were allowed simultaneous access to two bottles during each session. One bottle contained THC (or vehicle) mixed into a saccharin solution whereas the other bottle contained only water. Use of a saccharin solution avoided the confound of having liquids in the two bottles that differed substantially with respect to caloric value. Flavors were not added to the saccharin solution at any phase of this experiment. Additional procedural details for each experiment are provided below.

2.4.1 Experiment 1: Flavor profile—After one week of acclimation, twelve experimentally naive male and female rats ($n=6/\text{sex}$) were given access to 50 ml of sucrose solution in 100 ml glass stopper vials (Lab Products Inc, Seaford, DE) during five 1-h sessions per week. Vial weight and volume of solution were measured before and after each session. Each rat used the same vial/stopper every day and all vials were washed after sessions with warm water and Virkon. After establishing stable drinking levels of a 10% sucrose solution vehicle (sucrose + water + vehicle of 7.8% polysorbate 80 and saline), a series of artificial and natural fruit flavors (LorAnn Oils) were added to the vehicle solution (0.5 ml flavor per 50 ml solution), with rats exposed to one flavor per week in 1-h sessions (flavors listed in Table 1). Rats were given access to unflavored sucrose solution during the first session of the week, and flavored solution for the remaining four sessions. Sixteen different flavors (plus unflavored sucrose vehicle solution) were evaluated over the course of

sixteen weeks. Flavors that did not alter volume consumed (compared to unflavored sucrose solution vehicle) were potential candidates as flavors to be used in Experiments 2 and 3.

2.4.2 Experiment 2: Voluntary oral THC ingestion—Twelve experimentally naive male and female rats ($n=6/\text{sex}$) were used, with procedures identical to those of Experiment 1, except that specific THC concentrations were paired with each flavor tested. Further, THC concentrations were weight-adjusted across the rats (within 20 g weight range), such that a rat consuming 50 ml of solution would attain an approximate THC dose of 0 (raspberry), 0.1 (apple), 1 (banana cream), 2.5 (coconut), 5 (blueberry), 7.5 (cranberry) or 10 (plum) mg/kg. Actual THC concentrations ranged from 0.002–0.2 mg/kg/ml (see Figure 1). As in Experiment 1, rats were given access to unflavored sucrose solution with vehicle during the first session of the week. For the remaining four sessions, each rat received sucrose solution with the flavor and concentration of THC designated for the week. After completion of initial four-day exposures to all THC concentrations and their associated flavors, a single 1-h session of exposure to each flavor alone was conducted. Consumption volume was measured at the end of each session throughout the experiment.

2.4.3 Experiment 3: Effects of i.p. THC on sucrose drinking—Following a one-week washout period, the rats from Experiment 2 were used again under identical procedures, except that each flavored solution was paired with a dose of THC administered via i.p. injection immediately before each 1-h drinking session. Flavor and THC (in mg/kg) combinations were raspberry (0), apricot (0.1), watermelon (0.3), peach (1), and mango (3). With exception of raspberry (paired with the vehicle dose in both Experiment 2 and 3), flavors used in Experiments 2 and 3 were distinct. During the first session of the week, rats did not receive an injection and were given access to unflavored sucrose solution. Prior to each of the remaining four sessions, rats received an i.p. injection of a single dose of THC and received access to sucrose solution that contained the dose's associated flavor. One dose was evaluated per week in ascending order. After completion of the THC dose-effect curve, a single 1-h session of exposure to each flavor without a preceding injection with THC or vehicle was conducted. Consumption volume was measured at the end of each session throughout the experiment.

2.4.4 Experiment 4: Two-bottle oral THC ingestion—Sixteen male and female rats had access to two bottles during daily 2-h sessions, during which house lights were illuminated. One contained 50 ml of a 0.1% saccharin, 5% vehicle, and water solution and the other bottle contained 50 ml of water. Vial volumes and weights were recorded before and after each session and total number of licks were recorded after sessions. Following the establishment of stable drinking levels, THC was added to the training solution (weight-adjusted for each 20 g body weight range), such that a rat consuming 50 ml of solution would attain a dose of 1 mg/kg THC (i.e., 0.02 mg/kg/ml). After stable drinking levels of the THC training solution had been established, dose effect-curves were determined with concentrations of 0, 0.006, 0.02, 0.06, and 0.1 mg/kg/ml THC. Each dose was tested for five consecutive sessions.

2.5 Data analysis

Volume of fluid consumed was measured during each 1-h test session in Experiments 1–3. For Experiment 1, average volume per session of each flavored solution for the 4-session exposure period was determined for each rat. Subsequently, the mean ($\pm$ SEM) of these averages was calculated across rats separately for each flavor such that data for each rat consisted of a single average amount of liquid consumed per flavor. Resulting data were analyzed through use of split-plot (sex $\times$ flavor) ANOVA. These data are presented in Table 1. For Experiments 2 and 3, the mean ($\pm$ SEM) volume consumed for the (1) first exposure and (2) flavor only sessions and (3) average volume for the last three exposure sessions were calculated separately for each flavor and sex. Resulting data were analyzed for each sex separately through use of split-plot [THC concentration $\times$ exposure session (session 1 vs. last 3-session average vs. flavor only tests)] ANOVAs. The data for Experiments 2 and 3 are presented in panels C (females) and D (males) of Figures 1 and 2, respectively. In addition, for the flavors selected for use in Experiments 2 and 3, data from Experiment 1 were re-analyzed using an identical statistical procedure as for Experiments 2 and 3 and are presented in Figures 1 and 2 as panels A (females) and B (males). For Experiment 4, the means ($\pm$ SEM) for volume of saccharin solution consumed and the number of licks for the first session and for the last three sessions were also calculated separately for each THC concentration and sex. Resulting data were analyzed for each sex and each dependent variable through use of separate split-plot [THC concentration $\times$ exposure session (session 1 vs. last 3-session average)] ANOVAs. For all experiments, Tukey post hoc tests ($\alpha=0.05$) were used, as appropriate, to determine differences between individual means. NCSS 11 Statistical Software (2016; NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss) was used for all analyses.

3.1 Results

Table 1 shows the results of the flavor profile evaluation (Experiment 1). Two primary findings emerged. First, with exception of three flavors (cherry, pineapple, and orange) that rats of both sexes found aversive, rats did not exhibit preference or aversion to any other flavors as compared to unflavored sucrose solution. Second, male rats consumed significantly higher volumes of sucrose solution and of most flavored solutions than female rats [$F(16,96)=2.48$, $p<0.05$]. Exceptions were raspberry, coconut, pineapple, and orange, for which significant sex differences in consumption were not observed. For the latter two flavors, the lack of differences may have been related to a floor effect since overall volume for these two citrus flavors was severely reduced for both sexes. Given the aversion to citrus and cherry flavors, they were not used in subsequent experiments.

Figure 1 shows the results of Experiment 2 (panels C and D), with flavor consumption data from rats in Experiment 1 presented for comparison purposes (panels A and B). The volume (in ml) of flavored sucrose solutions containing vehicle (panels A and B) or THC (panels C and D) ingested voluntarily by female and male rats (top and bottom panels, respectively) is shown for the first exposure session and as an averaged amount across the last three sessions of the four-session exposure period. In addition, consumption of sucrose solution by the THC-exposed rats in Experiment 2 during a subsequent “flavor only” session is also shown

(panels C and D). Female rats from Experiment 1 that received vehicle in flavored sucrose solutions drank similar amounts of solution across all flavors, but consumed a higher average volume during the last three sessions of each four-session exposure period than during the first session [Figure 1A; main effect of day: $F(1,30)=21.22$, $p<0.05$]. Males from Experiment 1 consumed less coconut-flavored and more blueberry- and cranberry-flavored solutions during the first session (as compared to raspberry) [Figure 1B; flavor $\times$ day interaction: $F(6,30)=3.14$, $p<0.05$], but drinking volume did not differ across flavors during the last three sessions. In the group of THC-exposed rats from Experiment 2 (Figure 1, panels C and D), males exhibited a small, but significant, increase in volume of 0.002 mg/kg THC consumed compared to vehicle drinking during the first exposure session [Figure 1D]; however, as THC concentration was increased, volumes dose-dependently decreased in both sexes upon initial exposure, with the exception of the 0.2 mg/kg/ml THC solution which was associated with a return to drinking comparable to vehicle levels [Figure 1C for females and 1D for males; flavor $\times$ day interactions for females and males, respectively: $F(12,60)=11.53$, $p<0.05$ and $F(12,60)=12.5$, $p<0.05$]. Although drinking volumes also showed dose-dependent decreases over the final three sessions of exposure to each THC concentration for both sexes, they were increased compared to the first exposure session at several concentrations (0.05 mg/kg/ml for females and 0.05, 0.1, and 0.15 mg/kg/ml for males). At the 0.2 mg/kg/ml THC concentration, drinking was decreased during the 3-session period as compared to the first session in females, but not in males. In addition, males drank greater volumes in both Experiments 1 and 2 (Figures 1B and 1D) and consumed higher doses of THC in Experiment 2 (Table 2) than females over the course of the 1-h sessions. Consumption of sucrose solution during the subsequent “flavor only” sessions was variable across flavor, but was similar to consumption amounts observed during the last 3 THC exposure sessions (i.e., post hoc tests revealed no significant main effect difference between consumption during the last 3 sessions and the flavor alone tests for either sex) [Figure 1C and 1D for females and males, respectively].

Figure 2 shows the results of Experiment 3 (panels C and D), with flavor consumption data from rats in Experiment 1 presented for comparison purposes (panels A and B). Female rats from Experiment 1 that received vehicle in flavored sucrose solutions drank similar amounts of solution across all flavors, but consumed a higher average volume during the last three sessions of each four-session exposure period than during the first session [Figure 2A; main effect of day: $F(1,20)=17.89$, $p<0.05$]. Males from Experiment 1 also consumed more sucrose solution during the last three sessions and more peach-flavored solution (compared to raspberry) [Figure 2B; main effects of day and flavor, respectively: $F(1,20)=34.81$, $p<0.05$ and $F(4,20)=3.13$, $p<0.05$]. In contrast, i.p. injections of THC (1 and 3 mg/kg) in Experiment 2 produced reductions in drinking in females during the first exposure session, with a further decrease observed over the final three exposure session period at the 1 mg/kg dose [Figure 2C; $F(8,40)=9.08$, $p<0.05$]. In contrast, males in Experiment 2 drank similar volumes during the first and subsequent sessions and exhibited decreased drinking only after receiving an i.p. injection of 3 mg/kg THC [Figure 2D; $F(8,40)=6.22$, $p<0.05$]. Consumption of sucrose solution during the subsequent “flavor only” sessions was consistently suppressed in females that had a history of injection with THC (compared to those that had received vehicle). In addition, these rats consumed significantly less sucrose solution across all

flavors during flavor alone tests as compared to when drug was present in the solution [Figure 2C; $F(8,40)=9.08$, $p<0.05$]. Male rats with a history of THC injection showed similar decreases in consumption of sucrose solution during these flavor alone tests [Figure 2D; $F(8,40)=6.22$, $p<0.05$].

Figure 3 shows the results of voluntary ingestion of THC delivered in a saccharin-sweetened solution. In females, decreased drinking was observed during the first exposure session at higher THC concentrations, with further decreases in drinking volumes were seen over the last three sessions [Figure 3A; $F(5,35)=6.86$, $p<0.05$]. In males, drinking showed a small, but significant, decrease upon first exposure to the lowest THC (0.006 mg/kg/ml) concentration [Figure 3B; $F(5,35)=14.06$, $p<0.05$]; however, the volume of saccharin solution consumed at higher THC concentrations was similar to that containing vehicle. In contrast, the volumes of saccharin solution containing higher THC concentrations during the subsequent 3-day exposure period exhibited significant decreases [Figure 3B; $F(5,35)=14.06$, $p<0.05$]. Consumption of water from the second bottle was notably low (approximately 10 times less than saccharin-sweetened THC solution) across all conditions and is shown for the vehicle condition only at the left side of each panel. Data on number of licks across concentration for each sex showed patterns similar to those observed with volume (data not shown).

4.0 Discussion

Although the reinforcing effects of synthetic cannabinoids have been shown in several i.v. rat self-administration studies (Deiana et al., 2007; Fadda et al., 2006; Fattore et al., 2001), efforts to demonstrate such effects with i.v. THC have been largely unsuccessful in this species (Lefever et al., 2014; Panlilio and Justinova, 2018). The results of the present study are consistent with these previous studies, in that rats also did not self-administer THC orally in a novel voluntary ingestion experimental design. Rather, upon initial exposure, increases in THC concentration resulted in dose-dependent decreases in drinking in rats of both sexes regardless of sweetener used (sucrose or saccharin) and regardless of the presence or absence of a flavor. Nevertheless, some interesting findings emerged which may serve to elucidate the complex relationship between pharmacological effects and environmental contingencies in mediating the observed behavioral effects of THC in this model.

To encourage rats to consume THC orally, sucrose or saccharin was added to the THC-containing vehicle solutions. Rats and humans show affinity for sweet flavors and will readily drink solutions containing either sweetener (Hoffman et al., 2016; Murray et al., 2016). Further, consumption of sucrose or saccharin solution is associated with disruptions in dopamine signaling in the nucleus accumbens (Wickham et al., 2018), an effect that is also observed concomitant with self-administration of abused substances such as cocaine (Saddoris et al., 2016). Indeed, rodents have shown enhanced preference for oral consumption of sucrose solution compared to self-administration of several abused substances, including cocaine, heroin, and nicotine (Cantin et al., 2010; Huynh et al., 2017; Madsen and Ahmed, 2015). Hence, sucrose- and saccharin-sweetened solutions provide strong incentive motivation to drink.

Despite the rewarding effects of sucrose solution consumption, initial exposure to higher doses of oral or i.p. THC produced decreases in drinking in rats of both sexes. Because each THC dose was paired with a unique flavor that the rat had never tasted, aversion to novel flavors – which is common in rats due to their lack of an emetic response (Parker, 2006) -- is a possible explanation of these initial decreases. However, this hypothesis is not supported by the observations that an initial decrease in drinking did not occur in a separate group of rats that received sucrose solution containing the same flavor, but no THC, and it did not occur for all flavor-THC dose combinations. Instead, direct pharmacological effects of the drug likely contributed to the observed decreases at higher oral THC concentrations of 0.15 and 0.2 mg/kg/ml and i.p. THC doses of 1 and 3 mg/kg. Oral THC doses consumed by rats at these concentrations approximate doses (~ 1–3 mg/kg; Table 1) that, when administered acutely via i.p. injection, produce observable behavioral effects in rats, including decreased sucrose solution drinking (present study) and discriminative stimulus effects (Barrett et al., 1995; Wiley et al., 2017). The finding that decreased drinking occurred at lower concentrations in females than in males is also consistent with previous findings showing that female rats are more sensitive than males to the discriminative stimulus and response rate suppressive effects of THC (Wiley et al., 2017).

Subsequent sessions of exposure to each THC concentration plus flavor resulted in divergent changes to drinking patterns that were dependent upon route of THC administration (oral or i.p.). In rats of both sexes, oral consumption of THC-containing sucrose solution increased compared to initial effects, albeit consumption still showed dose-dependent decreases compared to vehicle. At least three mechanisms may have contributed to these results with oral THC: acute tolerance developed to oral THC's initial suppressive effects on drinking; THC induced an increase of the incentive properties of sucrose (Olarate-Sanchez et al., 2015); or THC's aversive effects were weakened after first exposure (Davis and Riley, 2010). Subsequent tests with flavor alone, in which drinking did not decrease further, support the latter hypothesis, in that, voluntary consumption of oral THC was not effective in producing conditioned taste avoidance, an effect that has been shown previously with i.p. THC (Parker and Gillies, 1995 and see below).

In contrast to the partial reversal of THC's initial effect observed with repeated oral THC exposure, injected THC appeared to produce a sustained dose-dependent decrease in drinking over the entire 4-session exposure period. In males, this effect occurred only at the highest THC dose (3 mg/kg) initially and subsequently whereas females exhibited decreased drinking at 1 and 3 mg/kg THC. Further, the initial suppressive effect of these THC doses intensified over the course of the last three sessions in females, suggesting the development of conditioned taste avoidance, as has been reported previously with THC (Parker and Gillies, 1995). This hypothesis also receives support from the finding that later assessments with flavors only resulted in pronounced suppression of drinking in both sexes even at THC doses that did not produce decreased consumption during the four sessions of THC and flavor association.

Interestingly, in the voluntary saccharin drinking experiment, intensification of THC's suppressive effects occurred in rats of both sexes, an effect that bore more similarity to the enhanced decrease observed with injected THC in female rats than to the increases seen

during the last three sessions of voluntary oral consumption in the one-bottle sucrose solution assay. Procedural differences (e.g., number of bottles, type of sweetener, absence of added flavors) prevent definitive determination of specific factors that were responsible for the difference in the effects of oral THC in the two models, emphasizing the interplay of drug properties and environmental contingencies in the expression of behavioral effects of THC.

Two limitations of the study are especially important to note. First, use of the same animals in Experiments 2 and 3 raises the issue of potential carry-over effects in Experiment 3 resulting from the previous flavor conditioning or THC exposure. Second, because rats were exposed to several flavors, each of which was paired with a different THC dose, stimulus generalization across tastes conceivably could have occurred. While this possibility cannot be eliminated completely, the distinctly different patterns of consumption across the THC concentration/dose-effect functions and in the associated “flavor only” probe tests in the two experiments argues against it.

In summary, results of the present study demonstrate that the one- and two-bottle voluntary ingestion procedures used here were not successful in engendering oral self-administration of THC. However, use of this method provided additional insight into THC’s aversive effects. A primary finding was that voluntary oral consumption of THC failed to induce conditioned avoidance of the flavors with which it was paired, a robust effect that occurred with experimenter-administered THC in this study and previously in other labs. The disparity in THC effects was observed despite similar doses across route of administration, albeit rate of administration differed (i.e., bolus injection and oral consumption over a 1-h session). Delineation of the behavioral mechanisms underlying this difference is needed to determine its translational implications for the increasing number of consumers of THC-containing edibles associated with commercial availability in legalized cannabis markets.

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Figure 1.

Panels A and B (Experiment 1) show voluntary oral consumption of sucrose solution with vehicle and added flavors in adult female and male rats, respectively, during a 4-session test period. Consumption volume during the first session (filled circles) and mean consumption volume over the final 3 sessions (filled squares) are shown. Panels C and D (Experiment 2) show the voluntary oral consumption of sucrose solution with added concentrations of THC (each paired with a specific flavor) in adult female and male rats, respectively, during the first of 4 sessions (filled circles) and over the final 3 sessions (filled squares). In addition, consumption volumes during subsequent exposure to the flavor alone (without associated THC concentration) are shown (unfilled triangles). Each point represents the mean ($\pm$ SEM) of data for 6 rats. Asterisks (*) indicate significant interaction term and post hoc differences ($p < 0.05$) compared to respective vehicle. Dollar sign (\$) indicates significant interaction term and post hoc differences ($p < 0.05$) compared to first session exposure at the specific flavor/THC concentration. Pound sign (#) indicates significant main effect of session or flavor/THC concentration ($p < 0.05$) compared to first exposure.

Figure 2.

Panels A and B (Experiment 1) show voluntary oral consumption of sucrose solution with vehicle and added flavors in adult female and male rats, respectively, during a 4-session test period. Consumption volume during the first session (filled circles) and mean consumption volume over the final 3 sessions (filled squares) are shown. Panels C and D (Experiment 3) show voluntary oral consumption of sucrose solution following i.p. injection with doses of THC (each paired with a specific flavor) in adult female and male rats, respectively, during the first of 4 sessions (filled circles) and over the final 3 sessions (filled squares). In addition, consumption volumes during subsequent exposure to the flavor alone (without associated THC dose) are shown (unfilled triangles). Each point represents the mean ($\pm$ SEM) of data for 6 rats. Asterisks (*) indicate significant interaction term and post hoc difference ($p < 0.05$) compared to respective vehicle. Dollar sign (\$) indicates significant interaction term and post hoc difference ($p < 0.05$) compared to first session exposure at the specific flavor/THC concentration. Pound sign (#) indicates significant main effect of session or flavor/THC concentration ($p < 0.05$) compared to first exposure or raspberry/vehicle, respectively.

Figure 3.

Panels A and B (Experiment 4) show voluntary oral consumption of saccharin solution with added concentrations of THC in adult female and male rats, respectively, during the first of 4 sessions (unfilled squares) and over the final 3 sessions (filled squares). For comparison purposes, consumption of water from the second bottle for the vehicle condition only is shown at the left side of each panel. Each point represents the mean ($\pm$ SEM) of data for 6 rats. Asterisks (*) indicate significant difference ($p < 0.05$) compared to respective saccharin alone vehicle. Dollar sign (\$) indicates significant difference ($p < 0.05$) between volume consumed during the first session as compared to the final 3-session average at specific THC concentration.

Table 1.

Average Volume (ml) of Sucrose Solution Consumed

Flavor	Male		Female	
	Mean	SEM	Mean	SEM
Unflavored	35.7	1.5	26.0	1.7
Raspberry	32.2	1.4	25.9	2.0
Coconut	29.4	0.5	24.1	2.0
Grape	36.3	2.2	27.9	2.1
Banana Cream	36.9	2.3	27.8	2.3
Apple	35.9	1.0	26.7	2.4
Cherry	20.0 *	1.2	11.5 *	2.2
Plum	36.7	1.2	27.9	2.3
Blueberry	39.8	1.2	29.3	1.9
Strawberry	33.2	1.4	20.3	2.3
Cranberry	39.9	1.7	27.0	3.3
Apricot	37.0	1.0	25.9	2.6
Pineapple	11.2 *	2.0	6.7 *	0.8
Watermelon	37.4	1.0	27.4	2.2
Peach	39.6	1.6	27.4	2.1
Mango	39.3	1.2	27.8	2.9
Orange [†]	4.95 *	0.6	2.74 *	0.6

* Average volume significantly lower than sex-specific unflavored baseline. Significant sex difference for all flavors, except for raspberry, coconut, pineapple and orange.

[†] Flavor was dropped from study after two test days.

Table 2.Oral THC Dose[†] Consumed Across Available Concentrations in Experiments 2 and 4

Experiment 2	Female		Male	
THC Concentration	Session 1	Sessions 2–4	Session 1	Sessions 2–4
0.002 mg/kg/ml	0.05 (0.004)	0.05 (0.001)	0.05 (0.004)	0.05 (0.004)
0.02 mg/kg/ml	0.46 (0.03)	0.51 (0.02)	0.43 (0.03)	0.57 (0.03)
0.05 mg/kg/ml	0.40 (0.08)	0.89 (0.09)	0.64 (0.11)	1.11 (0.08)
0.1 mg/kg/ml	0.78 (0.19)	1.35 (0.14)	1.07 (0.28)	2.44 (0.12)
0.15 mg/kg/ml	0.35 (0.03)	1.40 (0.06)	0.88 (0.20)	2.75 (0.20)
0.2 mg/kg/ml	3.23 (0.47)	1.42 (0.13)	3.90 (0.41)	2.57 (0.14)
Experiment 4	Female		Male	
THC Concentration	Session 1	Sessions 2–4	Session 1	Sessions 2–4
0.006 mg/kg/ml	0.17 (0.008)	0.15 (0.007)	0.14 (0.01)	0.13 (0.01)
0.02 mg/kg/ml	0.57 (0.03)	0.46 (0.01)	0.52 (0.04)	0.42 (0.03)
0.06 mg/kg/ml	1.31 (0.04)	0.92 (0.06)	1.75 (0.18)	1.10 (0.12)
0.1 mg/kg/ml	1.69 (0.22)	1.49 (0.12)	2.75 (0.29)	1.30 (0.11)

[†]Values in table are mean (± SEM) oral THC doses (mg/kg) consumed.