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REVIEW

Preclinical Studies of Cannabinoid Reward, Treatments for Cannabis Use Disorder, and Addiction-Related Effects of Cannabinoid Exposure

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Cannabis use has become increasingly accepted socially and legally, for both recreational and medicinal purposes. Without reliable information about the effects of cannabis, people cannot make informed decisions regarding its use. Like alcohol and tobacco, cannabis can have serious adverse effects on health, and some people have difficulty discontinuing their use of the drug. Many cannabis users progress to using and becoming addicted to other drugs, but the reasons for this progression are unclear. The natural cannabinoid system of the brain is complex and involved in many functions, including brain development, reward, emotion, and cognition. Animal research provides an objective and controlled means of obtaining information about: (1) how cannabis affects the brain and behavior, (2) whether medications can be developed to treat cannabis use disorder, and (3) whether cannabis might produce lasting changes in the brain that increase the likelihood of becoming addicted to other drugs. This review explains the tactics used to address these issues, evaluates the progress that has been made, and offers some directions for future research.

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INTRODUCTION

In recent years, the medical and recreational use of cannabis has become increasingly accepted (Pacula and Smart, 2017; Rubens, 2014), and there is an increasing public perception that cannabis is relatively harmless (Pew Research Center, April 2015; Quinnipiac University Poll, April 20, 2017). However, cannabis use disorder is recognized in the Diagnostic and Statistical Manual of Mental Disorders, and about 3 out of 10 users meet the criteria for this diagnosis (Hasin *et al*, 2015). Thus, there is a need for scientific research to: (1) study the addiction-related effects of cannabis; (2) evaluate the effectiveness of medications developed to treat cannabis use disorder; and (3) evaluate the abuse potential of new medications designed to produce cannabis-like medicinal effects. Animal models of cannabis use and the effects of cannabis on the brain provide a means of conducting much of this research. In this review, we will discuss preclinical procedures that can be used to test new cannabinoid drugs for abuse potential, and to evaluate treatments that might aid in reducing cannabis use and preventing relapse. We will describe progress made in this area involving several different neurotransmitter systems.

It is common for drug abusers to have experience with multiple classes of drugs, taking more than one drug simultaneously or within a period of days or weeks (Kedia *et al*, 2007; Martin, 2008). A subset of cannabis users progresses to becoming regular users of drugs such as cocaine and heroin (Fergusson *et al*, 2006; Secades-Villa *et al*, 2015). There are probably multiple reasons for this progression, some of which can only be studied in humans. However, animal models of drug abuse provide a means of testing a specific kind of hypothesis: that exposure to one drug can alter the brain in such a way that the individual becomes more susceptible to the addictive effects of other drugs. In this review, we will discuss the preclinical findings showing that cannabinoid exposure produces lasting changes in brain processes that are involved in addiction, and that exposure to cannabinoids can alter the taking of other drugs later in life.

REINFORCING EFFECTS OF CANNABINOIDS

At least initially, addictive drugs are taken for their rewarding effects. Laboratory animals will work to

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self-administer most drugs that are addictive in humans, including opioids, psychostimulants, alcohol, and sedatives (Griffiths, 1980; Johanson and Balster, 1978; Katz and Goldberg, 1988; Schuster and Thompson, 1969). This correspondence allows drug self-administration procedures to be used to model many aspects of addiction that are difficult or impossible to study in humans (Panlilio and Goldberg, 2007). However, for many years, cannabinoids were an exception to this situation. Cannabis is widely used by humans, and the current DSM-5 defines cannabis use disorder by criteria that parallel those for other drugs of abuse (American Psychiatric Association, 2013). Approximately 4 million people in the United States meet the criteria for cannabis use disorder, and ~87 000 receive treatment for it (Center for Behavioral Health Statistics and Quality, 2016a, b). Gaoni and Mechoulam (1964) first isolated THC from hashish and identified it as the main psychoactive compound in cannabis in the early 1960s. However, despite many attempts at establishing procedures for THC self-administration in rats and rhesus monkeys (Carney *et al*, 1977; Deneau and Kaymakcalan, 1971; Harris *et al*, 1974; Kaymakcalan, 1972; Lefever *et al*, 2014; Li *et al*, 2012b; Mansbach *et al*, 1994; Pickens *et al*, 1973), robust self-administration behavior was not obtained (see Table 1). Some studies showed positive results, but ultimately did not lead to establishment of reliable models (John *et al*, 2017; Takahashi and Singer, 1979, 1980; van Ree *et al*, 1978). Eventually, there were breakthroughs when intravenous self-administration of the synthetic cannabinoid WIN 55,212-2 was demonstrated in mice (Martellotta *et al*, 1998) and rats (Fattore *et al*, 2001) and intravenous self-administration of THC was demonstrated in squirrel monkeys (Tanda *et al*, 2000).

Rodent Models

Martellotta *et al* (1998) were the first to show that under specific conditions, restrained, experimentally naive mice would perform an operant response (poking their nose into a hole) to receive intravenous injections of the synthetic cannabinoid WIN 55,212-2. They also showed that this behavior is mediated by the actions of the drug at the cannabinoid CB_1 receptor. Mice self-administered WIN 55,212-2 for only a single session in that study. A similar approach was later used by Navarro *et al* (2001) who showed that mice would self-administer WIN 55,212-2 and another cannabinoid agonist HU-210 in single sessions, and that this behavior could be prevented by pretreatment with the opioid antagonist naloxone. Fattore *et al* (2001) extended WIN 55,212-2 self-administration procedures to use rats over multiple sessions, allowing study of the acquisition of the behavior, its maintenance over time, and its extinction when drug delivery was discontinued. This procedure has been used in subsequent studies (see Table 1 for details) to investigate subject variables (strain and sex of the rats), procedural variables (dose of the cannabinoid), treatment drugs (potential therapeutics), and specific aspects of addiction (relapse) (Deiana *et al*, 2007; Fattore *et al*, 2007, 2010; Justinova *et al*, 2013; Kirschmann *et al*, 2017; Lefever *et al*, 2014; Mendizabal *et al*, 2006; Scherma *et al*, 2016a; Struik *et al*, 2017). However, compared with self-administration of classical drugs of abuse such as cocaine and heroin, self-administration of WIN 55,212-2 by rodents tends to be more sensitive to variables such as training conditions and genetic strain, and it is generally harder to obtain. For example, in some studies Sprague-Dawley rats self-administered WIN 55,212-2, but in other studies they did not (see Table 1). Furthermore, cannabinoid self-administration in rodents may not be useful for predicting the abuse potential of specific cannabinoids, as robust self-administration of THC does not occur in rodents even after they have learned to self-administer WIN 55,212 (Lefever *et al*, 2014). It is not clear to what extent such findings represent a failing of the rodent procedures as a model of cannabinoid reward as opposed to reflecting salient facets of intravenous cannabinoid delivery, such as psychotomimetic and anxiogenic effects (Englund *et al*, 2012). It should also be noted in general that synthetic cannabinoids such as WIN 55,212-2 differ in important ways from THC in terms of receptor efficacy and secondary messenger effects (Laprairie *et al*, 2014), and hence results obtained with synthetic compounds might not generalize to THC and other phytocannabinoids.

Like THC (Justinova *et al*, 2013; Solinas *et al*, 2007a; Tanda *et al*, 1997), WIN 55,212-2 increases dopamine release in the shell, but not the core, of the nucleus accumbens (Fadda *et al*, 2006; Lecca *et al*, 2006), and this effect on reward circuitry of the brain is presumably involved in cannabinoid reward. Another synthetic cannabinoid, JWH-018, has also been found to be self-administered by rodents and to increase dopamine release preferentially in the nucleus accumbens shell (De Luca *et al*, 2015). JWH-018 and other synthetic cannabinoids have been detected in 'spice' drugs abused by humans. Like WIN 55,212 but unlike the partial CB_1 receptor agonist THC, JWH-018 acts as a full agonist at CB_1 receptors (De Luca *et al*, 2015). The endocannabinoid 2-AG is also self-administered by rats, although at lower rates than observed with other cannabinoids or commonly abused drugs such as cocaine, heroin, or nicotine, and also with lesser effects on dopamine levels in the nucleus accumbens shell (De Luca *et al*, 2014). There are sex and strain differences in WIN 55,212-2 self-administration (Deiana *et al*, 2007; Fattore *et al*, 2007) and the reinstatement models of relapse to cannabinoid use after a period of abstinence (Fattore *et al*, 2010; Justinova *et al*, 2003; Spano *et al*, 2004), with intact females rats showing a higher level of vulnerability than males or ovariectomized females.

Non-Human Primate Models

Dr Steven Goldberg and his colleagues at the Intramural Research Program of the National Institute on Drug Abuse

TABLE 1 Intravenous Self-Administration Studies with Cannabimimetics in Laboratory Animals

Study	Subjects Gender Drug Experience	Reinforcer	Dose/infusion	Vehicle	Schedule	Procedure	Results IVSA	Results Reinstatement (drug priming-induced)
Rodent studies								
Van Ree *et al* (1978)	Wistar rats FEM Naive	THC	7.5–300 μg/kg	Tween 20 (1%)/ saline	FR1	Initial forced injections No food restriction IVSA acquisition	+/− Low incidence of lever pressing 40% Rats responded	
Takahashi and Singer (1979,1980)	Wistar rats Male Naive	THC	6.25–50 μg/kg	Tween 80 (0.6%)/ saline	FR	Automatic food pellet delivery (FT-1 min) IVSA acquisition and maintenance	+/− Behavior maintained only with food pellet delivery and food deprivation	
Martellotta *et al* (1998)	CD1 mice Male Naive	WIN 55,212-2	10–500 μg/kg	Cremophor (10%)/ heparinized saline	FR1	Restrained mice used for single IVSA session	+/− Max resp. at 100 μg/kg	
Ledent *et al* (1999)	CD1 mice-WT or CB1 KO Male Naive	WIN 55,212-2	100 μg/kg	Cremophor (10%)/ heparinized saline	FR1	Restrained mice used for single IVSA session	+WT mice −CB1 KO mice	
Navarro *et al* (2001)	CD1 Mice Naive	WIN 55,212-2 HU-210	10–100 μg/kg 5 μg/kg	Tween 80/ heparinized saline	FR1	Restrained mice used for single IVSA session	+/− WIN Max resp. at 50 and 100 μg/kg +/− HU-210 Max resp. at 5 μg/kg	
Fattore *et al* (2001)	L-E rats Male Naive	WIN 55,212-2	6.25–50 μg/kg	Tween 80/ heparinized saline	FR1	IVSA acquisition, maintenance, extinction	+ Max resp. at 12.5 μg/kg 87% Rats acquired	
Spano *et al* (2004)	L-E rats Male Naive	WIN 55,212-2	12.5 μg/kg	Tween 80/ heparinized saline	FR1	IVSA acquisition and extinction Drug-induced reinstatement	+	+ WIN + Heroin − Cocaine
Fadda *et al* (2006)	L-E rats L-H rats Male Naive	WIN 55,212-2	12.5 μg/kg	Tween 80/ heparinized saline	FR1	IVSA acquisition Simultaneous microdialysis	+ ↑ DA in NAc shell	
Lecca *et al* (2006)	S-D rats Male Naive	WIN 55,212-2	12.5 μg/kg	Tween 80 (0.3%)/ saline	FR1-2	IVSA acquisition, maintenance, extinction Simultaneous microdialysis	+ 90% Rats acquired ↑ DA (NAc shell > core)	
Mendizabal *et al* (2006)	CD1 mice C57BL/6 J mice – WT or pro-dynorphin KO Male Naive	WIN 55,212-2	3.125–12.5 μg/kg	Tween 80/saline	FR1	Freely moving mice IVSA acquisition, maintenance	+ 90% rats acquired WT: max resp. at 12.5 μg/kg KO: max resp. at 6.25 μg/kg	
Deiana *et al* (2007)	L-E rats L-H rats S-D rats Male Naive	WIN 55,212-2	6.25–25 μg/kg	Tween 80/ heparinized saline	FR1	IVSA acquisition, maintenance, extinction	+L-E rats +L-H rats −S-D rats Max resp. at 12.5 μg/kg	
Fattore *et al* (2007)	L-E rats L-H rats S-D rats FEM (OVX or intact) Male Naive	WIN 55,212-2	12.5 μg/kg	Tween 80/saline	FR1	IVSA acquisition, maintenance, extinction	+L-E rats Intact FEM > males or OVX FEM +L-H rats Intact FEM > males or OVX FEM −S-D rats	
Solinas *et al* (2007)	L-E rats Male Naive	WIN 55,212-2	12.5–25 μg/kg	Tween 80/saline	FR1-5	IVSA acquisition and maintenance	+	
Fattore *et al* (2010)	L-H rats FEM (OVX or intact) Male Naive	WIN 55,212-2	12.5 μg/kg	Tween 80/ heparinized saline	FR1	IVSA acquisition, maintenance, extinction Drug-induced reinstatement Cue-induced reinstatement	+ Intact FEM > males or OVX FEM	+ WIN Intact FEM > males or OVX FEM + Cue Intact FEM > males or OVX FEM
Justinova *et al* (2013)	L-H rats Male Naive	WIN 55,212-2	12.5 μg/kg	Tween 80/ heparinized saline	FR1	IVSA acquisition, maintenance Drug-induced reinstatement	+	+ WIN

Table 1 (Continued)

Study	Subjects Gender Drug Experience	Reinforcer	Dose/infusion	Vehicle	Schedule	Procedure	Results IVSA	Results Reinstatement (drug priming-induced)
De Luca *et al* (2014)	S-D rats Male Naive	2-AG	12.5–50 μg/kg	Tween 80 (2%)/ ethanol (2%)/saline	FR1-2	IVSA acquisition, extinction, reacquisition	+ Max resp. at 25 μg/kg 90% Rats acquired	
Lefever *et al* (2014)	L-E rats Male WIN SA	THC	3–100 μg/kg	Polysorbate 80 (1%)/saline	FR3	IVSA (Substitution for WIN55,212)	–	
Vallée *et al* (2014)	CD1 mice Naive	WIN 55,212-2	12.5 μg/kg	Tween 80/saline	FR1 PR	IVSA acquisition, maintenance	+	
De Luca *et al* (2015)	S-D rats C57BL/6 Mice Male Naive	JWH-018	Rats: 10–20 μg/kg Mice: 15–30 μg/kg	Tween 80 (2%)/ ethanol (2%)/saline	FR1-3 (rats) FR1 and PR (mice)	IVSA acquisition, extinction, reacquisition	+ Rats Max resp. at 20 μg/kg, 90% acquired + Mice Max resp. at 30 μg/kg, 90% acquired	
Scherma *et al* (2016)	L-H rats Male Naive	WIN 55,212-2	12.5 μg/kg	Tween 80/ heparinized saline	FR1	THC exposure in adolescence IVSA acquisition, maintenance	+ THC-exposed > vehicle-exposed rats	
Struik *et al* (2017)	L-H rats Male Naive	WIN 55,212-2	12.5 μg/kg	Tween 80/saline	FR1	Prior NDL exposure IVSA acquisition, maintenance, extinction Drug-induced reinstatement Cue-induced reinstatement	+ NDL exposed > vehicle exposed rats	+ WIN NDL exposed = vehicle exposed +Cue NDL exposed = vehicle exposed
Kirschmann *et al* (2017)	S-D rats Male Naive	WIN 55,212-2	12.5 μg/kg	Tween 80/saline	FR1	Short (2 h) and long (6 h) access IVSA in adolescence Cue-induced reinstatement	+ 2 h intake = 6 h intake	+ Cue + Incubation of craving
Non-human primate studies								
Deneau and Kaymakcalan (1971; Kaymakcalan (1972)	Rhesus m. Male FEM Naive	THC	100-400 μg/kg	Tween 80 (0.2–0.8%)/saline	FR	Automatic THC injections Cocaine IVSA training IVSA acquisition	– Naive subjects +/– Responding occurred only after physical dependence to THC developed or after cocaine SA	
Pickens *et al* (1973)	Rhesus m. Phencyclidine SA	THC	25–100 μg/kg	PVP/saline	FR1	IVSA substitution for phencyclidine	–	
Harris *et al* (1974)	Rhesus m. Male Naive	THC	25–300 μg/kg	PVP/saline	FR	Naive then cocaine SA—substitution for cocaine Automated THC injections	– Naive subjects – When substituted for cocaine – After chronic THC exposure	
Carney *et al* (1977)	Rhesus m. Male Cocaine SA	THC	3–300 μg/kg	EL-620/ Ethanol/ saline	FR10	IVSA substitution for cocaine	–	
Mansbach *et al* (1994)	Rhesus m. Male Cocaine SA	THC CP 55,940	17–100 μg/kg 0.3–3 μg/kg	EL-620/ Ethanol/ saline	FR	IVSA substitution for phencyclidine	– THC – CP 55,940	
Tanda *et al* (2000)	Squirrel m. Male Cocaine SA	THC	1–8 μg/kg	Tween 80(0.4–1%)/ Ethanol (0.4–1%)/ saline	FR10	IVSA (Substitution for cocaine), extinction, reacquisition	+ Max resp. at 4 μg/kg	
Justinova *et al* (2003)	Squirrel m. Male Naive	THC	1–16 μg/kg	Tween 80 (0.4-1%)/ Ethanol (0.4-1%)/ saline	FR10	IVSA acquisition, extinction, reacquisition	+ Max resp. at 4 μg/kg	
Justinova *et al* (2004)	Squirrel m. Male THC or cocaine SA	THC	2–8 μg/kg	Tween 80 (0.4–1%)/ Ethanol (0.4–1%)/ saline	FR10	IVSA maintenance	+ Max resp. at 4 μg/kg	
Justinova *et al* (2005)	Squirrel m. Male Naive or THC SA	AEA MethAEA	2.5–160 μg/kg 2.5–80 μg/kg	Water-soluble emulsion or Tween 80 (0.125–4%)/ Ethanol/Saline	FR10	IVSA acquisition, maintenance, extinction	+AEA Max resp. at 40 μg/kg +MethAEA Max resp. at 40 μg/kg	

Table 1 (Continued)

Study	Subjects Gender Drug Experience	Reinforcer	Dose/infusion	Vehicle	Schedule	Procedure	Results IVSA	Results Reinstatement (drug priming-induced)
Justinova *et al* (2008a)	Squirrel m. Male THC or AEA SA	AEA THC URB597	3–100 μg/kg 1–8 μg/kg 1–100 μg/kg	AEA, THC: Tween 80 (0.125–4%)/ Ethanol/Saline URB597: Tween 80 (5%)/ PEG (5%)/Saline	FR10	IVSA acquisition, maintenance, extinction Drug-induced reinstatement	+ AEA Max resp. at 30 μg/kg + THC Max resp. at 4 μg/kg − URB597	+ THC − URB597
Justinova *et al* (2008b)	Squirrel m. Male Amphetamine or cocaine SA	THC	10–80 μg/kg at the end of the session	Tween 80(0.4–1%)/ Ethanol (0.4–1%)/ saline	SOS FI 30 min (FR10:S)	IVSA acquisition, maintenance, extinction Drug-induced reinstatement Cue-induced reinstatement	+ Max resp. at 40 μg/kg at the end of the session	+THC +Morphine +AEA +MethAEA +AM404 − Cocaine +Cue
Justinova *et al* (2011a)	Squirrel m. Male THC or AEA SA	THC AEA	0.5–8 μg/kg 1–80 μg/kg	Tween 80(1%)/ Ethanol (1%)/ saline	FR10	IVSA maintenance, extinction Drug-induced reinstatement	+THC Max resp. at 4 μg/kg + AEA Max resp. at 40 μg/kg	+THC
Justinova *et al* (2011b)	Squirrel m. Male AEA or nicotine SA	2-AG	0.1–100 μg/kg	Tween 80(5%)/ Ethanol (3%)/sterile water (Stock solution 1.5 mg/ml)	FR10	Substitution for AEA or nicotine IVSA acquisition, maintenance, extinction	+ Max resp. at 3 μg/kg	
Li *et al* (2012b)	Rhesus m. Females Males Naive	THC	3.2–32 μg/kg	EL-620/ Ethanol/ saline	FR30	Substitution for heroin IVSA acquisition	−	
Justinova *et al* (2013)	Squirrel m. Male THC SA	THC	0.5–16 μg/kg	Tween 80(1%)/ Ethanol (1%)/ saline	FR10	IVSA maintenance, extinction Drug-induced reinstatement Cue-induced reinstatement	+ Max resp. at 4 μg/kg	+THC + Cue
Justinova *et al* (2014)	Squirrel m. Male THC SA	THC	0.1–32 μg/kg	Tween 80(1%)/ Ethanol (1%)/ saline (Stock solution 0.4 mg/ml)	FR10	IVSA maintenance	+ Max resp. at 4 μg/kg	
Justinova *et al* (2015)	Squirrel m. Male AEA, THC, or cocaine SA	URB694	0.3–30 μg/kg	AEA: Tween 80 (1%)/ Ethanol (1%)/ saline URB694: DMSO (5%)/Tween 80 (5%)/saline	FR10	Substitution for AEA, THC, or cocaine IVSA acquisition, maintenance, extinction Drug-induced reinstatement	+ Regardless of SA history Max resp. at 1 μg/kg	+THC − URB694
Schindler *et al* (2016a)	Squirrel m. Male THC SA	THC	1–8 μg/kg	Tween 80(1%)/ Ethanol (1%)/ saline (Stock solution 0.4 mg/ml)	FR10	IVSA maintenance Drug-induced reinstatement Cue-induced reinstatement	+ Max resp. at 4 μg/kg	+THC +Nicotine +Cocaine +Cue
Schindler *et al* (2016b)	Squirrel m. Male AEA, THC or cocaine SA	AM404 VDM11 AEA	1–100 μg/kg 0.3–56 μg/kg 2.5–80 μg/kg	AM404, AEA: Tween 80(2%)/ Ethanol (2%)/ saline VDM11: Tween 80 (2%)/ Ethanol (6%)/ saline	FR10	Substitution for AEA or cocaine IVSA acquisition, maintenance, extinction Drug-induced reinstatement	+AM404 Max resp. at 10 μg/kg + VDM11 Max resp. at 10 μg/kg + AEA Max resp. at 40 μg/kg	+AEA + THC + AM404
John *et al* (2017)	Rhesus m. Male Naive or METH or cocaine SA Cynomolgus m. Male Cocaine SA	THC CP 55,940	0.01–10 μg/kg 0.001–3 μg/kg	Tween 80 (1%)/ Ethanol (1%)/ Saline	FR10 (Rhesus) SOS FI 600- s [FR30:S] (Cynomolgus)	Rhesus: Food SA training. THC, and CP 55,940 substituted for food pellets Cynomolgus: THC substituted for cocaine	− THC FR: low rates of resp. only after chronic THC exposure +/− THC SOS: 50% of animals resp. above vehicle +/− CP 55,940	

Abbreviations:: 2-AG, 2-archidonoylglycerol; AEA, anandamide; DA, dopamine; DMSO, dimethyl sulfoxide; EL-620, emulphor; FEM, female; FR, fixed ratio; FT-1, fixed-time 1 min schedule; Inf., infusion; IVSA, intravenous self-administration; KO, knockout; L-E, Long-Evans; L-H, Lister-Hooded; m., monkeys; METH, methamphetamine; NDL, nandrolone; OVX, ovariectomy; PEG, polyethylene glycol; PR, progressive ratio; PVP, polyvinylpyrollidone; Resp., responding; SA, self-administration; SOS, second-order schedule; S-D, Sprague-Dawley; WT, wild type; WIN, WIN 55,212-2.

Symbols: +/−, <50% Animals responded, low incidence of lever pressing, or special conditions (eg, single session use, movement restriction); +, reliable self-administration behavior or significant reinstatement of drug seeking; −, no self-administration above vehicle levels or no reinstatement of drug seeking; >, higher self-administration rates, faster acquisition, or preferential dopamine increase; =, identical effect; ↑, increase; ↓, decrease.

(Tanda *et al*, 2000) developed the first procedure for obtaining robust THC self-administration in animals, showing that squirrel monkeys will reliably self-administer intravenous injections of THC under a fixed-ratio 10 schedule, in which 10 lever-pressing responses are required for each injection. The monkeys in this first study had a history of cocaine self-administration, but later studies showed that drug-naive squirrel monkeys will also acquire self-administration of THC or the endocannabinoid anandamide (Justinova *et al*, 2003; Justinova *et al*, 2005), responding at rates that were even higher than in monkeys with cocaine experience. Anandamide- and nicotine-trained groups of monkeys were later used to demonstrate self-administration of another endocannabinoid, 2-AG, that in this model had rewarding effects comparable to THC, anandamide, nicotine, and cocaine under the same conditions (Justinova *et al*, 2011b). The level of 2-AG taking did not depend on the particular self-administration history. The reinforcing effects of THC, anandamide, and 2-AG are mediated by cannabinoid CB_1 receptors, and these drugs are self-administered over a range of doses, producing inverted U-shaped dose–response curves typical of all self-administered drugs.

As with other drugs of abuse, the procedures used with cannabinoid self-administration in squirrel monkeys can be modified to focus on specific aspects of drug use (Panlilio *et al*, 2007). A second-order schedule of THC self-administration in monkeys (Justinova *et al*, 2008b) is used to model the effects of drug-associated cues in the human drug-abuse environment that typically guide and reinforce the sequences of behavior required to obtain, prepare, and self-administer drugs. Under this schedule, lever responding during the hour-long session produces only brief presentations of the visual cues that have been associated with drug delivery; this maintains long sequences of THC-seeking behavior that are eventually rewarded at the end of the session by delivery of THC and a longer cue presentation. Thus, high rates of THC-seeking behavior can be studied in the absence of direct pharmacological effects of THC, but with the behavior ultimately maintained by the effects of the drug. To model relapse to cannabis use induced by re-encountering the drug after a period of abstinence, delivery of the drug is discontinued during a period of imposed abstinence until the drug-seeking response drops to a very low level; then, the response can be reinstated by presenting a free priming injection of the drug. Or, to study the relapse-triggering effects of re-encountering cannabinoid-associated cues after a period of abstinence, both THC and its associated visual cues are discontinued during the abstinence period, and then the drug-seeking response is reinstated by again presenting the visual cues for responding.

In a recent study, John *et al* (2017) allowed Old World primates (rhesus and cynomolgus monkeys) to self-administer THC or the synthetic cannabinoid CP 55,940 using parameters similar to the ones used in squirrel monkeys, including the drug vehicle and the schedules of reinforcement. They obtained cannabinoid self-administration in only 7 of 13 monkeys, and only under certain conditions, such as when subjects were exposed to THC until tolerance developed to the suppressant effects of THC on food-rewarded responding. These findings suggest that it is not simply the training parameters that are responsible for the robust cannabinoid self-administration obtained in squirrel monkeys, but that the squirrel monkey species is particularly sensitive to cannabinoid reward and/or insensitive to cannabinoid-induced aversive effects. The reasons for this sensitivity, and whether it is because of commonalities between the cannabinoid systems of squirrel monkeys and humans, remain to be determined.

In summary, rodent models of cannabinoid reward have many limitations, and cannabinoid self-administration in squirrel monkeys has clear advantages. As described in the next section, THC self-administration procedures in squirrel monkeys, including the drug-priming and cue-induced reinstatement models of relapse, can be used to identify and validate mechanisms that might be exploited for the treatment of cannabis use disorder (Justinova *et al*, 2004, 2008b, 2011a, 2013, 2014; Schindler *et al*, 2016a, b). Self-administration procedures also provide a means of assessing the abuse potential of new drugs that directly or indirectly affect the cannabinoid system of the brain (Justinova *et al*, 2008a, 2015; Schindler *et al*, 2016b) (see Table 1 for details).

POTENTIAL TREATMENT TARGETS FOR CANNABIS USE DISORDER

Cannabis use disorder is recognized by DSM-5 (American Psychiatric Association, 2013) under the category of Cannabis-Related Disorders that also includes cannabis intoxication and the newly incorporated condition, cannabis withdrawal. The diagnostic criteria for cannabis use disorder and cannabis withdrawal are essentially the same as those for other drugs such as opioids and tobacco, recognizing that cannabis users can develop the same general symptoms common to all substance use disorders (Budney, 2006). These criteria fit into four main groupings: impaired control over substance use, social impairment, risky use, and pharmacological criteria (tolerance and withdrawal). The severity of cannabis use disorder can change over time and can range from mild to moderate to severe based on the number of symptom criteria endorsed.

A separate set of diagnostic criteria has been developed for cannabis withdrawal syndrome that occurs when use is abruptly discontinued or substantially reduced after a prolonged period of heavy cannabis use. The withdrawal syndrome can include irritability, anxiety, insomnia, decreased appetite, restlessness, depressed mood, as well as a range of physical symptoms like abdominal pain, sweating, fever, chills, or headache. It is

typically not as severe as opioid or alcohol withdrawal, probably because of the slow elimination of THC from the body (Huestis, 2005), but can cause considerable distress and functional impairment of normal daily activities (Budney and Hughes, 2006; Haney, 2005). Withdrawal-associated dysphoria can make quitting difficult for chronic users and contributes to relapse to cannabis use. According to the National Survey on Drug Use and Health (Center for Behavioral Health Statistics and Quality, 2016a), the prevalence of cannabis use disorder in the US population aged $\geqslant 12$ years is 1.5% (4 million people), making it the most prevalent substance use disorder behind alcohol and tobacco. As the effects of simply discontinuing chronic THC administration in rodents and non-human primates are subtle (Beardsley *et al*, 1986; Maldonado, 2002), cannabis withdrawal is typically modeled in THC-dependent rodents by acutely precipitating withdrawal with a CB_1 receptor antagonist to produce more pronounced symptoms (Panagis *et al*, 2008). However, precipitated THC withdrawal has not been used extensively for medication development, and most of the medications that have been tested clinically for cannabis withdrawal have involved drugs already approved for other purposes (Haney *et al*, 2004, 2008, 2010, 2013; Levin *et al*, 2011; Mason *et al*, 2012; Trigo *et al*, 2016a, b).

The demand for treatment for cannabis use disorder is higher than for any other substance except alcohol (Center for Behavioral Health Statistics and Quality, 2016b), but there are no approved medications for this purpose. Psychosocial therapies are effective for some, but the rates of nonresponse and relapse hover at $\sim$70% (Balter *et al*, 2014). Thus, effective medications to complement the psychotherapeutic approaches would be highly beneficial. The pharmacotherapies that have shown the most promise in clinical studies are CB_1 receptor agonists, the antiepileptic gabapentin, and *N*-acetylcysteine (Gorelick, 2016; Marshall *et al*, 2014). Here, we will review therapeutic targets that were investigated in preclinical models and that might be exploited for medication development. Although it might be useful to do so in the future, procedures have not been developed to model the specific criteria of cannabis use disorder in animals. No single model can capture all aspects and stages of addiction, and hence the general strategy is to model certain aspects of drug abuse to predict whether a specific treatment or target substrate could be valuable (Acri and Skolnick, 2013). Specifically, compounds that decrease cannabinoid self-administration or prevent drug seeking in animal models of relapse might be effective for achieving and maintaining abstinence in humans. The general tactics used for these purposes are to decrease the reinforcing effects of cannabinoids, prevent withdrawal symptoms, and block the conditioned effects of drugs and environmental cues that induce craving and relapse. The following sections review the main targets for development of medications to treat cannabis use disorder (see Table 2).

Cannabinoid Receptors

Recent advances in our understanding of cannabinoid receptors suggest ways that the endogenous cannabinoid system might be manipulated more effectively and safely to produce therapeutic effects. Cannabinoid CB_1 receptors are constitutively active (Bouaboula *et al*, 1997; Landsman *et al*, 1997), and this means that they can become spontaneously activated in the absence of an agonist. Drugs that act as inverse agonists shift the equilibrium toward the resting state of constitutively active receptors, thus producing a pharmacological response of their own that is opposite to the one induced by an agonist. When cells have endogenous agonist tone, then a neutral agonist would produce the same effect as an inverse agonist, by antagonizing the endogenous agonist tone (Fong, 2014), and this has been shown to be the case with CB_1 receptors (Turu *et al*, 2007). Constitutive activity of cannabinoid receptors was first noticed when rimonabant (SR141716A)—which acts at the CB_1 receptor as a competitive antagonist against endocannabinoids and exogenously added agonists (Howlett *et al*, 2011)—was found to have inverse agonist properties, reversing the effects of constitutive activity of CB_1 receptors (Bouaboula *et al*, 1997; Howlett *et al*, 2011; Landsman *et al*, 1997).

The constitutive activity of CB_1 receptors has important implications for the development of therapeutics. Inverse agonists can produce 'stronger' effects than neutral antagonists, partly because of different affinities for receptors in resting state *vs* activated state (Meye *et al*, 2014). Recently, it was demonstrated in mouse brain slices that constitutive CB_1 activity occurs in the ventral tegmental area (VTA), where CB_1 inverse agonists, but not CB_1 neutral antagonists, increase GABAergic transmission onto VTA dopamine neurons, and that this increase is prevented by pretreatment with a neutral antagonist (Meye *et al*, 2013). The same study also showed CB_1 receptor constitutive activity on glutamatergic synapses in the basolateral amygdala. These brain areas play important roles in appetitive behavior, depression, and anxiety. Importantly, preclinical evidence suggests that neutral antagonists of the CB_1 receptor (eg, AM4113) can produce therapeutic effects such as decreasing food intake (Abrantes *et al*, 2009; Gueye *et al*, 2016; Meye *et al*, 2013; Sink *et al*, 2010) and blocking the abuse-related effects of THC (Justinova *et al*, 2008b; Schindler *et al*, 2016a), effects similar to those of the inverse agonist rimonabant, but do not seem to produce anxiety and depression-like effects like those that that led to rimonabant being abandoned as a medication (Gueye *et al*, 2016). When Schindler *et al* (2016a) compared the effects of AM4113 and rimonabant in squirrel monkeys, both treatments decreased THC self-administration and reinstatement, and furthermore both treatments decreased nicotine self-administration and reinstatement. Thus, CB_1 neutral antagonists might be effective for the treatment of cannabis and tobacco dependence without producing the undesirable side effects of their inverse agonist counterparts. Overall, these findings suggest that safe and effective cannabinoid CB_1 therapeutics can be

TABLE 2 Summary of Preclinical Behavioral Studies Investigating Pharmacological Targets for Treatment of CUD

Study	Target/action	Tested ligand	Methodology	Results
Tanda *et al* (2000)	Cannabinoid CB_1 receptor Inverse agonist	Rimonabant	THC IVSA Squirrel monkeys	↓ THC taking
Justinova *et al* (2008b)	Cannabinoid CB_1 receptor Inverse agonist	Rimonabant	THC IVSA Reinstatement Squirrel monkeys	↓ THC seeking ↓ THC-induced reinstatement ↓ Cue-induced reinstatement
Vallée *et al* (2014)	Cannabinoid CB_1 receptor Signaling-specific NAM	Pregnenolone	WIN55,212 IVSA CD1 mice	↓ WIN taking
Schindler *et al* (2016a)	Cannabinoid CB_1 receptor Inverse agonist Neutral antagonist	Rimonabant AM4113	THC IVSA Reinstatement Squirrel monkeys	↓ THC taking ↓ THC-induced reinstatement ↓ Cue-induced reinstatement
Navarro *et al* (2001)	μ-Opioid receptor Antagonist	Naltrexone	WIN 55,212 IVSA CD1 mice	↓ WIN taking
Justinova *et al* (2004)	μ-Opioid receptor Antagonist	Naltrexone	THC IVSA Squirrel monkeys	↓ THC taking
Justinova *et al* (2011a)	A_{2A} adenosine receptor Antagonist	MSX-3	THC and AEA IVSA Reinstatement Squirrel monkeys	↓ THC and AEA taking (1 mg/kg) ↑ THC and AEA taking (3 mg/kg) − On THC-induced reinstatement
Justinova *et al* (2014)	A_{2A} adenosine receptor Antagonist (presynaptic)	SCH442416	THC IVSA Squirrel monkeys	↓ THC taking
Solinas *et al* (2007a)	α7 Nicotinic acetylcholine receptor Antagonist	MLA	WIN 55,212 IVSA THC discrimination Rats	↓ WIN taking ↓ THC discriminative-stimulus effects
Justinova *et al* (2013)	α7 Nicotinic acetylcholine receptor KMO inhibition → ↑kynurenic acid—Negative allosteric modulator	Ro 61-8048	THC IVSA Reinstatement Squirrel monkeys WIN 55,212 IVSA Reinstatement Rats	↓ THC and WIN taking ↓ THC- and WIN-induced reinstatement ↓ Cue-induced reinstatement
Justinova *et al* (2008a) Justinova *et al* (2015)	Anandamide FAAH inhibition	URB597 URB694	THC, AEA IVSA Squirrel monkeys	− URB597 IVSA + URB694 IVSA − URB597-induced reinstatement − URB694-induced reinstatement

Abbreviations: AEA: anandamide; FAAH: fatty acid amide hydrolase; IVSA: intravenous self-administration; MLA: methyllycaconitine; NAM: negative allosteric modulator; WIN: WIN 55,212-2.
Symbols: −, no effect; ↓, decrease or blockade; ↑, increase or potentiation.

developed based on an understanding of the consequences of *in vivo* constitutive activity on hedonic, motivational, and other condition-specific processes. With regard to practical applications, it should be noted that the effectiveness of an antagonist therapy depends on compliance with treatment, and that CB_1 neutral antagonists have the potential to induce withdrawal symptoms in cannabis-dependent individuals. Thus, cannabinoid neutral antagonist therapy would probably be most useful for the strengthening of already-established abstinence behaviors and preventing relapse in motivated individuals.

Another exciting advance that could lead to improved treatments for cannabis use disorder and other psychiatric conditions involves the development of drugs that affect cannabinoid receptors in another new way, allosterically. The classic primary neuronal binding site of the CB_1 receptor is the orthosteric binding site, where endogenous and synthetic cannabinoid ligands (including the inverse agonists and neutral antagonists mentioned above) directly affect the response of the cell. Cannabinoid receptors can also contain allosteric binding sites that are discrete from the orthosteric site (Ross, 2007). Allosteric modulators are ligands that bind to these allosteric sites and alter the affinity and/or efficacy of orthosteric ligands (Bosier *et al*, 2010). Thus, allosteric modulators have no effect on neuronal signaling in the absence of the orthosteric ligand, but positive allosteric modulators (PAMs or allosteric enhancers) amplify the orthosteric agonist's (or inverse agonist's) effect, and negative allosteric modulators (NAMs) decrease the orthosteric ligand's effect. In contrast with allosteric modulators, allosteric agonists are ligands that bind at allosteric sites but possess their own efficacy, directly influencing receptor coupling without requiring the presence of an orthosteric ligand. Ligands that act as both direct agonists and allosteric modulators are called ago-allosteric modulators (Schwartz and Holst, 2007). Newly developed CB_1 allosteric ligands can have complex effects, and one label cannot always precisely convey the mechanism of action, thus making ligand classification and terminology difficult (Keov *et al*, 2011). For example, the most studied CB_1 allosteric modulator, Org27569, has an unusual pharmacological profile, enhancing orthosteric agonist binding (PAM) but inhibiting

agonist signaling efficacy (NAM) (Price *et al*, 2005). Despite consistent effects *in vitro* (Ahn *et al*, 2013; Shore *et al*, 2014), Org27569 does not always alter the actions of CB_1 orthosteric agonists *in vivo* (Ding *et al*, 2014; Gamage *et al*, 2014). Org27569 did block reinstatement of cocaine- and methamphetamine-seeking behavior (cue or priming-induced) in rats (Jing *et al*, 2014), and Org27569 or similar ligands could potentially be useful for weight control (Baillie *et al*, 2013; Horswill *et al*, 2007), but have not been yet used in animal models of cannabis abuse or relapse. However, promising effects have been obtained with another compound acting as a signaling-specific NAM of CB_1, pregnenolone, a precursor of all steroid hormones. Pregnenolone blocks behavioral and somatic effects of THC, effectively functioning as a negative feedback mechanism protecting the brain from CB_1 overactivation (Vallée *et al*, 2014). Importantly, pregnenolone blocked the effects of THC without disrupting endogenous cannabinoid signaling (Straiker *et al*, 2015), an effect that may be advantageous from a medication development perspective. However, pregnenolone as a medication has many disadvantages that prevent its use in humans (conversion to active steroids, short half-life, and poor bioavailability), prompting development of pregnenolone derivatives (Piazza *et al*, 2012) that are now called synthetic signaling-specific inhibitors of the CB_1. These derivatives are not converted into active steroids and have improved half-life and oral bioavailability. One of them is set to enter clinical testing as a potential treatment for cannabis use disorder (Busquets-Garcia *et al*, 2017). Thus, exploitation of allosteric modulation of CB_1 receptors, particularly the targeting of desirable signaling pathways, presents an opportunity for development of new CB_1 ligands with functional selectivity and improved side-effect profile (Khajehali *et al*, 2015; Kulkarni *et al*, 2016a; Qiao *et al*, 2016).

Opioid Receptors

The opioid receptor antagonist naltrexone has efficacy for treating alcohol abuse and may have general behavioral effects that are helpful in treating substance use disorders (Aboujaoude and Salame, 2016). Bidirectional modulatory interactions between the endogenous opioid and cannabinoid systems have been extensively studied in many preclinical models (Lopez-Moreno *et al*, 2010; Parolaro *et al*, 2010; Robledo *et al*, 2008; Scavone *et al*, 2013). In animal models of the abuse-related effects of cannabinoids, the opioid receptor antagonists naltrexone and naloxone have been found to block many neurochemical and behavioral effects of cannabinoids in rodents (Braida *et al*, 2001, 2004; Solinas and Goldberg, 2005; Solinas *et al*, 2004b), including THC-induced dopamine release in the nucleus accumbens shell (Chen *et al*, 1990; Tanda *et al*, 1997) and self-administration of synthetic cannabinoids (Braida *et al*, 2001; Navarro *et al*, 2001). In squirrel monkeys, naltrexone blocked the rewarding effects of THC during all 5 days of treatment (Justinova *et al*, 2004), but only blocked cue-driven THC-seeking behavior under a second-order schedule for 2 of the 5 days (Justinova *et al*, 2008b). In several human laboratory studies that investigated acute interactions (Cooper and Haney, 2010; Haney, 2007; Haney *et al*, 2003), the effects of naltrexone on cannabis self-administration and the subjective and cardiovascular effects of cannabis were not promising, as the effects of THC were mostly enhanced. However, chronic naltrexone administration attenuated the direct reinforcing and positive subjective effects of cannabis in daily cannabis smokers (Haney *et al*, 2015). Thus, the efficacy of chronic naltrexone maintenance as a treatment of cannabis use disorder should be further examined in clinical testing.

Adenosine Receptors

Adenosine A_{2A} receptors can physically interact with CB_1 receptors in the striatum to form heteroreceptor complexes that mediate the psychomotor (Carriba *et al*, 2007) and rewarding effects of cannabinoids (Justinova *et al*, 2011a, 2014). CB_1 receptors are also colocalized in corticostriatal glutamatergic terminals with presynaptic A_{2A} receptors, and in dendrites of GABAergic medium spiny neurons with postsynaptic A_{2A} receptors (Ferré *et al*, 2009, 2010). In squirrel monkeys, a presynaptic A_{2A} antagonist attenuated the reinforcing effects of THC in squirrel monkeys, and a postsynaptic A_{2A} antagonist enhanced them (Justinova *et al*, 2014), but antagonism of presynaptic A_{2A} receptors did not alter the reinstatement of THC seeking (Justinova *et al*, 2011a). Adenosine–cannabinoid interactions are complex, depending not only on the contributions of presynaptic *vs* postsynaptic receptors and the formation of heteromers with CB_1 receptors (or lack thereof), but also on whether they interact with other receptors (eg, adenosine A_1, dopamine D_2, metabotropic glutamate mGluR5) (Ferre *et al*, 2010; Tebano *et al*, 2012). Thus, A_{2A}/CB_1 heteromers could potentially be targeted for the treatments of cannabis use disorder, but the desired pharmacological profile for this purpose is unknown.

Nicotinic Acetylcholine Receptors

There is bidirectional crosstalk between the nicotinic cholinergic system and the endocannabinoid system, which are both involved in numerous physiological processes including reward (reviewed by (Scherma *et al*, 2016b). Pharmacological manipulation of nicotinic acetylcholine receptors (nAChRs) can modulate the rewarding effects of cannabinoids and, reciprocally, pharmacological manipulation of CB_1 receptors can modulate the reward-related effects of nicotine (Gamaleddin *et al*, 2015; Justinova *et al*, 2013; Schindler *et al*, 2016a; Solinas *et al*, 2007a, b; Valjent *et al*, 2002). Solinas *et al* (2007a) dissected the role of specific nAChR subtypes, finding differences between homomeric α7nAChRs and heteromeric $\alpha4\beta$*nAChRs that do not contain α7 subunits in rats. They found that the selective orthosteric α7nAChR antagonist methyllycaconitine

decreased THC-induced dopamine elevations in the nucleus accumbens shell, blocked the discriminative-stimulus effects of THC, and reduced self-administration of the synthetic cannabinoid WIN 55,212. On the other hand, a selective $\alpha4\beta^*$nAChR antagonist did not alter any of the abuse-related effects of cannabinoids. Thus, a treatment that increases $\alpha7$nAChR activity might be a useful pharmacological approach for the treatment of cannabis use disorder. However, orthosteric cholinergic antagonists like methyllycaconitine can have serious central (cognitive impairment) and peripheral (impaired angiogenesis) side effects (Arias *et al*, 2009; Roegge and Levin, 2006). A negative allosteric modulator might be better tolerated, as it would have no effect without the presence of an orthosteric ligand. Selective negative allosteric modulators of $\alpha7$nAChRs have not been developed, but the tryptophan metabolite kynurenic acid (KYNA) acts as an endogenous negative allosteric modulator of $\alpha7$nAChRs (Hilmas *et al*, 2001), and endogenous KYNA levels can be pharmacologically enhanced by inhibition of kynurenine 3-monooxygenase (KMO) that shunts the kynurenine metabolism pathway toward increased synthesis of KYNA (Amaral *et al*, 2013; Moroni, 1999).

The effects of the KMO inhibitor Ro 61-8048 (Rover *et al*, 1997) on neurochemical and behavioral effects of cannabinoids have been studied in rats and squirrel monkeys (Justinova *et al*, 2013). Treatment with Ro 61-8048 significantly increased levels of KYNA in the nucleus accumbens shell and VTA of rats and attenuated THC-induced dopamine elevations in these brain areas without affecting basal dopamine levels. Ro 61-8048 also decreased WIN 55,212 self-administration in rats and THC self-administration in monkeys. In models of relapse, the KMO inhibitor blocked reinstatement of extinguished THC seeking induced by the presentation of THC, WIN 55,212, or THC-associated cues. The reduction of the neurochemical and behavioral effects of THC by Ro 61-8048 was reversed by treatment with PNU120596 (a positive allosteric modulator at $\alpha7$nAChRs) (Hurst *et al*, 2005) confirming that the effects of KMO inhibition were due to the actions of KYNA on $\alpha7$nAChRs. Ro 61-8048 did not produce nonspecific impairment of operant behavior, as it did not alter food-maintained behavior in rats or food or cocaine self-administration in separate groups of monkeys. As increased levels of KYNA have been linked to cognitive deficits (Chess *et al*, 2007; Koola, 2016; Pocivavsek *et al*, 2011), it is encouraging from a translational perspective that the increases in brain KYNA levels achieved in the study of Justinova *et al* (2013) did not adversely impact working memory in rats or monkeys. Recent studies showed that KMO inhibition could also be an effective approach for treatment of other substance use disorders, including nicotine, cocaine, and alcohol (Secci *et al*, 2017; Vengeliene *et al*, 2016). In these studies (Justinova *et al*, 2013; Secci *et al*, 2017; Vengeliene *et al*, 2016), Ro 61-8048 effectively counteracted two major triggers of relapse, drug priming (with nicotine or THC) and cue-induce reinstatement of the seeking of THC, nicotine, alcohol, or cocaine in rats or monkeys. The fact that KMO inhibition has positive effects in models involving all of these abused substances suggests that it might be particularly useful for prevention of relapse in polydrug abusers. Development of KMO inhibitors as treatments for neurodegenerative diseases (Amaral *et al*, 2013; Smith *et al*, 2016b; Zwilling *et al*, 2011) is underway and could facilitate clinical testing of KMO inhibitors for the treatment of substance use disorders in the future.

Manipulation of Endogenous Cannabinoids

The most effective pharmacological treatments for substance use disorders to date have been replacement therapies using receptor agonists (methadone or nicotine) or partial agonists (buprenorphine or varenicline) for opioid or tobacco users (Cahill *et al*, 2013; Mattick *et al*, 2014). Administration of the cannabinoid CB_1 receptor agonist nabilone could be useful for treating cannabis use disorder (Haney *et al*, 2013). It should be noted that THC itself is a partial agonist of the CB_1 receptor, but that a lower efficacy partial CB_1 receptor agonist might reduce withdrawal and block rewarding effects of THC, producing therapeutic effects comparable to those of buprenorphine in opioid users. Taking a different tack, treatments that manipulate levels of endogenous cannabinoid ligands might provide a safer alternative to medications that directly bind to cannabinoid receptors, based on the hypothesis that enhancing natural endocannabinoid signaling would produce fewer side effects than would activating CB_1 receptors indiscriminately throughout the brain. Drugs that block the transport of anandamide or that block the metabolism of anandamide or 2-AG have been investigated as possible treatments for several types of pain and for neuropsychiatric disorders, including addiction (Berardi *et al*, 2016; Gamaleddin *et al*, 2011; Gobbi *et al*, 2005; Jayamanne *et al*, 2006; Justinova *et al*, 2015; Kathuria *et al*, 2003; Pryce *et al*, 2013; Ramesh *et al*, 2011; Scherma *et al*, 2008, 2012; Schlosburg *et al*, 2009). Increasing endocannabinoid levels could, for example, alleviate withdrawal symptoms or function as a replacement therapy in patients with cannabis use disorder. Although the CB_1 activation produced by inhibition of endocannabinoid transport or metabolism is proposed to be region and activity dependent, concerns have been raised regarding the abuse potential of certain drugs that increase endogenous levels of endocannabinoids. For example, the anandamide transport inhibitor AM404 can maintain high rates of self-administration and reinstate extinguished cannabinoid-seeking behavior in squirrel monkeys (Schindler *et al*, 2016b). FAAH inhibitors, which increase levels of anandamide by preventing its breakdown, are more promising than transport inhibitors as potential medications. The most-studied FAAH inhibitor, URB597, has been evaluated in separate groups of squirrel monkeys with histories of anandamide, THC, or cocaine self-administration, and none of these monkeys self-administered URB597 (Justinova *et al*, 2008a). Moreover, priming injection of URB597 did not reinstate extinguished drug-seeking

behavior in any of these three groups, unlike THC, which produced significant reinstatement of drug seeking in all of them. URB597 is a parent compound of another FAAH inhibitor, URB694, that has an improved pharmacological profile (higher potency, better solubility and stability); both drugs increase levels of anandamide and also the endogenous PPAR-α agonists OEA and PEA in monkey brain (Justinova *et al*, 2008a, 2015). Both drugs also block nicotine self-administration and priming-induced and cue-induced reinstatement of nicotine seeking in monkeys (Justinova *et al*, 2015). However, in contrast to URB597, URB694 had moderate reinforcing effects in anandamide, THC, and cocaine-experienced monkeys, although like URB597 it did not reinstate extinguished nicotine or THC seeking when given alone (Justinova *et al*, 2015). The self-administration of URB694 was attenuated by not only a CB_1 receptor antagonist but also by PPAR-α antagonist, suggesting that PPAR-α might be able to modulate the rewarding effects of some cannabinoid-related drugs. In rats, URB597 or URB694 do not increase dopamine release in the nucleus accumbens shell, and their discriminative-stimulus effects do not resemble those of THC (Gobbi *et al*, 2005; Justinova *et al*, 2015). Based on these results, these two FAAH inhibitors should have a minimal risk of abuse potential. However, FAAH inhibitors are a chemically diverse group of compounds, and they should be evaluated individually for side effects (Panlilio *et al*, 2016). The FAAH inhibitor PF-04457845 maintains high levels of self-administration and partially reinstates THC-seeking behavior in squirrel monkeys, and in rats it significantly increases dopamine levels in the nucleus accumbens shell (all unpublished results). PF-04457845 is currently being evaluated clinically as a treatment for cannabis withdrawal (NCT01618656) and did not show any cannabis-like adverse effects in previous clinical trials (Huggins *et al*, 2012; Li *et al*, 2012a). It remains to be seen whether FAAH inhibitors will prove effective for treatment of cannabis use disorder and whether preclinical results from squirrel monkeys or rats have translational value for prediction of cannabinoid-like abuse potential in humans.

Most Promising Targets and Tools for Further Development

Despite the clinical failure of the inverse agonist rimonabant, preclinical studies indicate several ways that the cannabinoid CB_1 receptor should still be a target for medication development. First, animal studies suggest that novel neutral antagonists would not produce the adverse effects of rimonabant, but could be efficacious for the treatment of cannabis use disorder, obesity, and tobacco use disorder (Gueye *et al*, 2016; Schindler *et al*, 2016a). Second, recent advances in CB_1 receptor pharmacology are leading to the development of new signaling-specific allosteric modulators that could have more desirable therapeutic and safety profiles than orthosteric antagonists (Vallée *et al*, 2014). Third, manipulation of the pharmacokinetic properties of CB_1 receptor agonists could also be helpful for implementation of replacement therapy for cannabis use disorder. For example, the latest efforts to improve the safety and drug-like profiles of CB_1 receptor agonists (Kulkarni *et al*, 2016b) are directed toward developing controlled-deactivation THC-based analogs that have structures based on THC, but faster onset/offset and shorter duration of action than currently existing THC analogues. Fourth, certain FAAH inhibitors enhance levels of endogenous cannabinoids but do not have cannabis-like rewarding or cognitive effects in animals and could be safe for the treatment of cannabis withdrawal. Other targets, including α7 nicotinic acetylcholine receptors, show promise in preclinical testing, but further progress is hampered by the current lack of clinically viable drugs for this target (see Table 2 for details).

ADDICTION-RELATED EFFECTS OF CANNABINOID EXPOSURE

It has long been recognized that drug use typically starts in adolescence and progresses through a series of stages, from tobacco and alcohol to cannabis, and then to psychostimulants and opioids (Kandel, 1975; Kandel and Faust, 1975; Kandel *et al*, 1992). This progression has been referred to metaphorically as a 'gateway' effect, because using one drug seems to lead to use of another. There are multiple reasons why this progression might occur (Degenhardt *et al*, 2010; Fergusson *et al*, 2015; Vanyukov *et al*, 2012), including the relative legal status, social acceptability, and availability of each class of drugs. An important possibility, which is hard to study directly in humans but can be evaluated systematically in animal models, is that exposure *per se* to one kind of drug can predispose the individual to becoming a habitual user of a different class of drugs.

Research in this area has become increasingly active over the past decade, mostly focusing on the effects of exposure to THC or synthetic cannabinoid agonists. As described below, this preclinical research is relevant to not only the classic progression from cannabis to 'hard' drugs in adolescents (Kandel, 1975), but also to effects of prenatal or adult exposure to cannabis, the effects of passive THC exposure on later cannabis use, and a 'reverse gateway' effect (Patton *et al*, 2005) in which cannabis use might increase the likelihood of developing tobacco use disorder. Perinatal exposure has also been studied and is important in light of high rates of cannabis use during pregnancy, possibly for antiemetic effects (Volkow *et al*, 2017; Westfall, 2004). These studies have shown that exposure to cannabinoid drugs can induce lasting changes in brain systems related to addiction and reward (including the endogenous cannabinoid, opioid, and dopamine systems) and can alter the rewarding effects of noncannabinoid drugs taken later in life.

Besides altering the brain pharmacologically, cannabinoid drugs could also affect behavior through learning mechanisms that are common to all rewards. For example, the general strategy of seeking and taking a drug for its

rewarding effects could facilitate trying other drugs. When cannabis becomes expensive or unavailable, users may switch to another class of drug as a substitute (Chandra and Chandra, 2015). The response topography used in smoking or injecting one drug could be transferred to another drug. In preclinical studies of drug self-administration, transfer is commonly used to facilitate drug training by using food reinforcement to initially establish a lever-pressing response. Although these behavioral mechanisms are important and can be investigated with animal models, they have not been systematically studied within the context of the etiology of drug abuse. For example, the acquisition and maintenance of THC self-administration was not appreciably different in monkeys that had previous experience self-administering cocaine (Tanda *et al*, 2000) as compared with drug-naive monkeys (Justinova *et al*, 2003) that had experience self-administering food pellets (with cocaine, food, and THC training procedures all using the same apparatus and operant schedule). However, these studies were not conducted as a single experiment that would allow formal conclusions concerning the effects of prior drug experience.

There are a number of procedural variables in animal models of gateway-like effects that (1) represent specific aspects of human drug use and (2) could be important determinants of behavior in both animals and humans. These include age of exposure (prenatal, perinatal, adolescent, or adult), regimen of exposure (modeling either light intermittent use or heavy escalating use of cannabis), and amount of time between exposure and testing. Age and sex differences have received considerable attention, but most studies have not involved more than one level of these other variables. Many studies of cannabinoid exposure have looked at changes in the brain or changes in behavior, and some have done both within the same subjects, but very few have attempted to demonstrate that the brain changes play a causal role in the behavioral changes. For example, besides studies where a cannabinoid antagonist was used to block the effects of cannabinoid exposure and establish that the effects of exposure are mediated by CB_1 receptors, we are aware of only one study (Tomasiewicz *et al*, 2012) where researchers tried to block a putative mechanism by which prior exposure to cannabinoids alters the self-administration of drugs. Regarding the studies discussed below, 'exposure' will refer to a period of cannabinoid administration, followed by a drug-free period of days or week before behavioral or physiological testing. Probably because of the difficulty of studying cannabinoid self-administration in rodents, the cannabinoid exposure in all of these studies was passive.

Effects of Cannabinoid Exposure on Addiction-Related Brain Function

Receptor density, functionality, and gene expression. Cannabinoid CB_1 receptors. Drug exposure can alter receptor systems in ways that produce a functional tolerance or sensitization to the effects of subsequent drug administrations. The endogenous cannabinoid system not only underlies the rewarding effects of cannabis, but can also interact with other drugs of abuse (Braida and Sala, 2002; Henderson-Redmond *et al*, 2016; Le Foll *et al*, 2008; Martin-Garcia *et al*, 2016; Parolaro *et al*, 2010) and the conditioned effects of drug-associated cues (De Vries and Schoffelmeer, 2005). Several studies in rats have shown that cannabinoid exposure can decrease CB_1 receptor expression and/or receptor functionality in brain areas related to reward, motivation, emotion, and cognition (Ginovart *et al*, 2012; Gonzalez *et al*, 2004; Lopez-Gallardo *et al*, 2012; Lopez-Rodriguez *et al*, 2014; Rubino *et al*, 2008, 2015). Rubino *et al* (2015) found that 3 days of THC exposure during early adolescence had time-dependent effects on the functionality of CB_1 receptors: functionality decreased soon after exposure, increased above control levels in mid to late adolescence, and decreased back to control levels in adulthood. The effects of cannabinoid exposure on CB_1 expression have been inconsistent across studies with respect to whether males and females are affected differentially (Lopez-Gallardo *et al*, 2012; Lopez-Rodriguez *et al*, 2014; Rubino *et al*, 2008); this inconsistency suggests that the effects of cannabinoid exposure on CB_1 receptors are not only sex dependent, but also influenced by other procedural or subject variables that are not fully understood. In several other studies, THC exposure did not affect CB_1 receptor density or functionality, possibly because of lighter THC exposure regimens (Ellgren *et al*, 2007, 2008; Morel *et al*, 2009) or to the rats being exposed at an earlier (Spano *et al*, 2007) or later age (Realini *et al*, 2011) than in the other studies.

μ-Opioid receptors and proenkephalin. Exposure to cannabinoids during the perinatal or adolescent period has been found to decrease the density and functionality of μ-opioid receptors and decrease preproenkephalin gene expression during adulthood in specific brain areas in male rats, but to increase μ-opioid binding or have no effect on μ-opioid functionality in female rats (Biscaia *et al*, 2008; Ellgren *et al*, 2007; Spano *et al*, 2007; Vela *et al*, 1998). Within the studies that directly compared males and females, these sex differences in μ-opioid receptors were consistent with the effects of cannabinoid exposure on morphine self-administration (Biscaia *et al*, 2003; Vela *et al*, 1998).

Dopamine. Signaling by dopamine cells in the nucleus accumbens is believed to be critical to the rewarding effects of most drugs of abuse, including cannabinoids (Cheer *et al*, 2004; Lecca *et al*, 2006; Tanda *et al*, 1997). Prenatal exposure to THC was associated with decreased expression of dopamine D_2 receptors in the nucleus accumbens of both fetal human brain and adult rat brain (DiNieri *et al*, 2011), a finding that supports the validity of cannabinoid exposure studies in rodents. Rats exposed to THC in adulthood showed changes in dopamine signaling (Cortright *et al*, 2011) and binding (Ginovart *et al*, 2012) in the nucleus accumbens, and rats exposed in adolescence showed changes

in dopamine receptor binding in the nucleus accumbens (in females) or prefrontal cortex (in males) (Zamberletti *et al*, 2012).

Other receptors and gene expression. Changes in other receptor systems related to drug abuse and addiction include decreased glutamate NMDA receptor density (Zamberletti *et al*, 2012) and increased serotonin transporter expression (Lopez-Rodriguez *et al*, 2014). Female rats exposed to THC in adolescence showed increased CREB activity in the nucleus accumbens, and low CREB activity in the hippocampus and prefrontal cortex that could affect the expression of many types of genes and might be responsible for depression-like behavior observed in the same study (Rubino *et al*, 2008).

Neuronal activity. Regional activity. Cannabinoid exposure can have lasting effects on basal levels of neuronal activity in reward-related regions and can also alter the effects of drugs on neuronal activity. THC exposure during the perinatal (Singh *et al*, 2006) or adult (Singh *et al*, 2005) period altered basal and heroin-induced neuronal activity in reward-related areas of rats during adulthood, and exposure in adulthood also increased the locomotor response to heroin. Changes in neuronal activity after cannabinoid exposure can be sex dependent (Higuera-Matas *et al*, 2008) and can dissipate after experience with opioid self-administration (González *et al*, 2003). Exposure to THC in adolescence also enhanced the neuronal response to the widely abused NMDA antagonist phencyclidine (Zamberletti *et al*, 2014).

Single-cell activity. In vivo electrophysiology techniques can be used to record the firing of individual neurons. Wu and French (2000) found that exposure to THC in adulthood produced tolerance to THC-induced hyperlocomotion, hypothermia, catalepsy, and dopamine cell firing in the substantia nigra pars compacta, but did not alter THC-induced dopamine cell firing in the VTA; they suggest that the lack of tolerance seen in the VTA is consistent with a lack of tolerance to the euphoric effects of cannabis in humans. However, rats exposed to higher doses of THC in adolescence did show tolerance to the cannabinoid agonist WIN 55,212-2 on the firing of dopamine cells in the VTA (Scherma *et al*, 2016a). Exposure to WIN 55,212-2 in either adolescence or adulthood can also induce tolerance to the effects of acute WIN 55,212-2 administration on cell firing in the VTA (Pistis *et al*, 2004); the firing of VTA cells in rats exposed in adolescence (but not those exposed in adulthood) also showed cross-tolerance to the effects of morphine, cocaine, and amphetamine. Exposure to WIN 55,212-2 in adolescence can also lead to depression-like behavior, decreased spontaneous firing of serotonin neurons, and increased spontaneous firing of noradrenergic neurons in rats (Bambico *et al*, 2010).

Neurochemical levels. Endocannabinoids. Rats exposed to low intermittent doses of THC during adolescence showed increased levels of the endogenous cannabinoid anandamide in the nucleus accumbens (Ellgren *et al*, 2008), but levels of 2-AG, the most abundant endocannabinoid in the brain, were not affected. Rats exposed twice daily to escalating doses of THC also showed increased levels of anandamide, in the prefrontal cortex, with no effect on levels of 2-AG or FAAH (which breaks down anandamide) at any of the time points tested (Rubino *et al*, 2015).

Dopamine. Several studies have used *in vivo* microdialysis techniques to investigate how cannabinoid exposure affects the ability of drugs to increase extracellular dopamine levels in the nucleus accumbens, which are relevant to rewarding effects and drug-induced hyperactivity. Rats that had been exposed to THC in adolescence showed an enhanced dopamine response in the accumbens core (Cadoni *et al*, 2008) and a blunted effect in the accumbens shell when given THC (Cadoni *et al*, 2008) or WIN 55,212-2 (Scherma *et al*, 2016a) in adulthood. A blunted effect of acute morphine was also seen after THC exposure, comparable to the effect seen after morphine exposure (Cadoni *et al*, 2008). Adolescent THC exposure did not alter the effects of acute THC administration on dopamine levels in the nucleus accumbens of Lewis or Fischer 344 rats (strains that are known to differ in their response to drugs of abuse), but it potentiated the effects of heroin in both strains (Cadoni *et al*, 2013). Exposure to THC in adulthood or to WIN 55,212-2 in adolescence did not alter the basal level of dopamine in the nucleus accumbens or the dopamine response to amphetamine or WIN 55,212-2 (Cortright *et al*, 2011; Ellgren *et al*, 2004).

Stress hormones. Like other drugs of abuse, cannabinoids can activate the hypothalamo–pituitary–adrenal axis (HPA) (Murphy *et al*, 1998). Disrupting development of the HPA could alter the response to drugs later in life (Burke and Miczek, 2014) and could conceivably affect addiction-related behavior such as stress-induced drug seeking (Erb *et al*, 1998; Sinha, 2008). Perinatal exposure to THC increased basal levels of corticosterone, corticotropin-releasing hormone, and adrenocorticotropic hormone in female rats, but decreased corticosterone levels in males (Rubio *et al*, 1998; Rubio *et al*, 1995); THC-exposed males also showed enhanced HPA activation and increased conditioned place preference compared with nonexposed controls when given access to a morphine-associated context. There is some indication that the effects of cannabinoid exposure on stress hormones might depend on the specific cannabinoid agonist. That is, basal corticosterone levels were not affected in rats of either sex that were exposed to CP 55,940 in adolescence (Biscaia *et al*, 2003), but exposure to the cannabinoid HU-210 in adolescence increased the HPA response in both male and female rats (Lee *et al*, 2014), with females showing a greater relative increase than males.

Other neurochemicals. THC exposure has also been found to alter levels of other addiction-related neurochemicals, including decreased levels of the δ-opioid agonist met-enkephalin in the nucleus accumbens core (Ellgren *et al*, 2008), decreased basal levels of GABA in the prefrontal

cortex, and increased glutamate levels in the prefrontal cortex and dorsal striatum when rats receive phencyclidine (Zamberletti *et al*, 2014).

Brain development. Volume, structure, and function. The endocannabinoid system plays a critical role in brain development (Fride, 2008). Work with animal models has shown that cannabinoid exposure during development can alter the structure and function of brain areas implicated in drug reward and addiction, even when rats are not exposed to cannabinoids until young adulthood. Although changes in the volume of a brain region do not necessarily correlate with changes in function, cannabinoid exposure has been shown to affect regional morphology of the brain (Keeley *et al*, 2015; Realini *et al*, 2011). Perinatal THC exposure can induce changes in genes that are involved in nervous system development (Economidou *et al*, 2007). THC exposure in young adult rats can increase the dendritic length and branching of medium spiny neurons in the nucleus accumbens shell and pyramidal cells in the medial prefrontal cortex (Kolb *et al*, 2006), and these effects are similar to those seen after exposure to amphetamine, cocaine, or nicotine (Patton *et al*, 2005). Exposure to low doses of THC in young adult rats can increase the levels of brain-derived neurotrophic factor (which plays a role in neuronal development and synaptic plasticity) in the nucleus accumbens and medial prefrontal cortex (Butovsky *et al*, 2005), and heavy THC exposure in adolescence can disrupt the physical maturation of the endocannabinoid system, altering endocannabinoid and glutamate signaling in the prefrontal cortex during adulthood (Rubino *et al*, 2015). THC given during a brief prenatal period was sufficient to interfere with glutamatergic and GABAergic neuron development (de Salas-Quiroga *et al*, 2015). Exposure to WIN 55,212-2 during a certain window of susceptibility (ie, in early or mid adolescence, but not in late adolescence or adulthood) disrupted the functional maturation of the prefrontal cortex in rats, leading to impaired GABAergic transmission and a lasting disinhibition of cortical cell firing (Cass *et al*, 2014). Exposure to the cannabinoid agonist CP 55,940 in adolescence and young adulthood produced lasting changes in the synaptic structure and function of the prefrontal cortex that could be responsible for cognitive deficits (Renard *et al*, 2016).

Effects of Cannabinoid Exposure on Behavior Relevant to Addiction

Locomotor activity. Opioid-induced activity. Drug-induced locomotor activity, particularly sensitization to repeated administration, has been studied as an indicator of incentive-motivational effects of drugs of abuse and dopaminergic signaling in reward-related brain regions (Kalivas and Stewart, 1991; Robinson and Berridge, 2008; Wise, 1987). Cannabinoid exposure has been found to enhance the locomotor effects of μ-opioid agonists in a number of studies. Rats exposed to THC during perinatal, adolescent, or adult stages show an enhanced locomotor response to morphine, heroin, or THC in adulthood (Cadoni *et al*, 2001; Cadoni *et al*, 2008; Panlilio *et al*, 2007; Pontieri *et al*, 2001; Singh *et al*, 2005). THC exposure did not enhance the locomotor-activating effects of heroin in Lewis or Fischer 344 rats (despite enhanced heroin-induced dopamine overflow in the nucleus accumbens of both strains) (Cadoni *et al*, 2013), but exposure to the cannabinoid agonist CP 55,940 did enhance morphine-induced locomotor activity in Lewis rats (Norwood *et al*, 2003). Consistent with interaction between the endogenous cannabinoid and opioid systems, exposure to morphine enhances the locomotor response to THC or the cannabinoid agonist WIN 55,212-2 (Cadoni *et al*, 2001, 2008).

Activity induced by psychomotor stimulants or dopamine agonists. The effects of cannabinoid exposure on locomotor activity induced by stimulants have been less consistent than the effects on locomotor activity induced by opioids, possibly showing more dependence on the treatment regimen and other procedural or subject variables. Most studies have shown no effect or a reduction in the effect of amphetamine after cannabinoid exposure during prenatal (Silva *et al*, 2012), adolescent (Ellgren *et al*, 2004), or young adult (Ginovart *et al*, 2012) periods, and THC exposure in young adulthood did not affect the level of locomotor activity induced by cocaine (Panlilio *et al*, 2007). However, 2 weeks of exposure to a high dose of THC increased the locomotor effects of amphetamine during cannabinoid withdrawal at 24 h after the last exposure (Gorriti *et al*, 1999), and adolescent exposure to the cannabinoid agonist HU-210 increased stereotypy behavior (indicating a highly sensitized dopamine response) during repeated amphetamine treatment in adult female rats but not males (Lee *et al*, 2014). Using an informative parametric experimental design, Cortright *et al* (2011) compared the effects of five different doses of intermittent THC exposure on amphetamine-induced locomotor activation and obtained inverted-U-shaped dose effect functions for THC when activity was measured either 2 days or 2 weeks after the last exposure. Intermittent exposure to a moderate dose of THC enhanced the locomotor-activating effects of the dopamine agonist apomorphine 2 days or 2 weeks after the last THC exposure (Cortright *et al*, 2011). Testing the effects of apomorphine and quinpirole on locomotor behavior suggests that perinatal exposure to THC (from the prenatal period though weaning) increased the sensitivity of presynaptic dopamine D_2 receptors (Moreno *et al*, 2003). Exposure to THC in young adulthood also altered locomotor activity dose-effect functions for quinpirole in a manner consistent with increased sensitivity of postsynaptic $D_{2/3}$ receptors (Ginovart *et al*, 2012).

Phencyclidine-induced activity. Phencyclidine, a dissociative anesthetic that acts as an antagonist at glutamate NMDA receptors, is often mixed with marijuana and smoked (Bush, 2013). Female rats exposed to THC in adolescence show a psychotic-like profile of behavior (ie, decreased social

interaction, impaired recognition memory, despair-like immobility in a forced-swim test), and they show enhanced locomotor activation and stereotypy when administered phencyclidine (Zamberletti *et al*, 2014). Compared with vehicle-exposed controls, male rats exposed to THC also showed enhanced effects of phencyclidine, but in the form of ataxia rather than locomotor activation (Zamberletti *et al*, 2016). These behavioral changes were associated with dysregulation of the prefrontal cortex in female rats and the hippocampus in male rats. Phencyclidine sensitivity is considered a model of positive symptoms of schizophrenia, and hence these findings are relevant to both cannabis-induced psychosis (Schuckit, 2006) and cannabis-induced alterations in the effects of other abused drugs.

Models of depression, anxiety, and memory. Emotional and cognitive effects of cannabinoid exposure could potentially lead to self-medication later in life or comorbidity of substance use disorders with other psychiatric disorders. Prenatal exposure to a low dose of THC produced learning and memory deficits in rats (Silva *et al*, 2012). Adolescent THC exposure affected behavior in several models of anxiety and learning, and these effects were dependent on both sex and strain of the rats (Long-Evans *vs* Wistar) (Keeley *et al*, 2015), suggesting a genetic influence on sensitivity to cannabinoid exposure. Adolescent (but not adult) exposure to the cannabinoid agonist CP 55,940 induced memory impairments and anxiety-like behavior (O'Shea *et al*, 2004). Biscaia *et al* (2003) observed sex-dependent anxiety-like effects of periadolescent exposure to CP 55,940. Realini *et al* (2011) found that THC exposure in adolescence but not adulthood produced depression-like symptoms and memory impairment; the depression-like symptoms were reversed by treatment with the FAAH inhibitor URB597, which increases endogenous levels of the endocannabinoid anandamide. Rubino *et al* (2008, 2009) found that female rats exposed to high doses of THC in adolescence later showed depression-like signs of anhedonia, behavioral despair, and impaired working memory. Bambico *et al* (2010) found that exposure to a low or high dose of THC in adolescence (but not adulthood) produced depression-like behavior, whereas a high dose also led to anxiety-like behavior. THC exposure during adolescence also interacts with early maternal deprivation to alter cognitive and emotional behavior in adulthood. Female rats that had been deprived of maternal contact for 1 day (on postnatal day 9) were resistant to memory impairments induced by THC exposure in adolescence, unlike rats that were not maternally deprived (Zamberletti *et al*, 2012); this suggests that adaptive developmental responses to maternal deprivation might protect against certain cannabinoid-induced deficits. In the same study, THC exposure also blocked the development of aggressive behavior in maternally deprived females and increased passive coping behavior in maternally deprived males.

Effects of Cannabinoid Exposure on Behavior in Animal Models of Drug Abuse

Opioid reward. Fixed-ratio 1 schedule. As shown in Table 3, most preclinical studies of the effects of cannabis exposure on behavior in animal models of drug reward have focused on opioids, specifically heroin and morphine. The results of these opioid studies have been fairly consistent across exposure regimens, sex, strain, and the age of subjects during the exposure period (including prenatal, adolescent, and adult). In most experiments involving opioid self-administration under a fixed-ratio 1 (FR1) schedule (where only one operant response is required per injection), cannabinoid increased the voluntary intake of morphine or heroin (Biscaia *et al*, 2008; Ellgren *et al*, 2007; Norwood *et al*, 2003; Solinas *et al*, 2004a; Spano *et al*, 2007; Tomasiewicz *et al*, 2012; Vela *et al*, 1998). The exceptions to this finding include the female rats in the study of Biscaia *et al* (2008), which showed high rates of morphine intake regardless of whether they were exposed to cannabinoids, and the rats in the study of Stopponi *et al* (2014), which did not show enhanced heroin intake but did show enhanced heroin seeking when treated with the adrenergic antagonist yohimbine in a model of relapse. The study by Tomasiewicz *et al* (2012) provides the most clear evidence for a specific mechanism by which cannabinoid exposure can affect later drug intake: (1) THC exposure increased proenkephalin expression when measured 30 days after the last THC treatment, (2) genetically engineered overexpression of proenkephalin in THC-naive rats mimicked the effects of THC exposure on heroin self-administration, and (3) genetically engineered knockdown of proenkephalin expression in the nucleus accumbens shell prevented the effects of THC exposure on heroin self-administration.

Progressive-ratio schedule. The FR1 schedule can indicate whether a drug has a reinforcing effect or not, but does not provide a measure of the strength of this effect because the level of drug intake is largely determined by pharmacokinetics and duration of effect (Norman *et al*, 2011; Panlilio *et al*, 2003). In contrast, progressive-ratio schedules provide a relatively direct index of the reinforcing efficacy or subjective value of the drug (Hursh and Silberberg, 2008; Stafford *et al*, 1998) by varying the price (ie, the number of responses required for each injection). As seen in Table 3, experiments with progressive-ratio schedules have consistently shown that cannabinoid exposure does not make opioid drugs more valuable as a reinforcer (González *et al*, 2003, 2004), even when the same rats show increased opioid intake under the FR1 schedule (Biscaia *et al*, 2008; Solinas *et al*, 2004a). If the results of these FR1 and progressive-ratio studies generalize to humans, people with a history of cannabis exposure who begin using opioid drugs might take larger or more frequent doses of opioids, but they would not be expected to experience stronger rewarding effects or go to greater lengths to obtain opioids.

Place conditioning. Compared with FR1 and progressive-ratio schedules of drug self-administration, place conditioning is a less direct measure of drug reward, but it provides information about conditioned effects of the drug and can also detect aversive drug effects (if the subjects avoid the drug-associated context). As seen in Table 3, in most place-conditioning studies cannabinoid exposure increased rats' preference for a context that had been associated with the effects of an opioid agonist (Cadoni *et al*, 2013; DiNieri *et al*, 2011; Morel *et al*, 2009; Rubio *et al*, 1998; Rubio *et al*, 1995; Singh *et al*, 2006). The notable exceptions are Lewis rats (Cadoni *et al*, 2013), in which THC exposure did not affect opioid place conditioning, and CD1 mice (Jardinaud *et al*, 2006) or maternally-deprived rats (Morel *et al*, 2009), in which THC exposure prevented opioid-conditioned place preference. Overall, these findings suggest that the impact of prior cannabis exposure on conditioning of opioid-associated cues that lead to drug seeking should be tested in clinical research.

Cannabinoid reward. There have been several cannabinoid exposure studies using place-conditioning procedures, but only one with cannabinoid self-administration (see Table 3). Rats that were exposed to THC in adolescence and then allowed to self-administer the synthetic cannabinoid WIN 55,212-2 in adulthood acquired cannabinoid self-administration behavior more rapidly and reached higher asymptotic levels of intake under an FR1 schedule (Scherma *et al*, 2016a); these results might be at least partly because of THC exposure inducing cross-tolerance to the effects of WIN 55,212-2 such that exposed rats required more drug to reach a satiating effect. In conditioned place-preference studies, THC exposure increased conditioning to a cannabinoid-associated context in two experiments using mice (Hyatt and Fantegrossi, 2014; Valjent and Maldonado, 2000), but WIN 55,212-2 exposure did not (Rodriguez-Arias *et al*, 2016). THC exposure had no effect on conditioning to a THC-associated context in two studies using rats (Hempel *et al*, 2016; Wakeford *et al*, 2016); these studies used a procedure that combined place conditioning and taste conditioning, but place conditioning did not occur in THC-exposed rats or in vehicle-exposed controls in either experiment. In the Valjent and Maldonado (2000) study, mice were given a single exposure injection 24 h before the first conditioning session; this exposure enhanced the conditioning of preference for a place associated with a low dose of THC, and it prevented the conditioning of aversion to a place associated with a high dose of THC. THC exposure also prevented the development of conditioned aversion to a place associated with a high dose of the cannabinoid agonist JWH-018 (Hyatt and Fantegrossi, 2014). These studies show that cannabinoids can have both rewarding and aversive effects, and they suggest that the salient effect of cannabinoid exposure might be to reduce unpleasant effects of the first conditioning injection of THC. The single-dose exposure procedure with a low conditioning dose of THC has been used (without vehicle controls) in subsequent experiments to promote the conditioning of place preference with THC (Castane *et al*, 2003; Valjent *et al*, 2002).

Psychostimulant, nicotine, and ethanol reward. In comparison with the numerous studies in which cannabinoid exposure increased opioid intake or conditioned preference for a place associated with opioid effects, there have been fewer studies conducted to determine whether cannabinoid exposure can alter the rewarding effects of psychostimulants, and within these studies the finding of altered rewarding effects of psychostimulants has been less consistent (see Table 3). Given that cocaine, amphetamine, and methylenedioxymethamphetamine (MDMA) differ substantially from each other in mechanism of action, and also that none of these drugs have been studied within this paradigm as extensively as morphine or heroin, these findings are clearly preliminary. Other drugs, including nicotine and ethanol, have received very little attention.

Amphetamine and apomorphine. Cortright *et al* (2011) found that THC exposure did not enhance amphetamine self-administration, but that it did increase apomorphine self-administration under a progressive-ratio schedule; this increase was 'modest' in comparison with the increase in progressive-ratio amphetamine self-administration seen in rats exposed to amphetamine itself.

MDMA. In studies of the conditioned preference for a place associated with MDMA ('ecstasy'), exposure to the cannabinoid agonist JWH-018 did not affect conditioning in NIH Swiss mice (Hyatt and Fantegrossi, 2014), but exposure to WIN 55,212-2 did enhance conditioning in OF1 mice (Rodriguez-Arias *et al*, 2016).

Cocaine. Rodriguez-Arias *et al* (2016) found that exposure to WIN 55,212-2 enhanced conditioning of preference for a place associated with a low dose of cocaine and also made preference for a cocaine-associated place more persistent in a model of relapse, but only in mice that had been characterized as high novelty seekers in a hole-board exploration test before any drug exposure. In studies of FR1 cocaine self-administration, THC exposure did not affect cocaine intake in male rats (Panlilio *et al*, 2007), but exposure to CP 55,940 enhanced cocaine intake in female rats during the first week when they were switched to cocaine after being pretrained with food reinforcement (Higuera-Matas *et al*, 2008); the cocaine intake of the other rats in the study (nonexposed females, exposed males, and nonexposed males) caught up to the CP 55,940-exposed females after the first week (Higuera-Matas *et al*, 2008). Under a progressive-ratio schedule, cocaine self-administration was lower in THC-exposed rats, indicating that cannabinoid exposure decreased the reward value of cocaine (Panlilio *et al*, 2007); testing with models of anxiety in the same study suggested that the lower reward value might have been because of THC exposure increasing aversive side effects of cocaine.

Nicotine. There is epidemiological evidence that cannabis use is associated with increased likelihood of becoming a regular user of tobacco (Agrawal *et al*, 2008; Patton *et al*, 2005; Vaughn *et al*, 2008); because tobacco smoking is both

TABLE 3 Effects of Previous Cannabinoid Exposure on Drug Self-Administration and Place Conditioning in Rodents

Study	Subjects	Exposure drug	Exposure regimen[a]	Age during exposure	Drug-free period[b]	Test drug[c]	Age at start of testing	FR1 self-admin.	PR self-admin.	Place conditioning[d]
Norwood *et al* (2003)	Lewis rats	CP	0.1 → 0.2 mg/kg/day for 16 days	Starting at PND 55–56	8 Days	Morphine	11 Weeks	Male ↑		
González *et al* (2003)	Wistar rats	THC	Dams given 5 mg/kg/day PO	Perinatal: GD5 through PND 24	⩾7 Weeks	Morphine … Food	⩾10 Weeks		Female = [e] Male = … Female = Male =	
Gonzalez *et al* (2004)	Wistar rats	THC	5 mg/kg/day for 7 days	Starting at 8–10 weeks	0 Days	Morphine			Male =	
Solinas *et al* (2004)	S-D rats	THC	2 → 8 mg/kg twice daily for 3 days	Starting at 10-12 weeks	8 Days	Heroin	12–13 Weeks	Male ↑	Male =	
Ellgren *et al* (2007)	L-E rats	THC	1.5 mg/kg/day, intermittent (8 out of 22 days)	PND 28–49	1 week	Heroin	8 Weeks	Male ↑		
Biscaia *et al* (2008)	Wistar rats	CP	0.4 mg/kg/day for 11 days	PND 35–45		Morphine	10 Weeks	Female = [f] Male ↑	Female = Male =	
Tomasiewicz *et al* (2012)	L-E rats	THC	1.5 mg/kg/day, intermittent (8 out of 22 days)	PND 28–49	5 Weeks	Heroin	12 Weeks	Male ↑ Male with *Penk* KD =		
Stopponi *et al* (2014)	Wistar rats	THC	2.5 → 10 mg/kg twice daily for 11 days	PND 35–45	30 Days	Heroin … Yohimbine reinstatement of heroin seeking	11 Weeks	Male = … Male ↑		
Vela *et al* (1998)	Wistar rats	THC	Dams given 5 mg/kg/day PO	Perinatal: GD5 through PND 1	>11 Weeks	Morphine … Food	>11 Weeks	Female ↑ Male = … Female = Male =		
Spano *et al* (2007)	L-E rats	THC	Dams given 0.15 mg/kg/day IV	Perinatal: GD5 through PND 2	8 Weeks	Heroin	9 Weeks	Male = /↑[g]		
Morel *et al* (2009)	L-E rats	THC[h]	5 → 10 mg/kg/day, intermittent (10 out of 14 days)	PND 35–48	7–14 Days	Morphine[i]	9 Weeks	Maternally deprived male ↓[j] Nondeprived male =		Maternally deprived male ↓ Nondeprived male ↑
Singh *et al* (2006)	Wistar rats	THC	5 mg/kg/day for 10 days	PND 4–14	1 Week	Heroin	3 Weeks			Male ↑
Cadoni *et al* (2013)	Lewis rats … Fischer rats	THC	2 → 8 mg/kg twice daily for 3 days	PND 38–42	30 Days	Heroin	10 Weeks			Lewis male = … Fischer male ↑
Rubio *et al* (1995)	Wistar rats	THC	Dams given 5 mg/kg/day PO	Perinatal: GD5 through PND 24	>7 Weeks	Morphine	>10 Weeks			Female = [k] Male ↑
Rubio *et al* (1998)	Wistar rats	THC	Dams given 1, 5 or 20 mg/kg/day PO	Perinatal: GD5 through PND 24	>7 weeks	Morphine	>10 Weeks			Female ↑[l] Male ↑
DiNieri *et al* (2011)	L-E rats	THC	Dams given 0.15 mg/kg/day IV	Perinatal: GD5 through PND 2	8 Weeks	Morphine	9 Weeks			Male ↑
Jardinaud *et al* (2006)	CD1 mice	THC	10 mg/kg/day for 10 days		15 Days	Morphine				Male ↓
Scherma *et al* (2016a, 2016b)	Lister-Hooded rats	THC	2.5 → 10 mg/kg twice daily for 11 days	PND 45–55	15 Days	WIN 55212-2	10 Weeks	Male ↑		
Valjent and Maldonado (2000)	CD1 mice	THC	1 Or 5 mg/kg (single injection)		1 Day	THC				Male ↑[m]
Wakeford *et al* (2016)	S-D rats	THC	3.2 mg/kg/day, intermittent (8 out of 22 days)	PND 28–50	40 Days	THC	13 Weeks			Male = [n]
Hempel *et al* (2016)	S-D rats	THC	3.2 mg/kg/day, intermittent (6 out of 12 days)	PND 71–82	3 Days	THC	12 Weeks			Male = [o]
Hyatt and Fantegrossi (2014)	NIH Swiss mice	THC	1 → 100 mg/kg/day, intermittent (5 out of 10 days)	Starting at 8 weeks	2 Days	JWH-018 (CB agonist) … MDMA	10 Weeks			Male ↑[p] … Male =

Table 3 (Continued)

Study	Subjects	Exposure drug	Exposure regimen[a]	Age during exposure	Drug-free period[b]	Test drug[c]	Age at start of testing	FR1 self-admin.	PR self-admin.	Place conditioning[d]
Rodriguez-Arias *et al* (2016)	OF1 mice	WIN	0.1 or 0.5 mg/kg/day for 5 days	PND 27–31	3 Days	MDMA	5 Weeks			Male ↑
Cortright *et al* (2011)	S-D rats	THC	3 mg/kg/day intermittent (5 out of 13 days)	Adult	10 Days	Amphetamine … Apomorphine		Male = … Male =	Male = … Male ↑	
Panlilio *et al* (2007)	S-D rats	THC	2 → 8 mg/kg twice daily for 3 days	Starting at 10–14 weeks	8 Days	Cocaine	12–14 Weeks	Male =	Male ↓	
Higuera-Matas *et al* (2008)	Wistar rats	CP	0.4 mg/kg/day for 11 days	PND 28–38	7 Weeks	Cocaine … Food	13 Weeks	Female ↑[q] Male = … Female = Male =		
Rodriguez-Arias *et al* (2016)	OF1 mice	WIN	0.1 or 0.5 mg/kg/day for 5 days	PND 26–30	3 Days	WIN … Cocaine	PND 34			Male = … Male ↑[r]
Panlilio *et al* (2013)	S-D rats	THC	2 → 8 mg/kg twice daily for 3 days	Starting at 10–12 weeks	8 Days	Nicotine	12–13 Weeks	Male ↑	Male =/↑[s]	
Economidou *et al* (2007)	Wistar rats	THC[t]	Dams given 5 mg/kg/day PO	Perinatal: GD 15–PND 9	8 Weeks	Ethanol[u]	9 Weeks	Male =		

Abbreviations: PND, postnatal day; GD, gestational day; FR1, fixed-ratio one; PR, progressive ratio; SA, self-administration; PO, per os; CB, cannabinoid; L-E, Long-Evans; S-D, Sprague-Dawley; *Penk* KD, knockdown of proenkephalin in nucleus accumbens shell; CP, CP-55,940; WIN, WIN 55212-2; MDMA, (±)-3,4-methylenedioxymethamphetamine.
Symbols: →, dose escalated over days; …, different experiment in same study; ↑, enhanced relative to vehicle-treated controls; ↓, diminished relative to vehicle-treated controls; =, no change relative to vehicle-treated controls.
[a]Treatments were intraperitoneal and given every day unless otherwise indicated.
[b]Time between last exposure and start of behavioral testing.
[c]Test drugs were intraperitoneal for place conditioning and intravenous for self-administration, unless otherwise noted.
[d]Results involve conditioned place preference unless otherwise indicated.
[e]Females had high morphine and food intake regardless of exposure treatment.
[f]Females had high morphine intake regardless of exposure treatment.
[g]THC exposure did not affect acquisition of responding, but decreased latency to the first response of the session, increased responding for a low dose of heroin, increased heroin intake when rats were acutely food deprived, and increased responding during extinction.
[h]Dronabinol.
[i]Oral self-administration of freely available morphine solution.
[j]THC exposure prevented escalation of intake seen in vehicle-treated maternal deprivation group.
[k]Nonsignificant trend for enhancement.
[l]Smaller effect than in males.
[m]THC-exposure potentiated conditioning of place preference when the training dose of THC was low and prevented conditioning of place aversion when the training dose of THC was high.
[n]Combined taste- and place-conditioning procedure. Place preference did not occur in exposed or nonexposed rats.
[o]Combined taste- and place-conditioning procedure. Place preference did not occur in exposed or nonexposed rats.
[p]THC-exposure potentiated conditioning of place preference when the training dose of JWH-018 was low and prevented conditioning of place aversion when the training dose of JWH-018 was high.
[q]Self-administration enhanced during first 7 days (acquisition phase), but not during last 14 days (maintenance phase).
[r]WIN exposure enhance cocaine induced place preference in groups classified as high or low novelty-seeking, but place preference was only reinstated (after extinction training) by a priming injection of cocaine in the high novelty-seeking group.
[s]THC exposure did not affect responding in a within-session PR test, but enhanced responding when the FR was increased across sessions.
[t]THC was combined with either 3% ethanol or 4.2% sucrose (as a control condition) in the only source of drinking water.
[u]Oral self-administration with lever press required for access.

deadly and highly resistant to change, this effect might be 'the most important health consequence of early frequent cannabis use' (Patton *et al*, 2005). Using procedures that paralleled earlier studies of the effects of THC exposure on the self-administration of heroin and cocaine (Panlilio *et al*, 2007; Solinas *et al*, 2004a), Panlilio *et al* (2013) found that THC exposure substantially increased the percentage of rats that developed intravenous nicotine self-administration on an FR1 schedule. In the same rats, THC exposure did not affect progressive-ratio nicotine self-administration when the price was raised within sessions, but it did increase self-administration when the price of nicotine was gradually raised across sessions. Thus, unlike heroin and cocaine, nicotine became more effective and more valued as a reward when rats had been exposed to THC. These findings suggest that increased incidence of tobacco use disorder in cannabis users could be due at least in part to effects of THC exposure *per se*.

Ethanol. Economidou *et al* (2007) exposed rats to THC, ethanol, or THC combined with ethanol during prenatal and postnatal development. None of these exposure regimens affected the later self-administration of oral ethanol on an FR1 schedule. Perinatal THC exposure also did not affect the extinction of ethanol seeking or the reinstatement of ethanol seeking induced by alcohol-associated cues or footshock stress.

CLINICAL IMPLICATIONS

Cannabis use disorder and withdrawal affect many people, but there are currently no medications approved for their treatment. Many existing drugs (eg, antipsychotics, antidepressants, anxiolytics, antiepileptics) have already been tested clinically for this purpose, but with mostly negative results (Balter *et al*, 2014; Gorelick, 2016; Panlilio *et al*, 2015), and hence intensive preclinical research is being conducted to identify new targets and develop viable treatments. Reinforcing effects of cannabinoids can be studied in human volunteers using behavioral and neuroscience techniques. For example, people can be allowed to smoke cannabis under controlled conditions, and then brain imaging techniques can be used to observe the effects on activity in specific brain regions (Quickfall and Crockford, 2006). These techniques can also be used to determine whether a new drug might have therapeutic value, for example by blocking certain effects of cannabis, and they could also be used to determine how the effects of cannabis are altered by other drugs of abuse. Of course, when developing medications for the treatment of cannabis use disorder, the most important test of therapeutic efficacy is to administer the treatment to cannabis users in a controlled experiment and compare the effects with placebo (see, eg, Haney *et al*, 2015; Vandrey *et al*, 2013). Thorough evaluation of the medication's side effects should also be conducted and weighed against potential and actual harm induced by cannabis use disorder in the individual, which can be serious but are typically less severe than the harm induced by alcohol, opioid, psychostimulant, or tobacco use disorder. In this light, it should be noted that, in addition to or instead of pharmacotherapy, contingency management and other forms of psychosocial therapy can be efficacious for reducing use of cannabis and other drugs (Holtyn *et al*, 2014; Schuster *et al*, 2016).

The preclinical evidence clearly indicates that cannabinoid exposure—especially during perinatal and adolescent development—can have lasting effects on addiction-related brain processes and behavior. However, it is not known how well the effects of passive cannabinoid injections (often at high doses) on brain and behavior in animals will translate to the effects of voluntary cannabis use in humans. Therefore, additional research should be conducted to identify the effects of self-administered cannabinoids in humans and, if warranted, to develop therapeutic interventions. For obvious ethical reasons, the effects of cannabis exposure on subsequent drug use cannot be studied experimentally in humans, and hence most of the information about the effects of exposure in humans have been obtained with classical methods of epidemiology, using retrospective or longitudinal observation and self-report. However, cannabis users can be compared with matched controls using minimally invasive techniques that parallel the methods used in preclinical studies. For example, imaging techniques can be used to assess the effects of cannabis exposure on neurotransmitter binding (Hirvonen *et al*, 2012), brain morphology (Smith *et al*, 2016a), and neuronal activity (Smith *et al*, 2004). Imaging techniques and behavioral measures could also be used to determine whether noncannabinoid drugs affect brain function differently in people who have been exposed to cannabis.

FUTURE RESEARCH DIRECTIONS

The reinforcing effects of cannabinoids have not been studied as extensively as those of drugs such as opioids and cocaine, and the mechanisms are not as well understood. It is still not clear why THC is not robustly self-administered by rodents. The answer to this question might be that the relative strengths of rewarding effects and aversive effects (and the brain functions that underlie these effects) differs across species and across individuals. If so, better animal models of cannabinoid reward might be obtained by developing new animal strains that have cannabinoid systems that more closely resemble those of humans. We expect that the development of treatments for cannabis use disorder will be facilitated by recent advances in knowledge concerning cannabinoid receptors and the development of exciting new pharmacological tools such as allosteric modulators. On the other hand, uncontrolled proliferation of synthetic cannabinoid agonists represents a new and potentially devastating problem. These designer drugs come in many forms, and as clandestine manufacturers modify chemical structures, completely untested cannabinoids are released to the public.

It is clear that cannabinoid exposure can have lasting effects on the brain and behavior, but it is not yet clear which brain changes underlie the effects of cannabinoid exposure on drug self-administration and conditioned place preference. The influence of variables such as genetic strain, sex, and age during cannabinoid exposure have been varied systematically in some studies where brain changes were observed, but there is a general need for more cannabinoid-exposure experiments that directly compare the effects of these variables on drug self-administration. It would also be informative to explicitly compare the effects of exposure to cannabis, THC, and synthetic cannabinoids. Probably because of the difficulty of studying cannabinoid reward in animals, the possibility that previous exposure to noncannabinoid drugs can alter cannabinoid reward remains to be explored. Finally, it should be noted that the simultaneous use of cannabis with other drugs might enhance the rewarding effects of cannabis or the other drugs, and that this simultaneous use could potentially have more impact than prior exposure to cannabis. For example, tobacco is often used together with cannabis with the goal of enhancing the rewarding effects of cannabis (Amos *et al*, 2004), and 75% of cannabis users report that their first use of cannabis involved co-use of another drug (Olthuis *et al*, 2013). The question of whether simultaneous use of cannabis and other drugs increases the likelihood of addiction is important and should be studied systematically under controlled conditions.

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