Chapter 67

Does Agonist Efficacy Alter the In Vivo Effects of Cannabinoids: Girl, Could We Get Much "Higher"?

Roger S. Gifford[1,2], Jimit G. Raghav[1,2], Torbjörn U.C. Järbe[1,2]

[1]Center for Drug Discovery (CDD), Northeastern University, Boston, MA, USA; [2]Department of Pharmaceutical Sciences, Northeastern University, Boston, MA, USA

Abbreviations

2-AG 2-Arachidonoylglycerol
11-OH 11-Hydroxy
AEA Arachidonoylethanolamine/anandamide
CB1R Cannabinoid 1 receptor
CB2R Cannabinoid 2 receptor
DEA Drug Enforcement Agency
ED_{50} Median effective dose
G_i G-protein that inhibits cAMP
G_s G-protein that stimulates cAMP
GABA γ-Aminobutyric acid
GPCR G-protein-coupled receptor
GTPγS Guanosine 5′-*O*-[γ-thio]triphosphate
ICSS Intracranial self-stimulation
NIDA National Institute of Drug Abuse
SAMHSA Substance Abuse and Mental Health Services Administration
SAR Structure–activity relationship
Δ^8-THC (−)-*trans*-Δ^8-Tetrahydrocannabinol
Δ^9-THC (−)-*trans*-Δ^9-Tetrahydrocannabinol

INTRODUCTION

(−)-*trans*-Δ^9-Tetrahydrocannabinol (Δ^9-THC), the main psychoactive ingredient in marijuana, has potent psychoactive effects on users, with sedation and altered cognition produced by high doses. Marijuana has been used for millennia, and Substance Abuse and Mental Health Services Administration (SAMHSA) survey data stated that marijuana is the most widely used illicit drug in the United States, with 7.5% of the population over 12 reporting use in the past month (SAMHSA, 2014). But new synthetic recreational drugs that target the cannabinoid 1 receptor (CB1R) have sprung up on the market, and their effects are still not well understood. Such recreational compounds will collectively be referred to as "Spice" in this chapter, irrespective of their chemical class or pharmacological profile once the compounds were detected and made illegal by law enforcement measures. Most of marijuana's effects are mediated through the CB1R, a G-protein-coupled receptor found throughout much of the brain. Most of these Spice compounds act as full agonists at CB1R, and Δ^9-THC is a partial agonist at CB1R, producing roughly 40% of the in vitro maximal effect of a full agonist such as CP-55,940 (Brents et al., 2012). This has raised concern that the Spice compounds may produce effects much stronger than those of Δ^9-THC, and case reports have been made of kidney failure, seizures, and death, symptoms rarely if ever seen with marijuana or Δ^9-THC (Behonick et al., 2014; Bhanushali, Jain, Fatima, Leisch, & Thornley-Brown, 2013; Harris & Brown, 2013). In the laboratory, though, most in vivo experiments have not found clear differences regarding partial versus full CB1R agonists beyond potency, i.e., the effects of full agonists could be replicated by a partial agonist at high enough doses (Fan, Compton, Ward, Melvin, & Martin, 1994). Before the rise of Spice abuse around 2005, very little was known about the recreational use of CB1R full agonists. But since then, Spice use has quickly grown into one of the most commonly abused drugs worldwide, with 8% of American high school seniors reporting using it in 2012 (Johnston, O'Malley, Bachman, & Schulenberg, 2013). To evaluate whether Spice's higher efficacy is responsible for its potential toxicity we need to review the differences in vivo between full and partial CB1R agonists.

The endocannabinoid system (ECS) is composed of two receptors, CB1R and CB2R. Of these, CB1R is responsible for the majority of the psychotropic effects of cannabinoid drugs. CB2R is mainly involved with immune function and will not be covered in this chapter. CB1R predominantly works via the G_i pathway, leading to inhibition of cAMP. It is at this level that we define partial and full agonist, based upon their maximal effect upon activation of CB1R. Partial agonists are purportedly limited in their maximal effect compared to full agonists (Childers, 2006). A hypothetical example is shown in Figure 1.

CANNABINOID AGONIST CLASSIFICATION

According to the International Union of Basic and Clinical Pharmacology (Pertwee et al., 2010), cannabinoid agonists currently

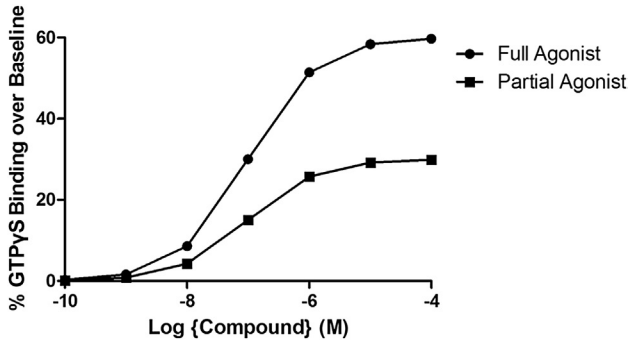

FIGURE 1 Example of efficacy differences at CB1R. Hypothetical representation of outcomes of full and partial agonists using an in vitro guanosine 5′-*O*-[γ-thio]triphosphate (GTPγS) receptor binding assay. Represented compounds have the same potency but different efficacies.

used for medicinal research and recreational use can be classified into four main groups as follows:

1. Classical cannabinoids: The compounds falling into this category have a dibenzopyran ring system. Δ^9-THC, the major psychoactive constituent of the marijuana plant, is the standard example. Other examples include Δ^8-THC, a minor isomer of marijuana that produces effects similar to but less potent than those of Δ^9-THC. The Δ^8-THC isomer is more chemically stable than the Δ^9-THC isomer and hence often used as the template for creating new ligands affecting the ECS (Jarbe & Gifford, 2014). HU-210 is a synthetic Δ^8-THC-derived cannabinoid that also falls in this category. HU-210 was the most potent cannabinoid compound synthesized at its creation, being 100–800 times more potent than Δ^9-THC, with a slow onset of effect but a long duration of action (Jarbe, Hiltunen, & Mechoulam, 1989; Mechoulam et al., 1988). HU-210 was classified as a Schedule I compound in 2008 after the US Drug Enforcement Agency found HU-210 in confiscated batches of Spice across the United States.
2. Nonclassical cannabinoids: Compounds in this class do not carry pyran rings and most of them are bicyclic ring compounds. CP-55,940 was the first compound synthesized in this class by the drug company Pfizer in 1974 (Melvin & Johnson, 1987), and it is still one of the most widely used compounds in the field of cannabinoid research. CP-47,497, a compound structurally similar to CP-55,940, was also synthesized by Pfizer (Weissman, Milne, & Melvin, 1982). This compound is more readily synthesized and later found its way into clandestine laboratories used for Spice production. CP-47,497 was placed on Schedule I in 2008.
3. Cannabinergic indoles: The compounds in this class have chemical structures different from the above cannabinoid agonists. WIN 55,212-2 was one of the first CB1R agonists to be synthesized in this class (Ward et al., 1990). Following this discovery, the National Institute of Drug Abuse sponsored further structure–activity relationship exploration of this class of compounds in collaboration with various investigators, resulting in a plethora of potent aminoalkylindoles and related indoles. JWH-018 (also known as AM678) was also an early compound in the aminoalkylindole series, and because of its potency at CB1R and the ease of synthesis, the compound made its way onto the streets as one of the earliest discovered Spice compounds (Atwood, Huffman, Straiker, & Mackie, 2010; Wiley et al., 1998). This eventually led to its ban in most countries of the world. Other potent CB1R agonists in this class that are now classified as Schedule I include JWH-073, AM2201, XLR-11, and UR-144.
4. Eicosanoids and derivatives: The two best known compounds in this class are arachidonoylethanolamine/anandamide (AEA) and 2-arachidonoylglycerol (2-AG), representing some of the body's endogenous cannabinoids. These compounds will not be discussed much in this chapter as, while they do activate CB1R (with AEA acting as a partial agonist and 2-AG as a full agonist), their pharmacological profiles do not completely match that of other cannabinoid drugs, and this extends to synthetic AEA analogues such as AM356 and AM1346 (Jarbe, Li, Liu, & Makriyannis, 2009; McMahon, Ginsburg, & Lamb, 2008).

The chemical structures of representative cannabinoid ligands from the above four classes are illustrated in Figure 2.

HERBAL INCENSE: NOT YOUR PARENTS' MARIJUANA?

While there has been extensive work, both clinical and observational, on the effects of marijuana and Δ^9-THC, very little was known about the compounds found in Spice until after reports of emergency room visits started surfacing. As such compounds had not originally been intended for use in clinical trials, no scientific data on the effects in humans were known and initially only a few in vivo animal studies had been performed. This lack of laboratory data precluded easy comparisons with marijuana and forced the study of Spice in humans to heavily rely on anecdotal reports of individuals who have used Spice, usually after their admission to a rehabilitation facility or a hospital.

Spice products are marketed in head shops all over the world, labeled as "not for human consumption." Various case studies reveal that the psychotropic effects of Spice can involve cases in which people experienced extreme paranoia along with panic attacks, while other anecdotal studies reported that the "high" obtained from Spice products is fairly similar to that obtained from smoking marijuana (Fattore & Fratta, 2011). Many of the users of Spice report that the onset of action of most Spice concoctions is faster than that of marijuana and the duration of the high is typically shorter compared to the marijuana-induced high. This may have some bearing on the sequence of tolerance development and dependence.

One 2013 survey concluded that there is a difference in the intensity of the highs caused by marijuana and Spice. In this study ($n = 14{,}966$), 17% of the sample reported using Spice, and 99% of all the subjects using Spice in the study reported being regular users of marijuana. The study concluded that all the surveyed marijuana users prefer marijuana over Spice (Winstock & Barratt, 2013). The main reason for their use of Spice was that it may go unnoticed in drug tests, whereas marijuana/Δ^9-THC does not. Finally, rarely do the users or the treatment providers know what dose, let alone what drug, is actually being taken, as labeling is scant to nonexistent. Overall, the anecdotal reports highlight side effects rarely seen with marijuana. Since Spice compounds are

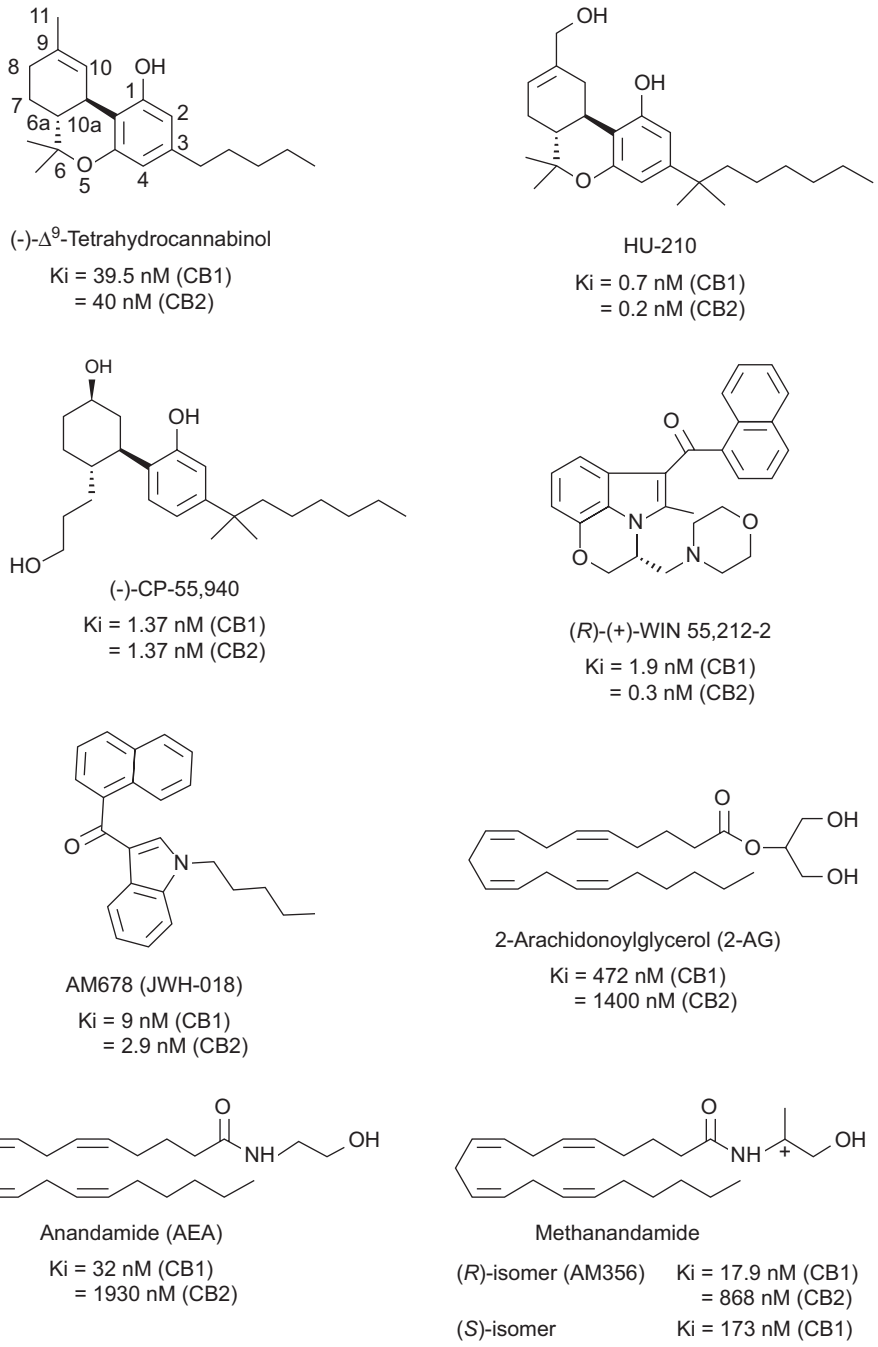

FIGURE 2 Structures of various cannabinoids from different classes. Chemical structures of Δ^9-THC and other ligands with affinity (Ki) for CB1R. AEA and 2-AG represent endogenous cannabinoids and AM356/methanandamide is a more stable analog of AEA.

almost entirely full CB1R agonists, it has been speculated that this may be the reason for the discrepancy between Spice products and marijuana/Δ^9-THC.

PRECLINICAL STUDIES

Almost all information about Spice use in humans is gained from recreational users' personal experiences, so objective data on Spice have to rely upon animal models. Very few studies have used Spice compounds though, as alternative synthetic full agonists were already in use in laboratories. Thus, most research on cannabinoid full agonists has used compounds that were not necessarily ever found in Spice. A common model used to test cannabinoid drugs is the "tetrad." By testing hypothermia, locomotor effects, analgesia, and catalepsy, a general picture of a cannabinoid's effects can be gained. Many early studies with full agonists, though, failed to find much difference between full agonists and Δ^9-THC (Fan et al., 1994). Paronis, Nikas, Shukla, and

Makriyannis (2012), however, reported that Δ^9-THC's maximal effect in a hypothermia assay was less than that of the full CB1R agonist AM2389. More importantly, the researchers also found that Δ^9-THC attenuated the hypothermia produced by AM2389 when the animals were pretreated with Δ^9-THC (Paronis et al., 2012). This antagonism could be overcome by increasing the AM2389 dose, thus displaying a theoretical hallmark effect of partial agonists combined with full agonists. One has to be careful, though, as other experiments failed to find this difference, and our own laboratory (unpublished) has found that the maximal effect of Δ^9-THC in hypothermia was on par with those of two full agonists (CP-55,940 and AM5983) and that problems of solubility limit the effectiveness of Δ^9-THC at the highest doses. In other tests of the tetrad, efficacy does not produce reliable differences of effect (Fan et al., 1994; Marshell et al., 2014). That Δ^9-THC will act inconsistently as a partial agonist in vivo may be due to uncontrolled variables, but what these conditions are is not known. Differences in species, handling, and previous drug history can all affect the results, and so what differences exist have not been ascertained. Since most of the experiments were carried out with varying methods, direct comparisons are difficult but suggestive that efficacy may be important under certain (unknown) conditions.

MARIJUANA DEPENDENCE STUDIES IN HUMANS

Marijuana, unlike other drugs of abuse such as morphine, barbiturates, and alcohol, is considered lacking a strong physical dependence component. For much of the early twentieth century it was widely believed that marijuana ingestion did not result in physical dependence. However, studies starting in the 1970s reported that people who consume marijuana chronically did indeed seek treatment for dependence (Haney, Ward, Comer, Foltin, & Fischman, 1999a). A few characteristics of marijuana dependence shared with other drugs of abuse is compulsive seeking behavior, limited self-control, and relapsing back to marijuana consumption despite the knowledge of the potential harm done by such dependence behavior (Maldonado, Berrendero, Ozaita, & Robledo, 2011). The dependence produced by marijuana is considered relatively mild compared to other drugs of abuse. Anxiety, insomnia, anorexia, and increased irritability are some of the symptoms associated with marijuana dependence (Gonzalez, Cebeira, & Fernandez-Ruiz, 2005). Some reports based on Δ^9-THC studies in humans strongly support the existence of such symptoms. A study with marijuana smokers was performed to elucidate withdrawal symptoms. In this study, subjects showed symptoms of withdrawal, which included increased anxiety, increased aggression, and reduction of appetite along with stomach pains (Haney, Ward, Comer, Foltin, & Fischman, 1999b). The general consensus about the time course of withdrawal seems to be that the withdrawal symptoms appear 24 h post-marijuana cessation, and the peak of these withdrawal symptoms lasts 1–4 days. The symptoms seem to dissipate within 1–2 weeks of abstinence (Cooper & Haney, 2008). There have also been case reports of people experiencing withdrawal from Spice, with symptoms appearing similar to those of marijuana withdrawal (Zimmermann et al., 2009).

DEPENDENCE AND WITHDRAWAL STUDIES IN ANIMALS

Δ^9-THC and most other cannabinoids to date are fat soluble, which is responsible for a "depot effect," wherein the drug is stored in fat tissues of the body and slowly released over time, providing a sustained release that makes withdrawal symptoms difficult to observe (Tanda & Goldberg, 2003). A widely used technique to elicit withdrawal and examine dependence in animals is by administering a CB1R antagonist (e.g., rimonabant) to animals chronically exposed to a cannabinoid agonist and thereby producing a state of withdrawal (Rodriguez de Fonseca, Carrera, Navarro, Koob, & Weiss, 1997). As of this writing there is no study available for cannabinoid dependence and withdrawal using Spice compounds such as JWH-018 or AM2201, although they have been studied with the aminoalkylindole WIN 55,212-2, wherein it produced effects similar to those of Δ^9-THC withdrawal (Aceto, Scates, & Martin, 2001).

TOLERANCE

Tolerance to cannabinoid agonists is well documented and can be formed in less than a week and may produce tolerance necessitating 50× more drug to achieve the same effect (Desai et al., 2013). While all reported cannabinoid agonists produce tolerance, one reason partial vs full CB1R agonists may differ in their effects lies in whether they produce the same amount of tolerance. One theory for why full agonists do not differ from partial agonists is that the receptor pool for CB1R is so large that maximal effect is achieved before saturation of all receptors (Gifford et al., 1999). As one cause of tolerance is downregulation of available receptors, the pool of receptors may decrease to the point at which high and low efficacy agonists may exert different maximal effects. Figure 3 shows an example of what this situation may look like. Data from Hruba, Ginsburg, and McMahon (2012) showed that chronic Δ^9-THC for 14 days significantly shifted the ED_{50} of Δ^9-THC drug discrimination for Δ^9-THC, CP-55,940, JWH-018, and JWH-073 by a factor of 9.2, 3.6, 4.3, and 5.6, respectively. Of these, both CP-55,940 and JWH-018's potency ratios were significantly different from that of Δ^9-THC. While the JWH-073 potency ratio difference did not reach significance compared to Δ^9-THC, the data are still supportive that tolerance for Δ^9-THC increased more than for the full agonist. This is not definitive, though, as the stronger tolerance to Δ^9-THC may just be due to Δ^9-THC being used to induce the tolerance, and other partial CB1R agonists were not examined. In support of this suggestion, other researchers (Desai et al., 2013) found that chronic administration of AM411, a partial CB1R agonist, did not produce differences in tolerance for Δ^9-THC, AM4054, and WIN 55,212, the last two of which are full CB1R agonists. These discrepancies highlight that induction of tolerance is highly dependent on both the protocol and the compound used, as tolerance is really a combination of various factors, from receptor downregulation to psychological mechanisms.

DRUG DISCRIMINATION

One way of measuring the subjective effects of drugs is through drug discrimination. Drug discrimination is a procedure by which animals are trained to perform a certain response to receive

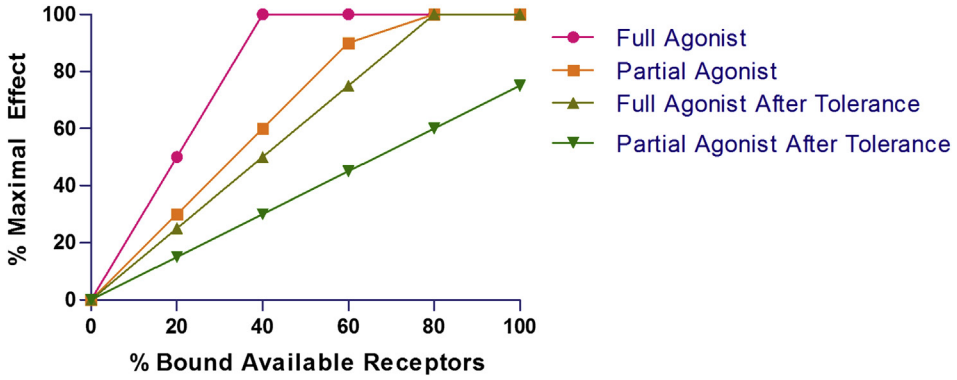

FIGURE 3 **Receptor pool interactions with full and partial agonists.** Example of how a large pool of receptors may cause a full agonist and a partial agonist to have the same maximal effect. The figure also includes an example of "tolerance" in which half the receptors have been lost, demonstrating how the partial agonist may differ from the full agonist under such circumstances.

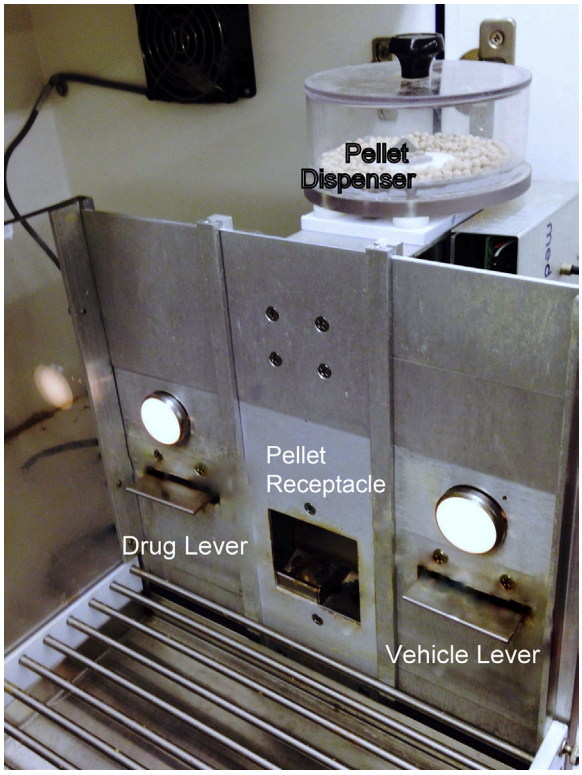

FIGURE 4 **Example drug discrimination chamber.** Pictured is an operant chamber used for drug discrimination. By pressing one of the two levers during training sessions and being rewarded with food pellets, animals learn to associate the respective lever with a certain condition (in this case left is the training drug and right is the placebo).

reinforcement after presentation of a specific stimulus, in this case either the presence or the lack of a certain drug. By training animals to recognize the effects of a specific drug, their response to another one can be measured to determine if it differs. Figure 4 shows an operant chamber used in such drug discrimination tests. Tests using both partial and full agonists as the training drug have demonstrated that animals generalize their response to the training drug to include other cannabinoid agonists, indicative that the subjective effects of CB1R agonists are all relatively similar (Jarbe, Deng, Vadivel, & Makriyannis, 2011; Jarbe, Li, Vadivel, & Makriyannis, 2010; Rodriguez & McMahon, 2014). To examine efficacy, Jarbe et al. (2012) took this a step further by using animals trained to discriminate between doses of AM5983, a full CB1R agonist. Theorizing that different doses of a full agonist may produce differing levels of tolerance, they found that while the EC_{50} for all compounds increased proportionally with training dose, the EC_{50} of the partial agonists Δ^9-THC and AM356 increased more rapidly with a higher training dose in comparison to the full agonist aminoalkylindole. This was attributed to the increased tolerance generated by regular exposure to a full agonist, which may help to whittle down the pool of CB1R available, requiring that partial agonists be given in proportionally greater doses to achieve similar levels of effect. Other researchers reported a similar outcome comparing the ED_{50} dose generalization curves for Δ^9-THC and CP-55,940 at two training doses of the full agonist CP-55,940 (De Vry & Jentzsch, 2003).

REINFORCEMENT

Research into the reinforcing value of cannabinoid drugs has been somewhat ambiguous, as many studies have reported inconsistent findings, some reporting no effect or negative reinforcement, while others found them to provide reinforcement. While the exact effect seems to be species/strain, dose, and prior history specific, there is strong evidence that cannabinoids play a role in reward processing and that they can be reinforcing under certain conditions. By reviewing the literature on reward and cannabinoids, Panagis, Mackey, and Vlachou (2014) found that, while there is discrepancy in the literature, the overall trends seem to indicate some differences between Δ^9-THC and synthetic full CB1R agonist compounds. For one, Δ^9-THC self-administration in naïve animals was accomplished only in 2013, as reviewed elsewhere (Justinova et al., 2013). By using low-dose intravenous perfusions into squirrel monkeys, reliably increased rates of Δ^9-THC self-administration were achieved compared to placebo sessions. So far, though, this feat has been accomplished only using squirrel monkeys and also in only one laboratory thus far. All other studies that reported Δ^9-THC self-administration included histories of either other drug exposure or severe food restriction. By using a synthetic full agonist like WIN 55,212-2, other laboratories have managed to produce synthetic cannabinoid self-administration

in both rats and mice, although it still remains difficult. Interestingly, rats self-administering WIN 55,212-2 did not sustain drug self-administration when THC was substituted for WIN 55,212-2 (Lefever, Marusich, Antonazzo, & Wiley, 2014). The failure to produce Δ^9-THC self-administration in rats, and the apparent difficulty involved in getting Δ^9-THC self-administration in any nonhuman model, is suggestive that full agonist synthetic cannabinoids may possess more reinforcing qualities than partial agonists, although discrepancies with other reinforcement models suggest something else may be occurring.

Conditioned place preference/avoidance assays are purported to reflect the inherent reinforcing qualities of a drug, such that rewarding drugs produce conditioned place preference and unpleasant ones produce aversion. Studies of cannabinoids find both outcomes, with both Δ^9-THC and full agonists producing place preference and aversion, depending on the exact protocol used (Panagis et al., 2014). For example, one research group (Hyatt & Fantegrossi, 2014) was able to produce conditioned place preference with Δ^9-THC at low doses and aversion at higher doses, but JWH-018 produced only conditioned place aversion at all doses tested. This could be overcome, though, by having the animals receive repeated exposure to Δ^9-THC. This caused low doses of JWH-018 to instead produce conditioned place preference. This is important, as it indicates that cannabinoid-naïve individuals may find Spice overtly strong and unpleasant and that only those with history of marijuana use may find it enjoyable.

One other approach to studying reward is by intracranial self-stimulation (ICSS), wherein an electrode is placed into the reward circuits of the brain and animals learn to perform a certain action to receive an electrical burst from the electrode. Typically, administration of rewarding drugs acts to lower the threshold of electrical stimulation necessary to act as reinforcement, while unpleasant/aversive drugs raise the threshold. Studies using Δ^9-THC show variable action, with it often acting to lower the threshold at low doses, but will instead increase the threshold at higher doses. Studies using full CB1R agonist compounds, though, were able only to raise the threshold for ICSS, with no documented case of actually lowering the threshold (Panagis et al., 2014). This appears counterintuitive to the fact that full agonists more readily act as reinforcers in self-administration studies.

IF NOT EFFICACY, WHAT, THEN?

While we have focused on efficacy as the underlying reason Spice may be more dangerous, there are numerous differences between Δ^9-THC/marijuana and Spice products that may be responsible for the discrepancies between the reported effects. One possibility is off-target effects, including those of possible metabolites, which have only lately begun to be investigated. For example, research (Brents et al., 2012) has shown that JWH-073 has multiple active metabolites that act as CB1R partial agonists, as well as one that acts as a CB1R antagonist. These partial agonists and the antagonist would muddy the effects in vivo, making comparisons difficult. This is true even of Δ^9-THC, whose main metabolite, 11-OH-Δ^9-THC, has been shown to be a full agonist (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990). Whether this is the reason for problems separating Δ^9-THC's effects from those of full agonists is not known. Possibly the best way to study this would be to use controlled deactivation cannabinoids, where CB1R agonists are specifically designed to have inactive metabolites, allowing for a better separation of the effects of full and partial agonists (Nikas et al., 2015; Sharma et al., 2013).

In addition to metabolites, functional selectivity of compounds may play a role. For example, Laprairie and colleagues tested six distinct cannabinoids from various chemical classes and found differing patterns of second-messenger recruitment for each, with CP-55,940 even producing some G_s activity, which usually produces a reaction opposite the standard G_i activity. In addition to second messengers, each ligand was also found to promote various amounts of arrestin to associate with CB1R, which is important in tolerance development and may explain some differences in tolerance production (Laprairie, Bagher, Kelly, Dupre, & Denovan-Wright, 2014).

In addition to differences in possible pharmacological targets/effects, the pharmacokinetics of the drug, including its method of administration, is very important. Nabilone is one of the two approved cannabinoid drugs in the United States, and since its approval, nabilone has been shown to act as a full agonist at CB1R. Its side effect profile typically mirrors that of dronabinol (i.e., Δ^9-THC), and there does not appear to be much difference in their safety profile. A major difference between nabilone and recreational synthetic cannabinoids, though, is its mode of administration. Interestingly, a review of potential abuse of nabilone found recreational use of nabilone to be rare (Ware & St Arnaud-Trempe, 2010). Users found nabilone to have more side effects and longer onset of effect than smoked marijuana. Possibly the side effects are due to the higher efficacy, but the lack of reported abuse is also likely to be related to its mode of administration. For further demonstration that mode of administration is important, see Marshell et al. (2014), who found that inhaled versus intraperitoneal (IP) injections of doses of JWH-018, JWH-073, and Δ^9-THC displayed different effects. Ignoring potency differences, one sees that inhalation produces none of the catalepsy observed after IP administration. Inhalation also reduced the maximal amount of analgesia produced, regardless of the agonist examined.

One other reason it may be so hard to detect partial CB1R agonists is that it may depend on the exact system used. For example Laaris, Good, & Lupica (2010), using γ-aminobutyric acid (GABA)/CB1R-expressing hippocampal axon terminals, found that both Δ^9-THC and WIN 55,212-2 had the same maximal effect on inhibitory postsynaptic currents. This is in contrast to glutamate/CB1R-expressing neurons from the same area, which showed Δ^9-THC to act as a partial agonist. The authors suggested that this is because the GABA neurons have higher levels of CB1R. This highlights the importance of the specific assay in defining full and partial agonists.

The main reason comparing marijuana to Spice is difficult is that marijuana is not composed solely of Δ^9-THC, but contains a myriad of other related compounds, which may have other pharmacological effects that may modulate the effects of marijuana (see Mechoulam, Fride, & Di Marzo, 1998; Russo, 2011). Furthermore, 11-OH-Δ^9-THC, a main metabolite of Δ^9-THC, has been studied for its cannabimimetic activity. Drug discrimination studies done with 11-OH-Δ^9-THC showed that it is generally more potent than Δ^9-THC in generalization studies done with Δ^9-THC as the training drug. 11-OH-Δ^9-THC, as well as 11-OH-Δ^8-THC, produces effects similar to those of Δ^9-THC also in humans, as summarized elsewhere (Balster & Prescott, 1992).

Other than Δ^9-THC, the only partial agonists studied to some extent in the literature are AEA, AM356, AM411, some other

phytocannabinoids, and the compound BAYER 59-3074. While AEA and its analogue AM356 have shown some differences from other cannabinoid agonists, such as not being fully antagonized by rimonabant and failing to affect animals at 100 mg/kg after chronic AM411 (Desai et al., 2013), these differences are not shared with Δ^9-THC. As mentioned previously, endocannabinoids and their analogues display some differences compared to other classes, regardless of efficacy (Jarbe, Lamb, Lin, & Makriyannis, 2001; Jarbe, Lamb, Liu, & Makriyannis, 2003). This lack of partial agonists tested means most assays compare only one partial agonist with a full agonist and thus cannot reliably indicate that efficacy is the reason for any difference in effect.

CONCLUSION

Overall, it seems that high/low efficacy may not be the most effective way to differentiate the important effects of various cannabinoids. While there definitely appears to be some discrepancies between Δ^9-THC and high-efficacy Spice compounds, the sparse research on partial agonists other than Δ^9-THC and AEA does not allow for a strong argument that CB1R efficacy is vital to understanding the effects of cannabinoid agonists. Instead, it is suggested that other differences between cannabinoids may be responsible for the differing reports of marijuana- and Spice-induced effects. Off-target effects, pharmacokinetics, metabolism, and ligands with biased agonism may all contribute to the effects of specific Spice compounds, and their inherent higher efficacy may be important only under specific circumstances, and whether the recreational use of Spice may produce these is still being determined.

APPLICATIONS TO OTHER ADDICTIONS AND SUBSTANCE MISUSE

While this research focuses on the differences among cannabinoid drugs, aspects of it may be helpful in guiding future research with other classes of drugs' partial and full agonists. It is important to note how efficacy can be altered depending on the assay/effect studied. These may also be relevant to research looking into the differences between inverse agonists and neutral antagonists, which also do not consistently produce different effects.

DEFINITION OF TERMS

Spice "Spice" was an early brand of herbal incense found to contain synthetic cannabinoids. The term has been generalized to refer to any herbal incense blend and/or the synthetic cannabinoid compounds found within it sold recreationally.

Drug discrimination This a behavioral technique for assessing "subjective" effects of drugs in animals or humans.

Cannabinoid This is a ligand that binds to and affects CB1R and/or CB2R. It may also refer to bioactive compounds of cannabis that do not necessarily bind to CB1R/CB2R.

Cannabinoid 1 receptor This was the first cannabinoid receptor discovered, responsible for the psychoactive effects of CB1R agonists and most observed effects of Δ^9-THC/marijuana.

Efficacy This refers to the maximal effect of a drug on a specific test.

Potency This refers to the dose of drug needed to achieve a certain level of effect.

Phytocannabinoid This is a cannabinoid drug derived from plants, especially marijuana.

Schedule I This is the top tier of compounds in the Controlled Substances Act. Placement in Schedule I means that there is a high potential of abuse and no established medical use.

Structure–activity relationship This refers to the relation between a molecule's chemical structure and its biological activity.

Conditioned place preference/avoidance This is a behavioral assay that utilizes two distinct environments wherein an animal learns to associate one environment with a specific stimulus, often a drug. The animal will then either prefer or avoid the associated environment based on the specific stimulus's reinforcing properties.

Intracranial self-stimulation This is a behavioral experiment wherein an electrode is inserted into certain brain areas where electrical stimulation acts as reinforcement.

KEY FACTS

Key Facts of Spice/Synthetic Marijuana

- Spice is a product typically sold as "herbal incense" or as a legal alternative to marijuana that was found to contain synthetic cannabinoid drugs.
- Spice was originally a specific brand of herbal incense and now is a generic term for any herbal incense.
- The exact contents of most Spice blends are not typically known and the drugs/dosage used are variable.
- Spice has been linked to various health problems not seen before with marijuana.
- As governments ban the use of certain compounds found in Spice, new synthetic cannabinoids have taken their place.

Key Facts of Cannabinoid Agonists

- Cannabinoid agonists of the CB1R produce similar effects in a dose-dependent manner, such as mood changes, altered memory, sedation, hypothermia, and analgesia.
- Spice compounds detected so far have all been full CB1R agonists; Δ^9-THC is a partial agonist.
- Most in vivo assays do not find a difference between partial and full agonists.
- Δ^9-THC is marketed medically as dronabinol. Nabilone is another approved cannabinoid medication and is a full agonist for CB1R.

SUMMARY POINTS

- Cannabinoid agonists can be differentiated between those with high and those with low efficacy.
- Spice is typically composed of synthetic full agonists for CB1R in comparison to marijuana/Δ^9-THC, a natural partial agonist for CB1.
- Case reports have found profound side effects from Spice never seen before from marijuana/Δ^9-THC.
- Most lab in vivo assays, though, do not find much difference between full and partial CB1R agonists.
- Some assays find differences only after long-term use of the drugs.
- Synthetic CB1R full agonists have many other differences with Δ^9-THC in addition to efficacy.

ACKNOWLEDGMENT

Preparation of this chapter was defrayed in part by National Institute on Drug Abuse Grant 5RO1DA 009064-19. We thank Dr K. Vemuri for providing the chemical structures displayed in Figure 2. The authors declare that the study sponsor did not have any role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the chapter for publication.

REFERENCES

Aceto, M. D., Scates, S. M., & Martin, B. B. (2001). Spontaneous and precipitated withdrawal with a synthetic cannabinoid, WIN 55212-2. *European Journal of Pharmacology*, *416*(1–2), 75–81.

Atwood, B. K., Huffman, J., Straiker, A., & Mackie, K. (2010). JWH018, a common constituent of 'Spice' herbal blends, is a potent and efficacious cannabinoid CB receptor agonist. *British Journal of Pharmacology*, *160*(3), 585–593. http://dx.doi.org/10.1111/j.1476-5381.2009.00582.x.

Balster, R. L., & Prescott, W. R. (1992). Delta 9-tetrahydrocannabinol discrimination in rats as a model for cannabis intoxication. *Neuroscience and Biobehavioral Reviews*, *16*(1), 55–62.

Behonick, G., Shanks, K. G., Firchau, D. J., Mathur, G., Lynch, C. F., Nashelsky, M., … Meroueh, C. (2014). Four postmortem case reports with quantitative detection of the synthetic cannabinoid, 5F-PB-22. *Journal of Analytical Toxicology*, *38*(8), 559–562. http://dx.doi.org/10.1093/jat/bku048.

Bhanushali, G. K., Jain, G., Fatima, H., Leisch, L. J., & Thornley-Brown, D. (2013). AKI associated with synthetic cannabinoids: a case series. *Clinical Journal of the American Society of Nephrology*, *8*(4), 523–526. http://dx.doi.org/10.2215/cjn.05690612.

Brents, L. K., Gallus-Zawada, A., Radominska-Pandya, A., Vasiljevik, T., Prisinzano, T. E., Fantegrossi, W. E., … Prather, P. L. (2012). Monohydroxylated metabolites of the K2 synthetic cannabinoid JWH-073 retain intermediate to high cannabinoid 1 receptor (CB1R) affinity and exhibit neutral antagonist to partial agonist activity. *Biochemical Pharmacology*, *83*(7), 952–961. http://dx.doi.org/10.1016/j.bcp.2012.01.004.

Childers, S. R. (2006). Activation of G-proteins in brain by endogenous and exogenous cannabinoids. *AAPS Journal*, *8*(1), E112–E117. http://dx.doi.org/10.1208/aapsj080113.

Cooper, Z. D., & Haney, M. (2008). Cannabis reinforcement and dependence: role of the cannabinoid CB1 receptor. *Addiction Biology*, *13*(2), 188–195. http://dx.doi.org/10.1111/j.1369-1600.2007.00095.x.

De Vry, J., & Jentzsch, K. R. (2003). Intrinsic activity estimation of cannabinoid CB_1 receptor ligands in a drug discrimination paradigm. *Behavioural Pharmacology*, *14*(5–6), 471–476.

Desai, R. I., Thakur, G. A., Vemuri, V. K., Bajaj, S., Makriyannis, A., & Bergman, J. (2013). Analysis of tolerance and behavioral/physical dependence during chronic CB1 agonist treatment: effects of CB1 agonists, antagonists, and noncannabinoid drugs. *Journal of Pharmacology and Experimental Therapeutics*, *344*(2), 319–328. http://dx.doi.org/10.1124/jpet.112.198374.

Fan, F., Compton, D. R., Ward, S., Melvin, L., & Martin, B. R. (1994). Development of cross-tolerance between delta 9-tetrahydrocannabinol, CP 55,940 and WIN 55,212. *Journal of Pharmacology and Experimental Therapeutics*, *271*(3), 1383–1390.

Fattore, L., & Fratta, W. (2011). Beyond THC: the new generation of cannabinoid designer drugs. *Frontiers in Behavioral Neuroscience*, *5*, 60. http://dx.doi.org/10.3389/fnbeh.2011.00060.

Gifford, A. N., Bruneus, M., Gatley, S. J., Lan, R., Makriyannis, A., & Volkow, N. D. (1999). Large receptor reserve for cannabinoid actions in the central nervous system. *Journal of Pharmacology and Experimental Therapeutics*, *288*(2), 478–483.

Gonzalez, S., Cebeira, M., & Fernandez-Ruiz, J. (2005). Cannabinoid tolerance and dependence: a review of studies in laboratory animals. *Pharmacology, Biochemistry, and Behavior*, *81*(2), 300–318. http://dx.doi.org/10.1016/j.pbb.2005.01.028.

Haney, M., Ward, A. S., Comer, S. D., Foltin, R. W., & Fischman, M. W. (1999a). Abstinence symptoms following oral THC administration to humans. *Psychopharmacology (Berlin)*, *141*(4), 385–394.

Haney, M., Ward, A. S., Comer, S. D., Foltin, R. W., & Fischman, M. W. (1999b). Abstinence symptoms following smoked marijuana in humans. *Psychopharmacology (Berlin)*, *141*(4), 395–404.

Harris, C. R., & Brown, A. (2013). Synthetic cannabinoid intoxication: a case series and review. *Journal of Emergency Medicine*, *44*(2), 360–366. http://dx.doi.org/10.1016/j.jemermed.2012.07.061.

Hruba, L., Ginsburg, B. C., & McMahon, L. R. (2012). Apparent inverse relationship between cannabinoid agonist efficacy and tolerance/cross-tolerance produced by Delta(9)-tetrahydrocannabinol treatment in rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics*, *342*(3), 843–849. http://dx.doi.org/10.1124/jpet.112.196444.

Hyatt, W. S., & Fantegrossi, W. E. (2014). Delta9-THC exposure attenuates aversive effects and reveals appetitive effects of K2/'Spice' constituent JWH-018 in mice. *Behavioural Pharmacology*, *25*(3), 253–257. http://dx.doi.org/10.1097/fbp.0000000000000034.

Jarbe, T. U., Deng, H., Vadivel, S. K., & Makriyannis, A. (2011). Cannabinergic aminoalkylindoles, including AM678=JWH018 found in 'Spice', examined using drug (Delta(9)-tetrahydrocannabinol) discrimination for rats. *Behavioural Pharmacology*, *22*(5–6), 498–507. http://dx.doi.org/10.1097/FBP.0b013e328349fbd5.

Jarbe, T. U., & Gifford, R. S. (2014). "Herbal incense": designer drug blends as cannabimimetics and their assessment by drug discrimination and other in vivo bioassays. *Life Sciences*, *97*(1), 64–71. http://dx.doi.org/10.1016/j.lfs.2013.07.011.

Jarbe, T. U., Hiltunen, A. J., & Mechoulam, R. (1989). Stereospecificity of the discriminative stimulus functions of the dimethylheptyl homologs of 11-hydroxy-delta 8-tetrahydrocannabinol in rats and pigeons. *Journal of Pharmacology and Experimental Therapeutics*, *250*(3), 1000–1005.

Jarbe, T. U., Lamb, R. J., Lin, S., & Makriyannis, A. (2001). (R)-methanandamide and Delta 9-THC as discriminative stimuli in rats: tests with the cannabinoid antagonist SR-141716 and the endogenous ligand anandamide. *Psychopharmacology (Berlin)*, *156*(4), 369–380.

Jarbe, T. U., Lamb, R. J., Liu, Q., & Makriyannis, A. (2003). (R)-Methanandamide and delta9-tetrahydrocannabinol-induced operant rate decreases in rats are not readily antagonized by SR-141716A. *European Journal of Pharmacology*, *466*(1–2), 121–127.

Jarbe, T. U., Li, C., Liu, Q., & Makriyannis, A. (2009). Discriminative stimulus functions in rats of AM1346, a high-affinity CB1R selective anandamide analog. *Psychopharmacology (Berlin)*, *203*(2), 229–239. http://dx.doi.org/10.1007/s00213-008-1199-3.

Jarbe, T. U., Li, C., Vadivel, S. K., & Makriyannis, A. (2010). Discriminative stimulus functions of methanandamide and delta(9)-THC in rats: tests with aminoalkylindoles (WIN55,212-2 and AM678) and ethanol. *Psychopharmacology (Berlin)*, *208*(1), 87–98. http://dx.doi.org/10.1007/s00213-009-1708-z.

Jarbe, T. U., Tai, S., LeMay, B. J., Nikas, S. P., Shukla, V. G., Zvonok, A., & Makriyannis, A. (2012). AM2389, a high-affinity, in vivo potent CB1-receptor-selective cannabinergic ligand as evidenced by drug discrimination in rats and hypothermia testing in mice. *Psychopharmacology (Berlin)*, *220*(2), 417–426. http://dx.doi.org/10.1007/s00213-011-2491-1.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2013). *Monitoring the Future National Results on Drug Use: 2012 Overview, Key Findings on Adolescent Drug Use*. Ann Arbor: Institute for Social Research, The University of Michigan.

Justinova, Z., Mascia, P., Wu, H. Q., Secci, M. E., Redhi, G. H., Panlilio, L. V., … Goldberg, S. R. (2013). Reducing cannabinoid abuse and preventing relapse by enhancing endogenous brain levels of kynurenic acid. *Nature Neuroscience*, *16*(11), 1652–1661. http://dx.doi.org/10.1038/nn.3540.

Laaris, N., Good, C. H., & Lupica, C. R. (2010). Delta9-tetrahydrocannabinol is a full agonist at CB1 receptors on GABA neuron axon terminals in the hippocampus. *Neuropharmacology*, *59*(1–2), 121–127. http://dx.doi.org/10.1016/j.neuropharm.2010.04.013.

Laprairie, R. B., Bagher, A. M., Kelly, M. E., Dupre, D. J., & Denovan-Wright, E. M. (2014). Type 1 cannabinoid receptor ligands display functional selectivity in a cell culture model of striatal medium spiny projection neurons. *Journal of Biological Chemistry*, *289*(36), 24845–24862. http://dx.doi.org/10.1074/jbc.M114.557025.

Lefever, T. W., Marusich, J. A., Antonazzo, K. R., & Wiley, J. L. (2014). Evaluation of WIN 55,212-2 self-administration in rats as a potential cannabinoid abuse liability model. *Pharmacology, Biochemistry, and Behavior*, *118*, 30–35. http://dx.doi.org/10.1016/j.pbb.2014.01.002.

Maldonado, R., Berrendero, F., Ozaita, A., & Robledo, P. (2011). Neurochemical basis of cannabis addiction. *Neuroscience*, *181*, 1–17. http://dx.doi.org/10.1016/j.neuroscience.2011.02.035.

Marshell, R., Kearney-Ramos, T., Brents, L. K., Hyatt, W. S., Tai, S., Prather, P. L., & Fantegrossi, W. E. (2014). In vivo effects of synthetic cannabinoids JWH-018 and JWH-073 and phytocannabinoid Delta9-THC in mice: inhalation versus intraperitoneal injection. *Pharmacology, Biochemistry, and Behavior*, *124*, 40–47. http://dx.doi.org/10.1016/j.pbb.2014.05.010.

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., & Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, *346*(6284), 561–564. http://dx.doi.org/10.1038/346561a0.

McMahon, L. R., Ginsburg, B. C., & Lamb, R. J. (2008). Cannabinoid agonists differentially substitute for the discriminative stimulus effects of Delta(9)-tetrahydrocannabinol in C57BL/6J mice. *Psychopharmacology (Berlin)*, *198*(4), 487–495. http://dx.doi.org/10.1007/s00213-007-0900-2.

Mechoulam, R., Feigenbaum, J. J., Lander, N., Segal, M., Jarbe, T. U., Hiltunen, A. J., & Consroe, P. (1988). Enantiomeric cannabinoids: stereospecificity of psychotropic activity. *Experientia*, *44*(9), 762–764.

Mechoulam, R., Fride, E., & Di Marzo, V. (1998). Endocannabinoids. *European Journal of Pharmacology*, *359*(1), 1–18.

Melvin, L. S., & Johnson, M. R. (1987). Structure-activity relationships of tricyclic and nonclassical bicyclic cannabinoids. *NIDA Research Monograph*, *79*, 31–47.

Nikas, S. P., Sharma, R., Paronis, C. A., Kulkarni, S., Thakur, G. A., Hurst, D., … Makriyannis, A. (2015). Probing the carboxyester side chain in controlled deactivation (−)-delta(8)-tetrahydrocannabinols. *Journal of Medicinal Chemistry*, *58*(2), 665–681. http://dx.doi.org/10.1021/jm501165d.

Panagis, G., Mackey, B., & Vlachou, S. (2014). Cannabinoid regulation of brain reward processing with an emphasis on the role of CB1 receptors: a step back into the future. *Frontiers in Psychiatry*, *5*, 92. http://dx.doi.org/10.3389/fpsyt.2014.00092.

Paronis, C. A., Nikas, S. P., Shukla, V. G., & Makriyannis, A. (2012). Delta(9)-Tetrahydrocannabinol acts as a partial agonist/antagonist in mice. *Behavioural Pharmacology*, *23*(8), 802–805. http://dx.doi.org/10.1097/FBP.0b013e32835a7c4d.

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P., Di Marzo, V., Elphick, M. R., … Ross, R. A. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB(1) and CB(2). *Pharmacological Reviews*, *62*(4), 588–631. http://dx.doi.org/10.1124/pr.110.003004.

Rodriguez de Fonseca, F., Carrera, M. R., Navarro, M., Koob, G. F., & Weiss, F. (1997). Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science*, *276*(5321), 2050–2054.

Rodriguez, J. S., & McMahon, L. R. (2014). JWH-018 in rhesus monkeys: differential antagonism of discriminative stimulus, rate-decreasing, and hypothermic effects. *European Journal of Pharmacology*, *740*, 151–159. http://dx.doi.org/10.1016/j.ejphar.2014.06.023.

Russo, E. B. (2011). Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *British Journal of Pharmacology*, *163*(7), 1344–1364. http://dx.doi.org/10.1111/j.1476-5381.2011.01238.x.

SAMHSA, (2014). *Results from the 2013 National Survey on Drug Use and Health: Summary of National Findings, NSDUH Series H-48, HHS Publication No. (SMA) 14-4863*. Rockville, MD: Substance Abuse and Mental Health Services Administration.

Sharma, R., Nikas, S. P., Paronis, C. A., Wood, J. T., Halikhedkar, A., Guo, J. J., … Makriyannis, A. (2013). Controlled-deactivation cannabinergic ligands. *Journal of Medicinal Chemistry*, *56*(24), 10142–10157. http://dx.doi.org/10.1021/jm4016075.

Tanda, G., & Goldberg, S. R. (2003). Cannabinoids: reward, dependence, and underlying neurochemical mechanisms–a review of recent preclinical data. *Psychopharmacology (Berlin)*, *169*(2), 115–134. http://dx.doi.org/10.1007/s00213-003-1485-z.

Ward, S. J., Baizman, E., Bell, M., Childers, S., D'Ambra, T., Eissenstat, M., … Pacheco, M. (1990). Aminoalkylindoles (AAIs): a new route to the cannabinoid receptor? *NIDA Research Monograph*, *105*, 425–426.

Ware, M. A., & St Arnaud-Trempe, E. (2010). The abuse potential of the synthetic cannabinoid nabilone. *Addiction*, *105*(3), 494–503. http://dx.doi.org/10.1111/j.1360-0443.2009.02776.x.

Weissman, A., Milne, G. M., & Melvin, L. S., Jr. (1982). Cannabimimetic activity from CP-47,497, a derivative of 3-phenylcyclohexanol. *Journal of Pharmacology and Experimental Therapeutics*, *223*(2), 516–523.

Wiley, J. L., Compton, D. R., Dai, D., Lainton, J. A., Phillips, M., Huffman, J. W., & Martin, B. R. (1998). Structure-activity relationships of indole- and pyrrole-derived cannabinoids. *Journal of Pharmacology and Experimental Therapeutics*, *285*(3), 995–1004.

Winstock, A. R., & Barratt, M. J. (2013). Synthetic cannabis: a comparison of patterns of use and effect profile with natural cannabis in a large global sample. *Drug and Alcohol Dependence*, *131*(1–2), 106–111. http://dx.doi.org/10.1016/j.drugalcdep.2012.12.011.

Zimmermann, U. S., Winkelmann, P. R., Pilhatsch, M., Nees, J. A., Spanagel, R., & Schulz, K. (2009). Withdrawal phenomena and dependence syndrome after the consumption of "spice gold". *Deutsches Arzteblatt International*, *106*(27), 464–467. http://dx.doi.org/10.3238/arztebl.2009.0464.