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Effect of Norbinaltorphimine on ⁹-Tetrahydrocannabinol (THC)-Induced Taste Avoidance in Adolescent and Adult Sprague-Dawley Rats

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Abstract

Rationale—The aversive effects of ⁹-tetrahydrocannabinol (THC) are mediated by activity at the kappa opioid receptor (KOR) as assessed in adult animals; however, no studies have assessed KOR involvement in the aversive effects of THC in adolescents. Given that adolescents have been reported to be insensitive to the aversive effects induced by KOR agonists, a different mechanism might mediate the aversive effects of THC in this age group.

Objectives—The present study was designed to assess the impact of KOR antagonism on the aversive effects of THC in adolescent and adult rats using the conditioned taste avoidance (CTA) procedure.

Methods—Following a single pretreatment injection of norbinaltorphimine (norBNI; 15 mg/kg), CTAs induced by THC (0, 0.56, 1.0, 1.8 and 3.2 mg/kg) were assessed in adolescent ($n = 84$) and adult ($n = 83$) Sprague Dawley rats.

Results—The KOR antagonist, norBNI, had weak and inconsistent effects on THC-induced taste avoidance in adolescent rats in that norBNI both attenuated and strengthened taste avoidance dependent on dose and trial. norBNI had limited impact on the final one-bottle avoidance and no effects on the two-bottle preference test. Interestingly, norBNI had no effect on THC-induced taste avoidance in adult rats as well.

Conclusions—That norBNI had no significant effect on THC-induced avoidance in adults and a minor and inconsistent effect in adolescents demonstrates that the aversive effects of THC are not mediated by KOR activity as assessed by the CTA design in Sprague Dawley rats.

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Keywords

⁹-tetrahydrocannabinol; taste avoidance; norbinaltorphimine; opioid; drugs of abuse; adolescent; adult; cannabinoid; addiction

1 Introduction

When an animal is injected with a drug following consumption of a novel taste, it will avoid that taste on subsequent exposures, presumably due to an association between the taste and the aversive effects of the drug (Garcia and Ervin 1968; Revusky and Garcia 1970; Rozin and Kalat 1971; for an alternative explanation, see Grigson 1997). This phenomenon, termed a conditioned taste avoidance (CTA), is a unique form of learning in that it is often acquired after a single taste-drug pairing, even if the taste and drug are separated by long delays (Revusky and Garcia 1970). Further, such learning appears to be relatively selective to taste in that exteroceptive cues, such as lights and tones, are not as readily associated with aversive drug effects (Rozin and Kalat 1971). CTA learning is thought to be evolutionarily shaped, given that rapid and selective associations over the long delays that naturally accompany digestion prevent the repeated consumption of poisoned foods (for a review, see Freeman and Riley 2009).

Initially demonstrated with radiation as the aversive stimulus (Garcia et al. 1955), CTAs have since been reported using a variety of drugs from a wide range of drug classes, including those that support self-administration (Gamzu 1977; Hunt and Amit 1987; Riley and Freeman 2004; see Riley and Tuck 1985 for a list of drugs that induce CTA). Research investigating its biological mechanism(s) has primarily focused on the related associative processes involved in such learning and has generally been limited to instances when emetics are used as the inducing agent (Lamprecht et al. 1997; Reilly 2008; Yamamoto 1993). Consequently, the mechanisms mediating the specific aversive effects of drugs of abuse are still relatively unknown. One drug that has received recent focus in this context is ⁹-tetrahydrocannabinol (THC), a partial cannabinoid 1 receptor (CB1) agonist and the primary psychoactive constituent of marijuana.

THC has been shown to induce taste avoidance at a wide range of doses (see Sengstake and Chambers (1976); see also Parker and Gillies 1995; Switzman et al. 1981, Wakeford & Riley, 2014), and although no work with the CTA design has addressed the biological mechanisms mediating its aversive effects, recent work with THC in the conditioned place avoidance (CPA) procedure has implicated activity at the kappa opioid receptor subtype (KOR). For example, adult mice pretreated with 10 mg/kg of the long-lasting KOR antagonist norbinaltorphimine (norBNI) fail to acquire place avoidance induced by 5 mg/kg THC, whereas non-pretreated mice acquire robust avoidance at the same dose (Zimmer et al. 2001). The same study demonstrated that knock-out mice lacking the gene coding for prodynorphin (the precursor for the endogenous KOR agonist dynorphin) display attenuated THC-induced place avoidance relative to intact animals. In a related study, Ghosland et al. (2002) examined the ability of THC (5 mg/kg) to induce CPA in three strains of adult knock-out mice lacking the genes coding for either the mu opioid-receptor subtype (MOR), the

delta opioid-receptor subtype (DOR) or KOR. While knock-out mice with selective deletions for MOR and DOR displayed THC-induced CPA, knock-out mice with the selective deletion for KOR did not. Finally, knock-out mice lacking the gene coding for the downstream regulatory element antagonistic modulator (DREAM) for prodynorphin demonstrate potentiated place avoidance at 1 and 5 mg/kg THC versus intact mice receiving the same dose (Cheng et al. 2004). DREAM acts as a transcriptional repressor for the prodynorphin gene, and thus, mice lacking this gene have upregulated KOR activity through increases in prodynorphin production.

Although the role of kappa activity in the aversive effects of THC has been demonstrated in adult animals, it has not been examined in adolescents. Adolescent rats acquire THC-induced taste avoidance (albeit weaker than those seen in adults; Schramm-Sapota et al. 2007), yet they are relatively insensitive to manipulations of the KOR system. Low to intermediate doses of the KOR agonist, U62-066, which reliably induce taste avoidance in adult rats, are incapable of producing such effects in adolescents (Anderson et al. 2014). Only high doses of U62-066 are effective at producing avoidance in adolescent rats (Anderson et al. 2013). If adolescents are insensitive to manipulations of the KOR system, yet still demonstrate robust THC-induced taste avoidance, it is possible that the aversive effects of THC in adolescents are mediated by a different mechanism than those in adults.

The goal of the present study was to characterize the role of the KOR system in mediating the aversive effects of THC in adolescent and adult Sprague-Dawley rats as measured through the CTA design. If the KOR system has no role in mediating the aversive effects of THC in adolescents, it might be predicted that antagonism of kappa activity in adolescent rats will have minimal (or no) effects on THC-induced taste avoidance (relative to those seen in adults). In order to assess KOR involvement in the aversive effects of THC, adolescent (Experiment 1) and adult (Experiment 2) rats were treated once with the long-term KOR antagonist norBNI 24 hours prior to taste avoidance conditioning with 0, 0.56, 1.0, 1.8 or 3.2 mg/kg THC.

2 Methods

Subjects—The subjects were 167 experimentally naïve, male Sprague Dawley rats (Harlan Sprague-Dawley; Indianapolis, IN). They arrived at the facility on postnatal day 21 (PND 21) and were group-housed in polycarbonate bins (23 × 44 × 21 cm, $n = 2-3$ per bin) and maintained on a 12:12 light-dark cycle (lights on at 0800 h) at an ambient temperature of 23°C. During CTA adaptation, conditioning and testing procedures (see below), animals were temporarily transferred to individual hanging wire-mesh test cages (24.3 × 19 × 18 cm) located in the same room as their group-housed bins. All procedures occurred during the light phase, and unless otherwise stated, food and water were available ad libitum. This study was approved by the Institutional Animal Care and Use Committee at American University and followed the National Research Council's *Guide for the Care and Use of Laboratory Animals* (2011) and the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (2003).

Drugs and Solutions—Norbinaltorphimine (norBNI, synthesized at the Chemical Biology Research Branch of the National Institute on Drug Abuse) was dissolved in sterile H₂O at a concentration of 15 mg/ml and administered subcutaneously (SC) at a dose of 15 mg/kg. Sterile H₂O was administered as vehicle to control animals at a volume equal to the 15 mg/kg dose of norBNI. ⁹-tetrahydrocannabinol (THC, generously supplied by the National Institute on Drug Abuse, NIDA) was dissolved in a solution of 5% ethanol, 5% Cremophor (Sigma-Aldrich) and 90% saline at a concentration of 1 mg/ml and administered intraperitoneally (IP) at a dose of 0.56, 1.0, 1.8 or 3.2 mg/kg. The vehicle was also prepared as a 5% ethanol, 5% Cremophor (Sigma-Aldrich) and 90% saline solution and was administered to control animals at a volume equal to the highest dose of THC administered (3.2 mg/kg). All drug, vehicle and saline solutions were filtered through a 0.2 µl syringe filter to remove any possible contaminants before being administered. Sodium saccharin (0.1%; Sigma-Aldrich) was prepared daily as a 1 g/L solution in tap water.

2.2 Experiment 1: Adolescents

Phase I: Habituation—Abbreviated CTA procedures designed to maintain proper growth rates in adolescent animals were utilized as published previously (Hurwitz et al. 2012; Wetzell et al. 2014). Beginning on PND 22, subjects ($n = 84$) were weighed daily to acclimate them to experimenter handling. On PND 28, they were deprived of water for 24 h prior to the start of water habituation. On PND 29, they were transferred to test cages and given 45-min access to water presented in graduated 50 ml Nalgene centrifuge tubes each affixed with a rubber stopper and sipper tube, after which they had ad-libitum access to water in their home bins for 23¼ h. This 2-day cycle (23¼-h water deprivation/45-min test cage access followed by 24-h water access) was repeated two more times (final day on PND 33).

Phase II: Drug pretreatment—Animals were ranked according to average water consumption on all habituation cycles and assigned to one of two groups [norBNI ($n = 42$) and Vehicle ($n = 42$)], such that mean water intake was comparable among groups. On PND 34 (approximately 24 h prior to conditioning, see below), subjects assigned to the norBNI group were injected with norBNI (15 mg/kg) and subjects assigned to the Vehicle group were injected with the norBNI vehicle at an equal volume. The timing of the norBNI injection was based on research by Munro and colleagues (2012) demonstrating that SC administration of norBNI produced long-lasting antagonistic effects that were selective to KOR.

Phase III. Taste avoidance conditioning—Following the injection of norBNI or vehicle, animals were deprived of water for 23¼ h (PND 34). A novel saccharin solution was then presented instead of water for 45 min on PND 35. Following saccharin access, animals in each pretreatment group (norBNI and Vehicle) were immediately rank-ordered according to saccharin consumption and assigned to one of five groups [0 ($n = 18$), 0.56 ($n = 16$), 1.0 ($n = 16$), 1.8 ($n = 16$), 3.2 ($n = 18$)], such that mean saccharin intake was comparable. This procedure yielded a total of 10 groups; N0, N0.56, N1.0, N1.8, N3.2, V0, V0.56, V1.0, V1.8 and V3.2, where N or V refers to the pretreatment group (norBNI or Vehicle) and the number refers to the dose of THC administered during conditioning. THC

doses were chosen based on their range from potentially rewarding to aversive (Braidia et al. 2004; Wakeford and Riley 2014). Within 20 min of saccharin access, subjects were injected with drug or vehicle and given ad-libitum water access in their home bins for 23¼ h. This 2-day cycle was repeated three more times for a total of four conditioning trials (final day on PND 41).

Phase IV. Two-bottle test—Following the final two-day cycle during conditioning, animals were deprived of water on Day 42 for 24h prior to being given 45-min access to two Nalgene tubes (one containing tap water and the other containing the saccharin solution) in a two-bottle assessment of the CTA (Day 43). Bottle placement was counterbalanced to control for positioning effects on this test. Following this access, the animals were returned to their home bins and no injections were administered.

2.3 Experiment 2: Adults

The procedures for the adults were identical to those described above with the following exceptions: 83 subjects were brought into the facility on PND 21 and maintained on ad-libitum food and water with no manipulations until PND 76 when daily weighing began. Water bottles were removed on PND 83, and the habituation phase began on PND 84. The norBNI or vehicle injections were given on PND 89, and the four CTA conditioning trials occurred from PND 90 to 96. The final two-bottle test was performed on PND 98.

2.4 Statistical Analysis

Saccharin consumption on the four conditioning sessions for each age group was analyzed via a $2 \times 4 \times 4$ repeated measures ANOVA with between-subject factors of Pretreatment (norBNI or vehicle) and Dose (0.0, 0.56, 1.0, 1.8 and 3.2) and a within-subject factor of Trial (1-4). In the case of a three-way interaction, simple effects of Trial at each Pretreatment and Dose (multivariate analysis) and simple effects of Pretreatment at each Dose and Trial (univariate analysis) were assessed with Bonferroni-corrected multiple comparisons as warranted. Saccharin consumption on the final one-bottle avoidance test (Conditioning Trial 4) for each age group was analyzed via a 2×5 factorial ANOVA with between-subject factors of Pretreatment (norBNI or Vehicle) and Dose (0.0, 0.56, 1.0, 1.8 and 3.2). A two-way interaction was followed by univariate analyses for simple effects at each level of Pretreatment and Dose and followed by Bonferroni-corrected pairwise comparisons as needed. On the two-bottle CTA test, saccharin and water consumption was recorded and the percent saccharin of total fluid consumption (i.e., saccharin/saccharin + water) for each age group was then analyzed with a 2×5 factorial ANOVA with between-subject factors of Pretreatment (norBNI or Vehicle) and Dose (0, 0.56, 1.0, 1.8 and 3.2). A two-way interaction was followed by univariate analyses for simple effects at each level of Pretreatment and Dose and followed by Bonferroni-corrected pairwise comparisons as needed. Significance for all tests was set to $\alpha = 0.05$.

3 Results

3.1 Experiment 1: Adolescents

Taste avoidance conditioning and final one-bottle avoidance test—THC induced dose-dependent taste avoidance in both norBNI- and vehicle-pretreated adolescent rats (see Figure 1). There was no consistent effect of norBNI pretreatment. The $2 \times 5 \times 4$ repeated measures ANOVA revealed significant effects of Dose [$F(4, 74) = 69.018$] and Trial [$F(3, 222) = 33.037$], as well as significant Dose $\times$ Trial [$F(12, 222) = 20.722$], Pretreatment $\times$ Dose [$F(4, 74) = 4.419$] and Pretreatment $\times$ Dose $\times$ Trial [$F(12, 222) = 2.573$] interactions. Tests for simple effects of Trial at each Pretreatment and Dose were significant for Groups V0 [$F(3, 72) = 9.670$], V0.56 [$F(3, 72) = 3.908$], V1.8 [$F(3, 72) = 19.001$], V3.2 [$F(3, 72) = 27.542$], N0 [$F(3, 72) = 12.521$], N1.0 [$F(3, 72) = 7.122$], N1.8 [$F(3, 72) = 5.117$], and N3.2 [$F(3, 72) = 28.311$]; however, there was no effect of Trial for Groups V1.0 and N0.56.

Bonferroni-corrected multiple between-group comparisons revealed that all norBNI-pretreated groups conditioned with THC drank significantly less saccharin than vehicle-conditioned animals of the same pretreatment group (Group N0) by Trial 2, with the exception of animals conditioned with 0.56 mg/kg THC which did not significantly differ from Group N0 on any trial. Group N1 drank significantly less saccharin than Group N0.56 on Trials 2, 3 and 4. Group N3.2 drank significantly less saccharin than all groups except for Group N1 by Trial 2. Group N3.2 drank significantly less saccharin than Group N1 by Trial 3. All vehicle-pretreated groups conditioned with THC drank significantly less saccharin than vehicle-conditioned animals of the same pretreatment group (Group V0) by Trial 2, with the exception of animals conditioned with 1 mg/kg THC which didn't drink significantly less than Group V0 until Trial 4. Group V1.8 drank significantly less saccharin than Groups V0.56 and V1 by Trial 3, whereas group V3.2 drank significantly less saccharin than Groups V0.56 and V1 by Trial 2. Groups V0.56 and V1 as well as Groups V1.8 and V3.2 did not significantly differ from one another on any trial (see Figure 1).

The analysis of simple effects of Pretreatment during CTA acquisition at each dose revealed that Group N1 drank significantly less saccharin than their vehicle-injected counterparts (Group V1) on Trials 2 and 3 [$F(1, 74) = 12.571$ and $F(1, 74) = 6.064$ respectively]. However, this effect was reversed for the 1.8 dose groups in that Group N1.8 drank significantly more saccharin than its vehicle-injected counterpart (Group V1.8) on Trials 3 and 4 [$F(1, 74) = 10.781$ and $F(1, 74) = 6.178$ respectively].

The 2×5 factorial ANOVA on the final one-bottle avoidance test (Conditioning Trial 4; illustrated in Figure 2, upper panel) resulted in a two-way interaction of Pretreatment and Dose [$F(4, 74) = 3.045$] with a significant effect of Dose [$F(4, 74) = 66.455$], but not Pretreatment. Similar to the results of CTA acquisition, Group N1.8 drank more saccharin than its vehicle-pretreated counterpart (V1.8). There were no other significant pretreatment effects among groups on Trial 4.

Two-bottle test—THC induced a dose-dependent decrease in saccharin preference on the two-bottle test independent of pretreatment (see Figure 2, lower panel). The adolescent 2×5 factorial ANOVA for percent saccharin consumed during the two-bottle test revealed a

significant effect of Dose [$F(4, 74) = 17.243$]. There was no effect of Pretreatment, nor was there a Dose $\times$ Pretreatment interaction. In relation to the significant Dose effect (collapsed across Pretreatment; not illustrated), all THC-conditioned groups showed significantly less preference for saccharin than the vehicle-conditioned groups (Groups N0 and V0). Furthermore, animals conditioned with 0.56 mg/kg of THC (Groups N0.56 and V0.56) showed a significantly higher preference for saccharin than animals conditioned with 1.8 and 3.2 mg/kg of THC. Animals conditioned with 1.0, 1.8 and 3.2 mg/kg of THC were not significantly different from one another in their saccharin preferences (all p 's $< .05$).

3.2 Experiment 2: Adults

Taste avoidance conditioning and final one-bottle avoidance test—THC induced dose-dependent taste avoidance in both norBNI- and vehicle-pretreated rats with no differences between the two pretreatment treatments (see Figure 3). The $2 \times 5 \times 4$ repeated measures ANOVA revealed significant effects of Dose [$F(4, 72) = 54.927$] and Trial [$F(3, 216) = 78.776$], as well as a Dose $\times$ Trial interaction [$F(12, 216) = 21.914$]. There was no effect of Pretreatment, nor were there significant Pretreatment $\times$ Dose, Pretreatment $\times$ Trial or Pretreatment $\times$ Dose $\times$ Trial interactions (see Figure 4).

In relation to the significant Dose $\times$ Trial interaction (collapsed across Pretreatment; not illustrated), all groups conditioned with THC drank significantly less saccharin than groups conditioned with vehicle (Groups N0 and V0) by Trial 2. Groups injected with 1.8 and 3.2 mg/kg of THC drank significantly less than those injected with 0.56 and 1.0 mg/kg of THC by Trial 2 and these effects were maintained on Trials 3 and 4. Finally, subjects in Group 1.0 drank significantly less saccharin than Group 0.56 mg/kg on Trial 4 (all p 's $< .05$).

The 2×5 factorial ANOVA on the final one-bottle avoidance test (Conditioning Trial 4; illustrated in Figure 4, upper panel) did not result in a significant 2-way interaction of Pretreatment and Dose which precluded any post-hoc analyses on the final one-bottle avoidance data. Although there was no 2-way interaction, there were significant effects of Pretreatment [$F(1, 72) = 4.432$] and Dose [$F(4, 72) = 143.487$].

Two-bottle test—THC induced a dose-dependent decrease in saccharin preference on the two-bottle preference test independent of pretreatment (see Figure 4, lower panel). The 2×5 factorial ANOVA indicated a significant effect of Dose [$F(4, 72) = 59.606$]. There was no effect of Pretreatment, nor was there a Dose $\times$ Pretreatment interaction. In relation to the significant Dose effect (collapsed across Pretreatment; not illustrated), all THC-conditioned groups showed significantly less preference for saccharin than the vehicle-conditioned groups (Groups N0 and V0). Furthermore, animals conditioned with 0.56 mg/kg (Groups N0.56 and V0.56) of THC showed a significantly higher preference for saccharin than animals conditioned with 1.0, 1.8 or 3.2 mg/kg of THC. Animals conditioned with 1.0, 1.8 or 3.2 mg/kg of THC were not significantly different from one another in their saccharin preferences (all p 's $< .05$).

4 Discussion

Although the aversive effects of THC appear to be mediated by KOR activity in adults (Cheng et al. 2004; Ghozland et al. 2002; Zimmer et al. 2001), it is unknown to what extent, if any, kappa receptor activity is involved in such effects in adolescents. Given that adolescent rats display relative insensitivity to the aversive effects of the kappa agonist U62-066 (Anderson et al. 2014), the aversive effects of THC may be mediated differently in this age group. As described, norBNI's effect on taste avoidance in adolescents was inconsistent in that norBNI had no effect on avoidance induced by the lowest (0.56 mg/kg) and highest (3.2 mg/kg) doses of THC and opposite effects at the intermediate doses (strengthening of avoidance at 1.0 mg/kg and attenuation of avoidance at 1.8 mg/kg). For all cases in which norBNI significantly impacted THC-induced avoidance, the effects were modest and trial dependent. On the final one-bottle avoidance test, norBNI pretreatment significantly attenuated taste avoidance only in the group of animals conditioned with 1.8 mg/kg of THC. There was no evidence of any pretreatment effect of norBNI on the two-bottle assessment as vehicle and norBNI-treated subjects displayed comparable dose-dependent avoidance of the THC-associated saccharin. Such findings are consistent with the initial prediction that adolescents are relatively insensitive to activity at the kappa receptor and that taste avoidance induced by THC would be mediated by a non-KOR related mechanism. Similar assessments were made in adults, primarily to replicate previous research and confirm that THC's aversive effects were kappa mediated in this population when assessed using the CTA procedure. Interestingly, norBNI was without effect in the adult subjects on any analysis run (i.e., acquisition, final one-bottle avoidance test or two-bottle test).

One immediate issue is whether norBNI at this dose and under the specific parameters tested is behaviorally active. As noted, although norBNI had no consistent effect in adolescent subjects, there were significant changes in THC-induced avoidance between saline- and norBNI-treated animals indicative of a behaviorally active dose. Interestingly, recent work from a number of laboratories report that norBNI as administered in the present study is capable of affecting drug-induced taste avoidance, as well as other behavioral effects. Specifically, subcutaneous norBNI pretreatment at a similar dose range used here and administered 24 h prior to the beginning of experimental procedures significantly affects ethanol-induced taste avoidance in stressed mice (Anderson et al. 2013), ethanol intake in adult mice (Morales et al. 2014), cocaine self-administration in rats given 6-h access to cocaine (Wee et al. 2009) and heroin self-administration in rats given 6-h access to heroin (Schlosburg et al. 2013). Although norBNI has been reported to impact a variety of behavioral endpoints, it is important to note that others have also found null effects when assessing the role of KOR activity with norBNI administration in the behavioral effects of other drugs of abuse (Hutsell et al. 2015; Negus 2004).

Although the present findings with adolescents are novel in terms of assessing the role of KOR mediation of THC's aversive effects, the results with adults are quite different from those seen in other assessments (see Ghozland et al. 2002; Zimmer et al. 2001). In this context, it is important to note that a number of differences exist among the various assessments that may impact the effects of KOR antagonism on THC-induced avoidance.

First, all previous assessments of KOR mediation of the aversive effects of THC utilized C57BL/6J mice or genetically modified mice created from this strain (Cheng et al. 2004; Ghozland et al. 2002; Zimmer et al. 2001), whereas the present assessment utilized Sprague-Dawley rats. In this context, it is interesting to note that the two species have been shown to express qualitatively different affective responses to manipulations of CB1 activity, supporting the notion that a species difference might account for the discrepant findings found between past and present research (see Braida et al, 2001; Braida et al, 2004; Chaperon et al, 1998; Hutcheson et al, 1998; Sanudo-Pena et al, 1997; Singh et al, 2004). Secondly, although the present experiments used a single norBNI pretreatment injection 24 h prior to the start of THC conditioning to antagonize KOR, all previous experiments that utilized norBNI to block THC-induced CPA administered the drug between 90 to 150 min prior to each of the THC conditioning trials. The timing of the norBNI administration is important in that norBNI induces antagonism of *both* MOR and KOR, with MOR antagonism occurring immediately following administration and lasting 4 h and KOR antagonism beginning within 2 h of administration and lasting for up to 21 days (Munro et al, 2012). Finally, prior work on THC's aversive effects used place aversion conditioning (Cheng et al. 2004; Ghozland et al. 2002; Zimmer et al. 2001), whereas the present studies used the CTA design (for reports of THC-induced CTA, see Chambers and Sengstake 1976; Parker and Gillies 1995; Switzman et al. 1981; Wakeford and Riley 2014). While both designs have been commonly used in assessing aversive drug effects (for CPA, see Stewart and Grupp 1986; Van der Kooy et al. 1983; for CTA, see Hunt and Amit 1987; Riley 2011; Verendelev and Riley 2012), comparisons between the two designs suggest that conditioned taste avoidance is a more sensitive measure, indexing aversions at lower doses (Lett 1985; Mucha and Herz 1985). Consequently, it may be more difficult to impact avoidance when using the CTA design.

Interestingly, many drugs of abuse produce both taste avoidance and place preferences at the same doses (for a discussion, see Riley, 2011). That the place conditioning assessment can measure both affective drug properties suggests that the rewarding effects of drugs of abuse might overshadow their aversive effects in the place conditioning design, allowing the acquisition and expression of a conditioned place preference as opposed to a conditioned place avoidance. In this context, it might be the case that KOR activity plays a role in the modulation of THC's rewarding effects and thus the reported change in place avoidance induced by the blocking of KOR reflects changes in reward and not in THC's aversive effects. Such a possibility is supported by the fact that THC increases dopamine release at the nucleus accumbens (Jianping et al. 1990; Tanda et al. 1997; Ton et al. 1988) and increases dynorphin production and release (Houser et al. 2000; Mason et al. 1999; Welch and Eads 1999) which in turn inhibits mesolimbic dopamine (Manzanares et al. 1991; Margolis et al. 2006; Spanagel et al. 1992). Blocking KOR activity would increase DA and THC's rewarding effects. Such an interaction would not be evident in the CTA design that indexes the aversive effects of drugs.

Stating that KOR activity is not involved in THC's aversive effects as indexed in the CTA design says little as to what mechanism is mediating the avoidance. However, the inability to identify the mechanism mediating a drug's aversive effects is not limited to THC as the vast majority of research on the biology of taste avoidance learning is focused primarily on the

physiological mechanisms underlying the associative learning process itself and not the aversive drug effects (see Introduction). Consequently, the specific biological systems and pathways involved in many drugs' aversive effects simply have not been investigated in depth. Such is the case with THC. Thus, it will be important to assess the role of a number of biological systems in THC-induced CTA to determine which specific neurochemical mechanisms [e.g., glutamate (Chen et al. 2013; Pistis et al. 2002), opioid (Cheng et al. 2004; Ghozland et al. 2002; Zimmer et al. 2001), etc.] or various combinations, e.g., KOR and MOR activity (Cheng et al. 2004; Zimmer et al. 2001)] are important to this effect.

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Figure 1.

Mean (+/- SEM) saccharin consumption (ml) for each adolescent THC dose group throughout acquisition of taste avoidance induced by THC. Groups pretreated with norBNI are indicated by black symbols, whereas groups pretreated with VEH are indicated by the white symbols. The groups conditioned with VEH (i.e., N0.0 and V0.0 indicated by the black and white circles respectively) are repeated in each separate panel in order to allow an assessment of which THC-conditioned groups demonstrated significant taste avoidance in comparison with groups conditioned with VEH. * $p < .05$ norBNI pretreated compared with vehicle pretreated of the same THC conditioning dose. # $p < .05$ VEH-THC groups compared with VEH-VEH groups. ^ $p < .05$ norBNI-THC groups compared with norBNI-VEH groups.

Figure 2.

Mean (+/- SEM) saccharin consumption (ml) on Conditioning Trial 4 for adolescent subjects pretreated with norBNI or vehicle (top panel). * $p < .05$ norBNI pretreated compared with VEH pretreated of same THC conditioning dose. Percent saccharin of total fluid consumed (e.g., saccharin / saccharin + water) for adolescent subjects pretreated with norBNI or vehicle (bottom panel). There was no significant Pretreatment effect or interaction with Pretreatment as a factor.

Figure 3.

Mean (+/- SEM) saccharin consumption (ml) for each adult THC dose group throughout acquisition of taste avoidance induced by THC. Groups pretreated with norBNI are indicated by black symbols, whereas groups pretreated with VEH are indicated by black and white symbols. The groups conditioned with VEH (i.e., N0.0 and V0.0 indicated by the black and white circles, respectively) are repeated in each separate graph in order to allow an assessment of which THC-conditioned groups demonstrated significant taste avoidance in comparison with groups conditioned with VEH. There was no significant Pretreatment effect or interaction with Pretreatment as a factor.

Figure 4.

Mean ($\pm$ SEM) saccharin consumption (ml) on Conditioning Trial 4 for adult subjects pretreated with norBNI or vehicle (bottom panel). There was no significant Pretreatment effect or interaction with Pretreatment as a factor (top panel). Percent saccharin of total fluid consumed (e.g., saccharin / saccharin + water) for adult subjects pretreated with norBNI or vehicle (bottom panel). There was no significant Pretreatment effect or interaction with Pretreatment as a factor.