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Cannabinoid-induced lower lip retraction in rats

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Abstract

Rationale: Lower lip retraction (LLR) in rats has been described as a distinctive effect of 5-HT_{1A} agonists. In the course of evaluating behavioral effects of cannabinoid agonists in rats, LLR effects were evident following injection of several cannabinoid agonists.

Objectives: To pharmacologically characterize cannabinoid-induced LLR in rats.

Methods: Lower lip retraction was scored using a 3-point scale for up to 6 hours after injection of the cannabinoid agonists ⁹-tetrahydrocannabinol (⁹-THC; 1–10 mg/kg), AM7499 (0.01–1.0 mg/kg), or AM2389 (0.003–0.1 mg/kg), or, for comparison, the 5-HT_{1A} agonist 8-OH-DPAT (0.01–0.3 mg/kg). Next, antagonist effects of rimonabant (1–10 mg/kg) and WAY100635 (0.3 mg/kg) on LLR produced by cannabinoid or 5-HT_{1A} agonists were evaluated. Lastly, effects of 8-OH-DPAT were determined following pretreatment with AM2389 (0.003–0.01 mg/kg) or ⁹-THC (1 mg/kg).

Results: All three cannabinoid agonists produced LLR. Effects of AM2389 were attenuated by both rimonabant and WAY100635 whereas effects of 8-OH-DPAT were antagonized by WAY 100635 but not by rimonabant. Pretreatment with 1 mg/kg ⁹-THC or 0.01 mg/kg AM2389 shifted the 8-OH-DPAT dose-effect function for LLR to the left and isobolographic analysis of the data indicates CB1 and 5-HT_{1A} interactions can be supraadditive.

Conclusions: Cannabinoid agonists produce LLR in rats, an effect heretofore ascribed only to activity at 5-HT_{1A} receptors, via CB1 receptor-mediated actions. Co-administration of a cannabinoid agonist and the 5-HT_{1A} agonist 8-OH-DPAT results in a synergistic effect on LLR.

Keywords

Lower lip retraction; cannabinoid; ⁹-THC; AM2389; AM7499; 8-OH-DPAT

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Introduction

Activation of presynaptic 5-HT_{1A} receptors in rats produces a characteristic relaxation, or loss of tone, of the musculature of the lower lip, resulting in retraction of the lower lip and revealing the lower incisors. This phenomenon, termed “lower lip retraction” (LLR), is seen following administration of selective 5-HT_{1A} agonists such as buspirone, ipsapirone, and 8-OH-DPAT. In contrast, nonselective and indirect serotonergic agonists do not produce LLR unless tested in the presence of 5-HT antagonists that do not block 5-HT_{1A} receptors (Berendsen et al., 1989). The correspondence between the appearance of LLR and 5-HT_{1A} activation has been used to establish 5-HT_{1A}-activity of novel or nonselective compounds (Ahmad et al., 1997; Assié et al., 2010; Jones et al., 2010; Stefanowicz et al., 2016). More recently, LLR has been used, in conjunction with forepaw treading and flat body posture, to identify potential *in vivo* biased agonist effects of selective 5-HT_{1A} agonists (Jastrzebska-Wiesek et al., 2018). To our knowledge, no drug lacking affinity for 5-HT_{1A} receptors has been reported to produce LLR in rats, and we are aware of only one study demonstrating that a non-serotonergic drug, ethanol, may modify the LLR effects of a selective 5-HT_{1A} agonist (Kleven et al., 1995). However, in the course of completing other studies on the effects of cannabinoid ligands in rats, we noticed the appearance of LLR following injection of several novel cannabinoid agonists.

There is now abundant evidence that cannabinoids interact with other neurotransmitters, including 5-HT (Cheer et al., 1999; Townsend et al., 2002). In particular, cannabinoid modulation of 5-HT_{1A} activity has been invoked in describing mechanisms which may underlie potential antidepressant and anxiolytic effects of endocannabinoids (Bambico et al., 2010; Häring et al., 2015; Marco et al., 2004). Studies of 5-HT_{1A} and CB1 interactions have revealed both additive and inhibitory effects, depending on the behavioral or physiological response. For example, 5-HT_{1A} agonists were found to inhibit ⁹-THC-induced catalepsy in mice whereas pretreatment with the 5-HT_{1A} selective antagonist, WAY100635, appeared to enhance these effects of ⁹-THC (Egashira et al., 2006). A subsequent study similarly suggested opposing effects of 5-HT_{1A} and cannabinoid agonists, i.e., 8-OH-DPAT attenuated immobility produced by ⁹-THC in a forced swim assay (Egashira et al., 2008). On the other hand, other studies report that the relationship between cannabinoid and serotonergic mechanisms is more facilitatory in nature. Thus, pretreatment with 8-OH-DPAT increased the discriminative stimulus and response rate-decreasing effects of ⁹-THC in rhesus monkeys (McMahon, 2016). A reciprocal influence of cannabinoids on serotonergic activity was recently demonstrated in rats, with the observation that daily injections of the cannabinoid JWH-018 increased the hypothermic and behavioral effects of 8-OH-DPAT (Elmore and Baumann, 2018). In contrast, an earlier study suggested that the hypothermic effects of 8-OH-DPAT were eliminated, and the behavioral effects of a 5-HT_{2A} agonist DOI were enhanced, in rats treated daily with another cannabinoid agonist, HU-210 (Hill et al., 2006). Together, the results of these behavioral studies indicate that associations between cannabinoids and 5-HT_{1A} are found in several species, albeit the nature of these interactions vary.

Corresponding evidence from *ex vivo* studies confirm interactions between cannabinergic and serotonergic systems. Thus, the elimination of CB1 receptor function, in CB1 receptor

knockout mice, is accompanied by a functional decrease in activity of 5-HT_{2A/C} and 5-HT_{1A} receptors (Mato et al., 2007). Conversely, increasing the activity of endocannabinoids, via elimination of fatty acid amide hydrolase (FAAH) in FAAH knockout mice, results in an increased firing rate of serotonergic neurons in the dorsal raphe (Bambico et al., 2010). Similarly, 5-HT_{1A} receptor levels are increased in rat hippocampal tissue following administration of HU-210 (Zavitsanou et al., 2010). Cannabinoid and 5-HT interactions may be direct, as CB1 receptor mRNA and protein have been identified within serotonergic neurons (Häring et al., 2007), although indirect modulation of 5-HT neurons by cannabinoid agonists also has been proposed (Haj-Dahmane and Shen, 2011). In short, while a fair amount of data provide evidence for cross-talk between cannabinergic and serotonergic systems, the precise cellular mechanisms underlying these interactions remain to be determined.

In the present studies, we provide further evidence of cannabinoid and 5-HT_{1A} interactions using a pharmacologically selective measure. Specifically, we compared the effects of two synthetic cannabinoids - AM7499 (a relatively short-acting drug that binds CB1 and CB2 receptors with similar affinity; Kulkarni et al, 2016) and AM2389 (which has a relatively higher affinity for CB1 over CB2 receptors in vitro; Nikas et al, 2010) - to the effects of ⁹-THC and 8-OH-DPAT. Results demonstrate that cannabinoid agonists are able to produce LLR in rats, an effect previously attributed solely to activation of 5-HT_{1A} receptors. Antagonism studies demonstrate that the effects of the cannabinoid agonists are primarily mediated by CB1 receptors and combination studies with 8-OH-DPAT and AM2389 reveal greater than additive effects. These results indicate that agonists at these two receptor types may act synergistically in producing their common effects and suggest that measures of LLR can be useful in exploring cannabinoid and 5-HT_{1A} interactions.

MATERIAL and METHODS

Animals:

40 Female Sprague Dawley rats (Charles River Laboratories, Wilmington MA) weighing 200–250g at the beginning of the study were pair housed in a climate controlled room with food and water available *ad libitum*. Rats were acclimatized to the animal facility for at least 1 week prior to testing. They also were acclimatized to the study procedures prior to testing and then were studied repeatedly in groups of 6–8 animals (except where noted), with a minimum of 7 days between each drug test. All experiments were performed during the light portion of the light/dark cycle. All studies were approved by the Northeastern University Animal Care and Use Committee, in accordance with guidelines established by the National Research Council.

Procedures:

Rats were observed and scored for LLR in transparent chambers (9"×6" ×5") with mirrors placed outside at the bottom and back of the chambers. LLR was scored separately by two blinded observers 1–2 min apart, using a scale of 0–2, where 0 = absent (no teeth visible), 1 = partial (lower incisors partially visible) and 2 = full (lower incisors completely visible). Effects of drugs initially were determined using single dosing procedures and LLR was

scored before and up to 6 h after injections. Subsequently, complete dose-effect functions were obtained with 8-OH-DPAT and AM7499 using cumulative dosing procedures similar to those described previously (Berendsen et al., 1989) and incorporating inter-injection times of 15 or 60 min. Briefly, 12 or 57 min after injection of, respectively, 8-OH-DPAT or AM7499, LLR was scored and rats were injected with the next dose of the same drug, increasing the total cumulative dose by 0.5 to 1.0 log unit with each injection. Rats tested with cumulative doses of AM7499 did not receive more than three injections per day, thus full dose-effect functions for AM7499 were obtained by studying overlapping doses in different animals. For drug combination studies, drugs were administered 1 to 180 min prior to the determination of a full cumulative dose-effect function with 8-OH-DPAT or AM7499, or effects of single doses of AM2389.

Drugs:

8-OH-DPAT (8-hydroxy-N,N-dipropyl-2-aminotetralin) and WAY100635 [N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt] were purchased from Sigma-Aldrich (St. Louis, MO), and dissolved in distilled water. ⁹-THC and rimonabant were obtained from the National Institute on Drug Abuse (NIDA, Rockville, MD), AM2389 [9 β -hydroxy-3-(1-hexyl-cyclobut-1-yl)-hexahydrocannabinol] and AM7499 {butyl-2-[(6a*R*,9*R*,10a*R*)-1-hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[*c*]chromen-3-yl]-2-methylpropanoate} were synthesized at the Center for Drug Discovery, Northeastern University, as previously described (Kulkarni et al., 2016; Nikas et al., 2010). AM2389, AM7499, ⁹-THC and rimonabant were dissolved in 5% ethanol, 5% emulphor-620 (Rhodia, Cranbury, NJ) and 90% saline and were further diluted with saline. Injections were delivered s.c. in volumes of 1 ml/kg body weight; drug doses are expressed in terms of the weight of free base.

Statistical analysis:

Curve fitting, linear regression, and statistical analysis were performed on log-transformed values of doses using GraphPad Prism v.5.02 (GraphPad Software, LaJolla, CA). Data from individual subjects were grouped to obtain means and SEM, which were then analyzed using one- or two-way ANOVA followed by Dunnett's or Bonferroni's multiple comparison tests. Significance for all tests was set at $p < 0.05$. ED₅₀ values (i.e., doses required for LLR scores of 1.0) were calculated by nonlinear regression of group dose-effect functions, with minimum values constrained to 0, and maximum values constrained to 2. Potency ratios were calculated by dividing baseline ED₅₀ values by ED₅₀ values obtained after indicated pretreatments. Responses following combinations of AM2389 and 8-OH-DPAT were further analyzed according to principles of dose-equivalence (Tallarida, 2012; 2016). Isobolograms constructed using ED₅₀ values obtained from nonlinear regression of 8-OH-DPAT and AM2389 dose-effect functions were used to calculate expected additive ED₅₀ values. Combinations of 0.003 or 0.01 mg/kg 8-OH-DPAT and AM2389 (total doses of 0.006 or 0.02 mg/kg) were converted to log-transformed values and interpolation of the dose-effect data was used to calculate the observed ED₅₀ value.

RESULTS

Time course and control dose-effect functions:

Initial single dose studies with 8-OH-DPAT were completed in a group of six rats ($n=3/\text{dose}$) to confirm our methods. As shown previously (Koek et al., 1998), 8-OH-DPAT increased LLR with a rapid onset to action. Maximum LLR scores were obtained in all rats within 10 min after injection of 0.1 or 1.0 mg/kg 8-OH-DPAT (see Table 1). There was a full recovery to baseline by 90 min after 0.1 mg/kg 8-OH-DPAT whereas the higher dose of 1.0 mg/kg 8-OH-DPAT produced sustained LLR for up to 90 min after injection. Determination of a complete dose-effect function, using cumulative dosing procedures in a full group of animals ($n=8$), revealed an ED_{50} value (with 95% CI) of 0.021 mg/kg (0.017, 0.025).

The cannabinoid agonists Δ^9 -THC, AM2389, AM7499 all produced LLR following single s.c. injections, with effects significantly different from saline control values at multiple time points [Δ^9 -THC: $F_{(14,77)} = 7.05$; AM2389: $F_{(15,96)} = 38.93$; AM7499: $F_{(20,102)} = 17.56$]. The onset of LLR following Δ^9 -THC and AM2389 was relatively slow and maximum LLR scores were obtained 3 to 6 h after injection (Figure 1). AM7499 had a faster onset of action than Δ^9 -THC or AM2389, with significant LLR evident within 1 h after administration of 0.1 and 0.3 mg/kg AM7499. Maximum LLR scores of 2 were obtained in all rats that received 0.1 mg/kg AM2389 or 0.3 mg/kg AM7499 whereas only 1 out of 6 rats demonstrated full LLR following the highest dose (10 mg/kg) of Δ^9 -THC tested.

Dose-effect functions constructed from data obtained at 3 h after injection reveal graded, dose-related effects for each cannabinoid agonist, shown in Figure 2. Of the three cannabinoid agonists, AM2389 was the most potent, with an ED_{50} value (with 95% CI) of 0.021 mg/kg (0.016, 0.028); the ED_{50} values of AM7499 and Δ^9 -THC were, respectively, 0.034 mg/kg (0.024, 0.046) and 6.8 mg/kg (3.4, 13.5). The CB1 antagonist rimonabant, 1.0–10.0 mg/kg, had saline-like effects on LLR over a 6 h period with a maximum LLR score of 0.3 ± 0.2 obtained at 2 h following doses of 1 or 3 mg/kg rimonabant (data not shown). The 5-HT_{1A} antagonist WAY100635 (0.3 mg/kg) had no effects on LLR at 30 min after injection (mean LLR score = 0.0 ± 0.0).

Antagonism:

A dose of 0.3 mg/kg WAY100635 significantly antagonized the effects of 8-OHDPAT, shifting the dose-effect function of 8-OH-DPAT almost 25-fold to the right, as shown in Table 2 and Figure 3A. This same dose of WAY100635 had a lesser effect on AM7499, shifting the AM7499 dose-effect function slightly downward and increasing the ED_{50} value about 2.6-fold, from 0.07 mg/kg (0.05, 0.09) to 0.18 mg/kg (0.10, 0.32) as shown in Figure 3B. Results of 2-way ANOVA revealed significant main effects of WAY100635 [$F_{(1,50)}=9.1$] and AM7499 [$F_{(4,50)}=35.1$] with no interaction [$F_{(4,50)}=1.2$]. In addition, 0.3 mg/kg WAY100635 administered 1 min prior to 0.03 mg/kg AM2389 significantly attenuated the effects of AM2389 at 3 and 6 hours (Figure 4; [$F_{(1,15)}=28.26$]).

A high dose of rimonabant, 10.0 mg/kg injected 30 min prior to 8-OH-DPAT, had much lesser effects than WAY 100635, increasing the ED_{50} value of 8-OH-DPAT less than 2-fold (Figure 3A, Table 2). In contrast, lower doses of 1 and 3 mg/kg rimonabant, injected 30 min

prior to AM2389, antagonized LLR effects of 0.1 mg/kg AM2389 (Figure 4; $[F_{(2,60)}=81.93]$). The effects of 0.1 mg/kg AM2389 following either 1 or 3 mg/kg rimonabant were significantly different from the effects of 0.1 mg/kg AM2389 alone throughout the observation period, although in rats pretreated with 1 mg/kg rimonabant, LLR effects of 0.1 mg/kg AM2389 were evident at 4 and 6 hours after injection (Figure 4).

LLR Potentiation.

A 3 h pretreatment with low, ineffective doses of cannabinoid agonists - 0.003 and 0.01 mg/kg AM2389 or 1.0 mg/kg 9 -THC - resulted in leftward shifts of the 8-OHDPAT dose-effect function (Figure 5). The increased potency of 8-OH-DPAT in the presence of cannabinoid agonists is reflected in potency ratios greater than 1 (Table 2). Combinations of 0.003 mg/kg 8-OH-DPAT with 0.003 mg/kg AM2389, and 0.01 mg/kg 8-OH-DPAT with 0.01 mg/kg AM2389, maintain fixed 1:1 proportionate dosing relative to the ED_{50} values of the drugs. Accordingly, isobolographic analysis was completed to determine whether the interaction between the cannabinoid and 5-HT_{1A} agonists was additive, subadditive, or superadditive. This analysis revealed that the observed ED_{50} dose of the two drugs given in combination, 0.011 mg/kg, was half of the expected additive ED_{50} dose of 0.022 mg/kg. Calculation of the interactive index (T; Tallarida, 2012) resulted in a value of 0.54, less than 1.0 which would be expected of an additive interaction; thus, the interaction of 8-OH-DPAT and AM2389 can be characterized as superadditive.

DISCUSSION

The appearance of LLR in rats previously has been observed only after administration of 5-HT_{1A} agonists, including 8-OH-DPAT. This singular mechanism of action for LLR has encouraged its use as a tool to characterize selective 5-HT_{1A} receptor-mediated actions of novel ligands (Assié et al., 2010; Jastrzebska-Wiesek et al., 2018; Stefanowicz et al., 2016). Our results with 8-OH-DPAT and WAY100635 are consistent with those reported previously by other groups (Koek et al., 2000; Li et al., 2007). Using these same procedures, we found that three cannabinoid agonists - 9 -THC, AM2389, and AM7499 - also increased LLR in a dose-dependent fashion. Qualitatively, the effects of the cannabinoid agonists on LLR were indistinguishable from those of 8-OH-DPAT, with the exception that doses of 9 -THC up to 10 mg/kg did not produce full LLR in all animals. There were, however, timecourse differences among the drugs, as all three cannabinoids had slow onsets and long durations of action compared to 8-OH-DPAT. To our knowledge, this is the first report that LLR is produced by cannabinoid agonists and, more generally, the first finding that nonserotonergic drugs are able to produce LLR.

The LLR effects of cannabinoid agonists appear to be mediated predominantly by CB1 receptors. This is initially suggested by the range of doses of all three cannabinoid agonists that result in LLR, which are similar to those that produce other characteristic CB1-mediated effects in rats, such as antinociception, hypothermia or diuresis (Kulkarni et al., 2016; Paronis et al., 2013). CB1 receptor-mediation of LLR was confirmed through the use of rimonabant, a CB1 selective antagonist (Rinaldi-Carmona et al., 1995). Rimonabant dose-dependently antagonized the increase in LLR produced by the CB1 full agonist AM2389,

with 1 mg/kg rimonabant delaying the appearance of LLR following 0.1 mg/kg AM2389, and a higher dose fully blocking the effects of AM2389 over a 6 h period. Doses of 1 to 3 mg/kg rimonabant have been used previously to antagonize CB1-mediated discriminative stimulus and other behavioral effects of Δ^9 -THC and synthetic cannabinoid agonists in mice and rats (Chopda et al., 2013; Wiley et al., 2014). Despite being primarily mediated by CB1 receptors, a role for 5-HT_{1A} in cannabinoid-induced LLR is evident. Thus, a dose of WAY100635 that produced a 25-fold rightward shift of the 8-OH-DPAT dose-effect function also attenuated the LLR effects of an intermediate dose of AM2389, and shifted the AM7499 dose-effect function almost 3-fold to the right. In a reciprocal manner, 10 mg/kg rimonabant, higher than the dose needed to eliminate the effects of 0.1 mg/kg AM2389, shifted the function for 8-OH-DPAT rightward, albeit less than 2-fold. The relatively modest attenuation of effects of CB1 agonists by the 5-HT_{1A} antagonist, and vice-versa, are not representative of a competitive antagonism at the receptor level. Instead, these findings suggest that 5-HT_{1A} and cannabinoid agonists produce LLR primarily through distinctive receptor mechanisms, however, their actions may be modulated downstream of their respective receptors by activity within the complementary neurotransmitter systems.

Separate but coordinated actions of cannabinoid CB1 and 5-HT_{1A} agonism is further established by the enhanced effects of 8-OH-DPAT following AM2389 or Δ^9 -THC pretreatment. The dissimilar time-courses of action for the two classes of compounds dictated pretreatment times and limited the types of interaction studies that could be completed, i.e., the drugs could not be co-administered at fixed dose-ratios as has become common in studies of drug synergism (e.g., Minervini et al., 2017). Notwithstanding this limitation, it is clear that doses of Δ^9 -THC or AM2389 that were ineffective when given alone produced parallel and dose-related leftward shifts of the 8-OH-DPAT dose-effect function. Principles of dose-equivalence can be used to characterize the nature of the interaction between drugs that produce similar behavioral or physiological effects (Kimmel et al., 1997; Rawls et al., 2002; Ward et al., 2008). Here, isobolographic analysis indicates that combinations of CB1 agonists and 8-OH-DPAT are synergistic. This deviation from additivity is consistent with the idea that CB1 and 5-HT_{1A} agonists produce LLR through different mechanisms (Tallarida, 2006).

The finding that cannabinoid agonists have greater than additive effects with 8-OHDPAT in producing LLR is, to our knowledge, unique. In the first studies describing observations of LLR, Berendsen et al. (1989) evaluated the effects of more than 30 diverse drugs either alone or in combination with 8-OH-DPAT or other 5-HT_{1A}-selective ligands. In those studies, no drugs without 5-HT_{1A} affinity were found to add to the LLR effects of 8-OH-DPAT and only the adrenergic α_2 antagonist, idazoxan, intermittently interfered with 8-OH-DPAT-induced LLR. In contrast, several compounds were found to enhance the effects of 8-OH-DPAT on forepaw treading or flat body posture, two other behavioral effects associated with 5-HT agonists (Berendsen et al., 1989). This led to the conclusion that LLR is a highly selective 5-HT_{1A} effect, requiring direct agonist activity at 5-HT_{1A} receptors. In light of these findings, the present results are even more surprising, not only because they demonstrate that a different receptor system, CB1, can mediate LLR, but also because they reveal a superadditive relationship between cannabinoids and 8-OH-DPAT.

The cellular mechanisms that underlie the interactions between CB1 and 5-HT_{1A} have yet to be fully described. Results of some *in vitro* or *ex vivo* studies suggest a positive correlation between activity at these two receptors, with demonstrations that cannabinoid exposure increases endogenous levels of 5-HT or the density of 5-HT_{1A} receptors (Haj-Dahmane and Shen, 2011; Oakes et al., 2017; Zavitsanou et al., 2010). However, such findings are not uniform as other results suggest that cannabinoids presynaptically inhibit 5-HT release (Nakazi et al., 2000). Previous *in vivo* studies of interactions between cannabinoids and 5-HT have yielded similarly mixed results, with reports of both facilitatory and inhibitory effects of cannabinoids on 5-HT_{1A} activity, or of 5-HT_{1A} influences on cannabinoid activity. Of note, these studies employed behavioral or physiological endpoints such as open field activity (Bambico et al., 2010), swimming behavior (Egashira et al., 2008), or changes in body temperature (Elmore and Baumann, 2018; Hill et al., 2006), which each engage multiple neurotransmitter systems. Coupled with this, CB1 receptors are among the most abundant and widely distributed G-protein-coupled receptors in the brain, and cannabinoid ligands are relatively promiscuous with regard to their direct or indirect modulatory effects in different signaling pathways (Herkenham et al., 1991; De Petrocellis et al., 2000; Rawls et al., 2002; Solinas et al., 2010; Sun et al., 2007). Similarly, serotonin has been presented as the most abundant neurotransmitter in the brain, and at least 14 different 5-HT receptor subtypes have been identified (Carhart-Harris and Nutt, 2017; Jacobs and Azmitia, 1992). Moreover, among the 5-HT receptors subtypes, the two most prominent in psychopharmacology, 5-HT_{1A} and 5-HT_{2A}, often have directly opposing effects, or effects that maybe masked by actions at the alternate receptor (Carhart-Harris and Nutt, 2017; Li et al., 2007). If cannabinoids primarily affect serotonergic signaling by modulating the release of 5-HT, it is not difficult to grasp how in the use of nonselective behavioral measures might yield conflicting results. We propose that using a pharmacologically selective measure, such as LLR, may provide a more useful biomarker to evaluate *in vivo* interactions between CB1 and 5-HT_{1A} receptor activity.

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Fig 1.

Time course of lower lip retraction in rats. Panel A: Effects of Δ^9 -THC; Panel B: Effects of AM2389; Panel C: Effects of AM7499. Each point represents the mean of 6–8 rats; vertical bars indicate $\pm$ SEM. Ordinates: lower lip retraction score; Abscissae: Time since injection (in hours).

Fig 2.
Dose-effect functions for 8-OH-DPAT (injected cumulatively) and cannabinoid agonists (using data obtained 3 hours after injection, also shown in Figure 1). Each point represents the mean of 6–8 rats; vertical bars indicate $\pm$ SEM. Ordinates: lower lip retraction score; Abscissae: drug dose (in mg per kg).

Fig 3.

Panel A: Cumulative dose-effect functions for 8-OH-DPAT, obtained 30 min following pretreatment with vehicle, 10 mg/kg rimonabant or 0.3 mg/kg WAY100635. Points to the left over 'V' represents LLR scores after vehicle-only injections in the respective group of rats. Panel B: Cumulative dose-effect functions for AM7499 following 1 min pretreatment with 0.3 mg/kg WAY100635 or vehicle. Other details as in Figure 2.

Fig 4.

Time course of lower lip retraction produced by AM2389 after pretreatment with antagonists. Panel A: Effects of 0.03 mg/kg AM2389, alone or following 1 min pretreatment with 0.3 mg/kg WAY100635. Panel B: Effects of 0.1 mg/kg AM2389, alone or following 30 min pretreatment with 1 or 3 mg/kg rimonabant. Other details as in Figure 1.

Fig 5.

Panel A: Cumulative dose-effect functions for 8-OH-DPAT, obtained 3 h following pretreatment with vehicle, 0.003, or 0.01 mg/kg AM2389. Panel B: Cumulative dose-effect functions for 8-OH-DPAT, obtained 3 h following pretreatment with 1 mg/kg Δ^9 -THC or vehicle. Other details as in Figure 2.

Fig 6.

Isobologram constructed from data obtained with 8-OH-DPAT and AM2389. Points indicated near the axes represent the ED₅₀ values of each drug given alone; the solid line connecting these points represent predictive additive ED₅₀ values. The open square represent the predicted additive ED₅₀ value when the two drugs are administered in a 1:1 ratio (denoted by dashed line), and the open triangle represents the observed ED₅₀ value of the two drugs given in combination.

Table 1:

Mean ($\pm$ SEM) LLR scores at different times following single injections of 8-OHDPAT (n=3/dose).

8-OH-DPAT	5 min	10 min	15 min	20 min	30 min	60 min	90 min
0.1 mg/kg	1.7 $\pm$ 0.3	2.0 $\pm$ 0	2.0 $\pm$ 0	2.0 $\pm$ 0	2.0 $\pm$ 0	1.7 $\pm$ 0.1	0 $\pm$ 0
1.0 mg/kg	1.7 $\pm$ 0.3	2.0 $\pm$ 0	2.0 $\pm$ 0	2.0 $\pm$ 0	2.0 $\pm$ 0	2.0 $\pm$ 0	1.7 $\pm$ 0.3

Table 2:

ED₅₀ values (in mg/kg) and potency ratios compared to the vehicle pretreatment for 8-OH-DPAT after respective pretreatments (n=6–8 rats/group).

	Vehicle	3 mg/kg WAY100635	10 mg/kg Rimonabant	1 mg/kg ⁹ -THC	0.003 mg/kg AM2389	0.01 mg/kg AM2389
8-OH-DPAT ED₅₀ (95%CI)	0.021 (0.017, 0.025)	0.498 (0.390, 0.636)	0.037 (0.028, 0.049)	0.006 (0.004, 0.008)	0.012 (0.008, 0.018)	0.005 (0.004, 0.008)
Potency Ratio^a	NA	0.04	0.56	3.51	1.75	3.94

^aPotency ratios calculated as ED₅₀(vehicle) / ED₅₀(treatment); values <1 correspond to rightward shifts, values >1 = correspond to leftward shifts;
NA - not applicable