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REVIEW ARTICLE

Effects of β-caryophyllene, A Dietary Cannabinoid, in Animal Models of Drug Addiction

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Abstract: ***Background*:** β-caryophyllene (BCP) is a natural bicyclic sesquiterpene found in *Cannabis* and other plants. BCP is currently used as a food additive, although pharmacological studies suggest its potential therapeutic application for the treatment of certain brain disorders. The mechanisms of action of BCP remain uncertain, possibly including full agonism at the cannabinoid CB_2 receptor (CB_2R).

***Objective*:** The study aims to investigate BCP's potential as a new drug for the treatment of substance use disorders by reviewing preclinical studies with animal models.

***Results*:** BCP has been investigated in behavioral paradigms, including drug self-administration, conditioned place preference, and intracranial self-stimulation; the drugs tested were cocaine, nicotine, alcohol, and methamphetamine. Remarkably, BCP prevented or reversed behavioral changes resulting from drug exposure. As expected, the mechanism of action entails CB_2R activation, although this is unlikely to constitute the only molecular target to explain such effects. Another potential target is the peroxisome proliferator-activated receptor.

***Conclusion*:** Preclinical studies have reported promising results with BCP in animal models of substance use disorders. Further research, including studies in humans, are warranted to establish its therapeutic potential and its mechanisms of action.

Keywords: Cannabinoids, *Cannabis*, drugs, addiction, abuse, β-caryophyllene.

1. DRUG ADDICTION AND THE ENDOCANNABINOID SYSTEM

Drug addiction is a major health and social issue in most contemporary societies (WHO, 2020). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), it is characterized by the loss of inhibitory control and compulsive drug intake regardless of the long-term deleterious impact [1]. Long-term drug consumption results in molecular modifications in several brain areas, such as the mesolimbic system, amygdala, hippocampus, and prefrontal cortex [2, 3], which result in behavioral responses of binge and intoxication followed by withdrawal and negative affect, preoccupation and craving. All these neurochemical and behavioral modifications often lead to relapse and the inability to quit drug use [4, 5]. Currently, few medications can efficiently treat compulsive drug intake, withdrawal symptoms, and relapse [6]. Thus, preclinical research has been focusing on new strategies for pharmacologically targeting the brain reward system and related circuits disrupted in addiction [7]. Among these new potential targets is the endocannabinoid system (eCBS). The eCBS may be involved in various aspects of drug addiction, including reward, contextual memories precipitating drug seeking, drug tolerance, and withdrawal effects [8, 9]. The eCBS comprises two main cannabinoid receptor subtypes (CB_1R and CB_2R), their endogenous ligands, termed endocannabinoids, and the proteins and enzymes responsible for endocannabinoid transport, biosynthesis, and degradation [10]. CB_1R are densely expressed throughout brain regions related to reward and drug addiction [10-13], where they modulate the dopaminergic mesolimbic circuit and drug-associated reward [14]. Accordingly, CB_1R antagonism reduces dopamine release in the mesolimbic pathway, possibly by disinhibiting GABAergic afferent neurons projecting onto the ventral tegmental area (VTA) and nucleus accumbens [15]. As such, much effort was put into the development of CB_1R modulators and their investigation of drug-related responses. In fact, CB_1R antagonists and inverse agonists, such as rimonabant, taranabant, surinabant, and AM251, show robust effects in inhibiting drug-induced behaviors [15-17]. Unfortunately, however, the enthusiasm for these drugshas been dumped by the occurrence

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of clinically significant psychiatric side effects [18]. Contrary to CB_1R, CB_2R were initially considered a peripheral receptor, absent from neurons in the central nervous system [18, 19]. As a result, initial pharmacological studies focused on CB_2R agonists primarily as analgesic drugs devoid of central side effects. Later on, evidence from histological, genetic, electrophysiological, and pharmacological approaches challenged the view of CB_2R as a "peripheral receptor". CB_2R were identified in neurons in specific brain regions, including those involved in addiction, such as the midbrain VTA neurons [20-23]. In this pathway, CB_2R expression occurs in the cell bodies of dopaminergic neurons [20, 21]. Moreover, their pharmacological activation counteracts the dopamine release in the nucleus accumbens induced by drugs of abuse [20]. CB_2R agonists prevent drug reward [17, 24] and reduce the reinforcing effects of cocaine and ethanol [25-27]. In addition, CB_2R knockout mice were more likely to develop ethanol-induced conditioned place preference (CPP), higher sensitivity to withdrawal after the ingestion of acute doses of ethanol, and higher ethanol intake in comparison to wild type control group [28]. Thus, CB_2R activation could represent a novel mechanism for drug addiction treatment. Moreover, CB_2R agonists do not induce the psychiatric side effects related to the blockade of CB_1R. In fact, they reduce anxiety-like behaviors in rodents [29]. This review aims to discuss the effects of a CB_2R agonist, the compound (1R,9S,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene, β-caryophyllene (BCP). BCP is a natural bicyclic sesquiterpene found in *Cannabis* and other plants [29, 30]. It was approved as a food additive and classified as "generally recognized as safe" by the Food and Drug Administration (FDA). As a terpenoid, the synergistic interaction between BCP and eCBS is presumed to result in important pharmacological properties and therapeutic potential [31]. BCP acts as a CB_2R agonist with an insignificant affinity towards CB_1R [31, 32]. As such, its administration seems to be devoid of abuse potential [31-33] and of psychotropic side effects related to CB_1R binding [34]. In addition, preclinical results have shown that BCP induces anxiolytic, antidepressant [29], and analgesic effects [35]. Moreover, BCP has been investigated regarding its ability to counteract the effects of drugs of abuse [32, 36]. This paper will review preclinical studies investigating the potential use of BCP for the treatment of substance use disorders and discuss the involvement of CB_2R in such responses.

2. EFFECTS OF BCP IN ANIMAL MODELS OF ADDICTION

BCP has been investigated for its effects in animal models of addiction to ethanol, cocaine, nicotine, and methamphetamine. The main characteristics of each study are summarized in Table **1**. Regarding BCP effects in animal models of ethanol addiction, Al Mansouri and co-workers [26] found that BCP dose-dependently reduced ethanol consumption and preference in C57BL/6 mice in the two-bottle choice test in addition to decreased the acquisition of ethanol-induced CPP. Remarkably, AM630, a potent and selective CB_2R antagonist, inhibited both BCP effects, suggesting the role of CB_2R activation in BCP action. Overall, the results suggested intraperitoneal BCP injections reduced ethanol addiction-related responses in a CB_2R dependent manner. However, in contrast with the results from two bottle choice and CPP tests, BCP exacerbated sensitivity to very low doses of ethanol in the loss of righting reflex test besides inducing an increase in latency for mice recovering from the impairment in motor coordination induced by ethanol. These results are corroborated by a second study investigating the effects of BCP on the ethanol-induced loss of righting reflex test in Swiss-Webster mice [37]. In this paper, a high dose of BCP (300 mg/kg) increased sensitivity to the ethanol sedative effect and the reflex recovery time. Therefore, the effects of BCP on ethanol addiction seem to be dependent on the behavioral test. While BCP induced positive outcomes in two bottle choice and CPP tests in a CB_2R dependent manner, it was not able to improve ethanol-induced loss of righting reflex. Only one study focused on BCP effects on cocaine addiction [33]. While the intraperitoneal injection of BCP inhibited cocaine self-administration and shifted the cocaine dose-response curve downwards. BCP intragastric administration displayed a less effective response against cocaine self-administration in Long-Evans rats. BCP was also able to impair the reinstatement of cocaine seeking induced by a prime injection of the drug in rats previously extinguished from cocaine self-administration besides reducing the acquisition of cocaine-induced CPP. To evaluate the involvement of CB_2R on BCP effects, knockout mice for CB_2R (CB_2-KO) were used. However, the tests with the gene-knocked mice challenged the conception that BCP effects on cocaine self-administration could be mediated by cannabinoid receptors since CB_2-KO mice did not show attenuation of BCP effects. In contrast, the peroxisome proliferator activated receptors α and γ (PPARα or PPARγ) were suggested to be involved in the BCP mechanisms to reduce cocaine self-administration. Therefore, the effect of BCP on cocaine addiction may not be modulated by only CB_2R. However, more studies are needed to confirm these results. He and co-workers 2020 used pharmacological, transgenic, and optogenetic approaches to evaluate the effects of BCP in animal models of nicotine addiction [38]. Systemic administration of BCP dose-dependently inhibited nicotine self-administration and motivation for nicotine seeking in Long-Evans rats and C57BL/6J mice. This study suggests that the involvement of CB_2R on BCP's mechanism of action is dependent on BCP dose once the genetic inhibition of CB_2R in CB_2R knockout mice blocked the effects of 25, but not 50 mg/kg of BCP. BCP also attenuated reward in the intracranial self-stimulation test in mice and in an optogenetic VTA dopaminergic neuron stimulation model. This result suggests the involvement of dopamine in BCP reduction of brain reward. Thus, BCP action of reducing nicotine addiction may involve both CB_2R and non-CB_2R dependent mechanisms. Finally, He and co-workers 2021 focused on the effects of BCP on responses related to methamphetamine [39]. The authors used long-Evans rats and C57BL/6J mice in self-administration and electrical brain-stimulation reward tests. BCP attenuated methamphetamine self-administration and methamphetamine-primed reinstatement of drug seeking. The effects of BCP on methamphetamine infusions and in the progressive-ratio break-point level for methamphetamine self-administration were impaired by AM630, suggesting CB_2R involvement. However, the higher dose of BCP also inhibited methamphetamine self-administration in CB_2-KO mice. Lastly, BCP decreased methamphetamine-enhanced brain-stimulation reward. As for cocaine, these results suggest

Table 1. Summary of the main results found in the reviewed papers.

Dose BCP	Drug of Abuse	Behavioral Test	Result	CB_2R Blockade		Specie	Refs.
				Pharmacological	Genetic		
25, 50, and 100 mg/kg	Ethanol	Two-bottle choice	↓ (50, 100)	x (50)	No	Mice	Al Mansouri *et al.* 2014 [26]
		CPP	↓ (50)	x (50)	No		
		Loss of righting reflex	↑ (50)	x (50)	No		
30, 100, 178, and 300 mg/kg	Ethanol	Loss of righting reflex	↑ (300)	No	No	Mice	Oppong-Damoah *et al.* 2019 [37]
3, 10, 25, 50, and 100 mg/kg	Nicotine	Self-administration	↓ (10, 25, 50)	x (25)	x (25) = (50)	Mice and rat	He *et al.* 2020 [38]
		Seeking during extinction	↓ (25, 50)	No	No	Mice	
		Food self-administration	↓ (50)	No	= (50)	Mice	
		Electrical brain-stimulation	↓ (50, 100)	No	No	Rat	
		Optical brain-stimulation	↓ (50, 100)	No	No	Mice	
		Open field	-	No	No	Mice and rat	
		Rotarod	↓ (100)	No	No	Mice	
25, 50, and100mg/kg	Cocaine	Self-administration	↓ (50, 100)	= (100)	No	Rat	Galaj *et al.* 2020 [33]
			↓ (100)	No	= (100)	Mice	
		Reinstatement	↓ (25, 50)	No	No	Rat	
		BCP self-administration	-	No	No	Rat	
		CPP	↓ (50, 100)	No	No	Rat	
		Food self-administration	-	No	No	Rat	
		Electrical brain-stimulation	-	No	No	Rat	
		Optical brain-stimulation	↓ (50, 100)	No	No	Mice	
		Cocaine-induced hyperlocomotion	-	No	No	Rat	
10, 25, 50, and 100 mg/kg	Methampheta-mine	Self-administration	↓ (25, 50, 100)	x (50, 100)	No	Rat	He *et al.* 2021 [39]
			↓ (25, 50, 100)	No	x (25, 50)	Mice	
		Reinstatement	↓ (25, 50)	No	No	Rat	
		Cue-induced methamphetamine seeking	↓ (50, 100)	No	No	Rat	
		Electrical brain-stimulation	↓ (50, 100)	No	No	Rat	
		Methamphetamine-enhanced dopamine in NaC	↓ (50)	No	No	Rat	
		Open-field	-	No	No	Rat	

Note: ↓: BCP reduced the behavioral measure. ↑: BCP increased the behavioral measure. -: BCP did not have an effect. x: CB_2R blockade impared BCP effect. =: same effect in WT and CB_2KO or AM630. no: not measured. Number in parenthesis: dose of BCP with significant effect.

that the dose-dependent effects of BCP on methamphetamine-related behaviors are only partially mediated by CB_2R dependent mechanisms.

3. DISCUSSION

BCP has attracted attention as a potential drug candidate for treating substance use disorders, even though its mechanism of action has remained uncertain. Compared to synthetic CB_2R agonists (such as JWH133), BCP's presence in essential oils of plants and herbs makes it an easily accessible substance [30] with demonstrated safety in humans [40]. BCP effects have been investigated in experimental animals related to addiction to nicotine [38], ethanol [26, 37], cocaine [33], and methamphetamine [39]. With doses ranging from 25 to 100 mg/kg, BCP was able to prevent and/or reverse drug effects as evaluated in different animal models, such as self-administration, conditioned place preference, loss of righting reflex, and intracranial self-stimulation. To the best of our knowledge, there is no clinical study investigating BCP effects on drug abuse. However, Rose and co-workers (1994) evaluated the role of black pepper (which contains high levels of BCP) on nicotine smokers. They found that the group with the device containing black pepper essential

oil craved significantly less nicotine cigarettes than the control group [41]. Pharmacological and genetic blockade of CB_2R suggests that BCP effects may be dependent on CB_2R [26, 38]. Accordingly, the modulation of CB_2R was already shown to affect rewarding and reinforcing effects of drugs of abuse in different animal models [25, 42-44] besides regulating common neuropsychiatric comorbidities of addiction, such as anxiety [45, 54] and impulsivity [46]. Thus, the results from the literature support the therapeutic use of CB_2R agonists for drug use disorders. In addition, studies with optogenetic stimulation of dopaminergic neurons in the VTA suggested that dopamine may be involved in BCP effects [38]. Indeed, CB_2R is expressed in dopaminergic cell bodies in this brain region [20]. Thus, it is possible that BCP effects in preventing reward and reinforcing effects of drugs of abuse are modulated by CB_2R expressed in dopaminergic neurons located in the mesolimbic pathway. CB_2R mechanisms have been proposed to underlie the effects of BCP also in other mental disorders, such as anxiety and depression [29, 47]. However, the mechanism by which BCP modulates drug reward is not a consensus yet. Despite BCP displaying high selectivity for CB_2R compared to CB_1R, it also targets other brain receptors. As reported in Galaj and co-workers 2021 and He and co-workers 2021, BCP was effective in CB_2-KO mice, especially in high doses, challenging the conception of the CB_2R agonism as the only mechanism of action by which BCP acts in the addiction. The activation of PPARα or PPARγ might also contribute to its overall effects. Accordingly, these receptors counteract the effects of several drugs of abuse, such as nicotine [48], ethanol [49], and heroin [50], possibly by modulating dopamine release on the mesolimbic pathway [51]. It is worth mentioning that the direct antagonism of dopaminergic receptors did not show promising results for addiction treatment [52]. Indirect strategies to regulate dopaminergic activities have been investigated, especially for psychostimulant addiction treatment [53]. The ability of BCP to target different receptors able to modulate dopaminergic tonus on VTA, such as CB_2R and PPARα, provides a mechanistic rationale for the development of addiction pharmacotherapy based on this compound [53].

CONCLUSION

In conclusion, there is potential for BCP to be effective in the treatment of substance use disorders. However, the data is still limited to a few preclinical studies. Further studies in both animals and humans are needed to assess the possible outcomes of addiction, evaluating long-term effects, optimal dosing, alternative routes of administration, and different drugs. Finally, the role of CB_2R should be further investigated. Although BCP acts as a full agonist at this receptor, non-CB_2R mechanisms also seem to play a role in its effects, particularly at high doses.

LIST OF ABBREVIATIONS

CPP = Conditioned Place Preference

eCBS = Endocannabinoid System

FDA = Food and Drug Administration

VTA = Ventral Tegmental Area

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Association, A.P. Diagnostic and statistical manual of mental disorders: DSM-5. Arlington, VA. **2013**. Available from: https://www.amberton.edu/media/Syllabi/Fall%202021/Graduate/CSL6798_E1.pdf

[2] Wise, R.A.; Koob, G.F. The development and maintenance of drug addiction. *Neuropsychopharmacology,* **2014**, *39*(2), 254-262. http://dx.doi.org/10.1038/npp.2013.261 PMID: 24121188

[3] Everitt, B.J.; Robbins, T.W. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat. Neurosci.,* **2005**, *8*(11), 1481-1489. http://dx.doi.org/10.1038/nn1579 PMID: 16251991

[4] Koob, G.F.; Volkow, N.D. Neurobiology of addiction: a neurocircuitry analysis. *Lancet Psychiatry,* **2016**, *3*(8), 760-773. http://dx.doi.org/10.1016/S2215-0366(16)00104-8 PMID: 27475769

[5] Koob, G.F.; Le Moal, M. Addiction and the brain antireward system. *Annu. Rev. Psychol.,* **2008**, *59*(1), 29-53. http://dx.doi.org/10.1146/annurev.psych.59.103006.093548 PMID: 18154498

[6] Liu, J.; Li, J. Drug addiction: a curable mental disorder? *Acta Pharmacol. Sin.,* **2018**, *39*(12), 1823-1829. http://dx.doi.org/10.1038/s41401-018-0180-x PMID: 30382181

[7] Volkow, N.D.; Boyle, M. Neuroscience of addiction: Relevance to prevention and treatment. *Am. J. Psychiatry,* **2018**, *175*(8), 729-740. http://dx.doi.org/10.1176/appi.ajp.2018.17101174 PMID: 29690790

[8] Li, X.; Hempel, B.J.; Yang, H.J.; Han, X.; Bi, G.H.; Gardner, E.L.; Xi, Z.X. Dissecting the role of CB_1 and CB_2 receptors in cannabinoid reward *versus* aversion using transgenic CB_1- and CB_2-knockout mice. *Eur. Neuropsychopharmacol.,* **2021**, *43*, 38-51. http://dx.doi.org/10.1016/j.euroneuro.2020.11.019 PMID: 33334652

[9] Asth, L.; Santos, A.C.; Moreira, F.A. The endocannabinoid system and drug-associated contextual memories. *Behav. Pharmacol.,* **2022**, *33*(2&3), 90-104. http://dx.doi.org/10.1097/FBP.0000000000000621 PMID: 33491992

[10] Mechoulam, R.; Hanuš, L.O.; Pertwee, R.; Howlett, A.C. Early phytocannabinoid chemistry to endocannabinoids and beyond. *Nat. Rev. Neurosci.,* **2014**, *15*(11), 757-764. http://dx.doi.org/10.1038/nrn3811 PMID: 25315390

[11] Herkenham, M.; Lynn, A.B.; Little, M.D.; Johnson, M.R.; Melvin, L.S.; de Costa, B.R.; Rice, K.C. Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. USA,* **1990**, *87*(5), 1932-1936. http://dx.doi.org/10.1073/pnas.87.5.1932 PMID: 2308954

[12] Herkenham, M.; Lynn, A.B.; Johnson, M.R.; Melvin, L.S.; de Costa, B.R.; Rice, K.C. Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J. Neurosci.,* **1991**, *11*(2), 563-583. http://dx.doi.org/10.1523/JNEUROSCI.11-02-00563.1991 PMID: 1992016

[13] Tsou, K.; Brown, S.; Sañudo-Peña, M.C.; Mackie, K.; Walker, J.M. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience,* **1998**, *83*(2), 393-411. http://dx.doi.org/10.1016/S0306-4522(97)00436-3 PMID: 9460749

[14] Le Foll, B.; Goldberg, S.R. Cannabinoid CB1 receptor antagonists as promising new medications for drug dependence. *J. Pharmacol. Exp. Ther.,* **2005**, *312*(3), 875-883. http://dx.doi.org/10.1124/jpet.104.077974 PMID: 15525797

[15] Moreira, F.A.; Jupp, B.; Belin, D.; Dalley, J.W. Endocannabinoids and striatal function. *Behav. Pharmacol.,* **2015**, *26*(1 and 2 - Special Issue), 59-72. http://dx.doi.org/10.1097/FBP.0000000000000109 PMID: 25369747

[16] Parsons, L.H.; Hurd, Y.L. Endocannabinoid signalling in reward and addiction. *Nat. Rev. Neurosci.,* **2015**, *16*(10), 579-594. http://dx.doi.org/10.1038/nrn4004 PMID: 26373473

[17] Manzanares, J.; Cabañero, D.; Puente, N.; García-Gutiérrez, M.S.; Grandes, P.; Maldonado, R. Role of the endocannabinoid system in drug addiction. *Biochem. Pharmacol.,* **2018**, *157*, 108-121. http://dx.doi.org/10.1016/j.bcp.2018.09.013 PMID: 30217570

[18] Le Foll, B.; Gorelick, D.A.; Goldberg, S.R. The future of endocannabinoid-oriented clinical research after CB1 antagonists. *Psychopharmacology (Berl.),* **2009**, *205*(1), 171-174. http://dx.doi.org/10.1007/s00213-009-1506-7 PMID: 19300982

[19] Munro, S.; Thomas, K.L.; Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature,* **1993**, *365*(6441), 61-65. http://dx.doi.org/10.1038/365061a0 PMID: 7689702

[20] Zhang, H.Y.; Gao, M.; Shen, H.; Bi, G.H.; Yang, H.J.; Liu, Q.R.; Wu, J.; Gardner, E.L.; Bonci, A.; Xi, Z.X. Expression of functional cannabinoid CB_2 receptor in VTA dopamine neurons in rats. *Addict. Biol.,* **2017**, *22*(3), 752-765. http://dx.doi.org/10.1111/adb.12367 PMID: 26833913

[21] Zhang, H.Y.; Gao, M.; Liu, Q.R.; Bi, G.H.; Li, X.; Yang, H.J.; Gardner, E.L.; Wu, J.; Xi, Z.X. Cannabinoid CB_2 receptors modulate midbrain dopamine neuronal activity and dopamine-related behavior in mice. *Proc. Natl. Acad. Sci. USA,* **2014**, *111*(46), E5007-E5015. http://dx.doi.org/10.1073/pnas.1413210111 PMID: 25368177

[22] Gong, J.P.; Onaivi, E.S.; Ishiguro, H.; Liu, Q.R.; Tagliaferro, P.A.; Brusco, A.; Uhl, G.R. Cannabinoid CB2 receptors: Immunohistochemical localization in rat brain. *Brain Res.,* **2006**, *1071*(1), 10-23. http://dx.doi.org/10.1016/j.brainres.2005.11.035 PMID: 16472786

[23] Onaivi, E.S.; Ishiguro, H.; Gong, J.P.; Patel, S.; Meozzi, P.A.; Myers, L.; Perchuk, A.; Mora, Z.; Tagliaferro, P.A.; Gardner, E.; Brusco, A.; Akinshola, B.E.; Hope, B.; Lujilde, J.; Inada, T.; Iwasaki, S.; Macharia, D.; Teasenfitz, L.; Arinami, T.; Uhl, G.R. Brain neuronal CB2 cannabinoid receptors in drug abuse and depression: from mice to human subjects. *PLoS One,* **2008**, *3*(2), e1640. http://dx.doi.org/10.1371/journal.pone.0001640 PMID: 18286196

[24] Jordan, C.J.; Xi, Z.X. Progress in brain cannabinoid CB_2 receptor research: From genes to behavior. *Neurosci. Biobehav. Rev.,* **2019**, *98*, 208-220. http://dx.doi.org/10.1016/j.neubiorev.2018.12.026 PMID: 30611802

[25] Gobira, P.H.; Oliveira, A.C.; Gomes, J.S.; da Silveira, V.T.; Asth, L.; Bastos, J.R.; Batista, E.M.; Issy, A.C.; Okine, B.N.; de Oliveira, A.C.; Ribeiro, F.M.; Del Bel, E.A.; Aguiar, D.C.; Finn, D.P.; Moreira, F.A. Opposing roles of CB_1 and CB_2 cannabinoid receptors in the stimulant and rewarding effects of cocaine. *Br. J. Pharmacol.,* **2019**, *176*(10), 1541-1551. http://dx.doi.org/10.1111/bph.14473 PMID: 30101419

[26] Al Mansouri, S.; Ojha, S.; Al Maamari, E.; Al Ameri, M.; Nurulain, S.M.; Bahi, A. The cannabinoid receptor 2 agonist, β -caryophyllene, reduced voluntary alcohol intake and attenuated ethanol-induced place preference and sensitivity in mice. *Pharmacol. Biochem. Behav.,* **2014**, *124*, 260-268. http://dx.doi.org/10.1016/j.pbb.2014.06.025 PMID: 24999220

[27] Martín-Sánchez, A.; Warnault, V.; Montagud-Romero, S.; Pastor, A.; Mondragón, N.; De La Torre, R.; Valverde, O. Alcohol-induced conditioned place preference is modulated by CB2 cannabinoid receptors and modifies levels of endocannabinoids in the mesocorticolimbic system. *Pharmacol. Biochem. Behav.,* **2019**, *183*, 22-31. http://dx.doi.org/10.1016/j.pbb.2019.06.007 PMID: 31220547

[28] Ortega-Álvaro, A.; Ternianov, A.; Aracil-Fernández, A.; Navarrete, F.; García-Gutiérrez, M.S.; Manzanares, J. Role of cannabinoid CB_2 receptor in the reinforcing actions of ethanol. *Addict. Biol.,* **2015**, *20*(1), 43-55. http://dx.doi.org/10.1111/adb.12076 PMID: 23855434

[29] Bahi, A.; Al Mansouri, S.; Al Memari, E.; Al Ameri, M.; Nurulain, S.M.; Ojha, S. β-Caryophyllene, a CB2 receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice. *Physiol. Behav.,* **2014**, *135*, 119-124. http://dx.doi.org/10.1016/j.physbeh.2014.06.003 PMID: 24930711

[30] Sharma, C.; Al Kaabi, J.M.; Nurulain, S.M.; Goyal, S.N.; Kamal, M.A.; Ojha, S. Polypharmacological properties and therapeutic potential of β-caryophyllene: A dietary phytocannabinoid of pharmaceutical promise. *Curr. Pharm. Des.,* **2016**, *22*(21), 3237-3264. http://dx.doi.org/10.2174/1381612822666160311115226 PMID: 26965491

[31] Ben-Shabat, S.; Fride, E.; Sheskin, T.; Tamiri, T.; Rhee, M.H.; Vogel, Z.; Bisogno, T.; De Petrocellis, L.; Di Marzo, V.; Mechoulam, R. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur. J. Pharmacol.,* **1998**, *353*(1), 23-31. http://dx.doi.org/10.1016/S0014-2999(98)00392-6 PMID: 9721036

[32] Gertsch, J.; Leonti, M.; Raduner, S.; Racz, I.; Chen, J.Z.; Xie, X.Q.; Altmann, K.H.; Karsak, M.; Zimmer, A. Beta-caryophyllene is a dietary cannabinoid. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(26), 9099-9104. http://dx.doi.org/10.1073/pnas.0803601105 PMID: 18574142

[33] Galaj, E.; Bi, G.H.; Moore, A.; Chen, K.; He, Y.; Gardner, E.; Xi, Z.X. Beta-caryophyllene inhibits cocaine addiction-related behavior by activation of PPARα and PPARγ: repurposing a FDA-approved food additive for cocaine use disorder. *Neuropsychopharmacology,* **2021**, *46*(4), 860-870. http://dx.doi.org/10.1038/s41386-020-00885-4 PMID: 33069159

[34] Schmitt, D.; Levy, R.; Carroll, B. Toxicological evaluation of β-caryophyllene oil. *Int. J. Toxicol.,* **2016**, *35*(5), 558-567. http://dx.doi.org/10.1177/1091581816655303 PMID: 27358239

[35] Klauke, A.L.; Racz, I.; Pradier, B.; Markert, A.; Zimmer, A.M.; Gertsch, J.; Zimmer, A. The cannabinoid CB2 receptor-selective phytocannabinoid beta-caryophyllene exerts analgesic effects in mouse models of inflammatory and neuropathic pain. *Eur. Neuropsychopharmacol.,* **2014**, *24*(4), 608-620. http://dx.doi.org/10.1016/j.euroneuro.2013.10.008 PMID: 24210682

[36] Hashiesh, H.M.; Sharma, C.; Goyal, S.N.; Sadek, B.; Jha, N.K.; Kaabi, J.A.; Ojha, S. A focused review on CB2 receptor-selective pharmacological properties and therapeutic potential of β-caryophyllene, a dietary cannabinoid. *Biomed. Pharmacother.,* **2021**, *140*, 111639. http://dx.doi.org/10.1016/j.biopha.2021.111639 PMID: 34091179

[37] Oppong-Damoah, A.; Blough, B.E.; Makriyannis, A.; Murnane, K.S. The sesquiterpene beta-caryophyllene oxide attenuates ethanol drinking and place conditioning in mice. *Heliyon,* **2019**, *5*(6), e01915. http://dx.doi.org/10.1016/j.heliyon.2019.e01915 PMID: 31245644

[38] He, Y.; Galaj, E.; Bi, G.H.; Wang, X.F.; Gardner, E.; Xi, Z.X. β-Caryophyllene, a dietary terpenoid, inhibits nicotine taking and nicotine seeking in rodents. *Br. J. Pharmacol.,* **2020**, *177*(9), 2058-2072. http://dx.doi.org/10.1111/bph.14969 PMID: 31883107

[39] He, X.H.; Galaj, E.; Bi, G.H.; He, Y.; Hempel, B.; Wang, Y.L.; Gardner, E.L.; Xi, Z.X. β-caryophyllene, an FDA-approved food additive, inhibits methamphetamine-taking and methamphetamine-seeking behaviors possibly *via* CB2 and Non-CB2 receptor mechanisms. *Front. Pharmacol.,* **2021**, *12*, 722476. http://dx.doi.org/10.3389/fphar.2021.722476 PMID: 34566647

[40] Oliveira, G.L.S.; Machado, K.C.; Machado, K.C.; da Silva, A.P.S.C.L.; Feitosa, C.M.; de Castro Almeida, F.R. Non-clinical toxicity of β -caryophyllene, a dietary cannabinoid: Absence of ad-

verse effects in female Swiss mice. *Regul. Toxicol. Pharmacol.,* **2018**, *92*, 338-346. http://dx.doi.org/10.1016/j.yrtph.2017.12.013 PMID: 29258925

[41] Rose, J.E.; Behm, F.M. Inhalation of vapor from black pepper extract reduces smoking withdrawal symptoms. *Drug Alcohol Depend.,* **1994**, *34*(3), 225-229. http://dx.doi.org/10.1016/0376-8716(94)90160-0 PMID: 8033760

[42] Xi, Z.X.; Peng, X.Q.; Li, X.; Song, R.; Zhang, H.Y.; Liu, Q.R.; Yang, H.J.; Bi, G.H.; Li, J.; Gardner, E.L. Brain cannabinoid CB2 receptors modulate cocaine's actions in mice. *Nat. Neurosci.,* **2011**, *14*(9), 1160-1166. http://dx.doi.org/10.1038/nn.2874 PMID: 21785434

[43] Lopes, J.B.; Bastos, J.R.; Costa, R.B.; Aguiar, D.C.; Moreira, F.A. The roles of cannabinoid CB1 and CB2 receptors in cocaine-induced behavioral sensitization and conditioned place preference in mice. *Psychopharmacology (Berl.),* **2020**, *237*(2), 385-394. http://dx.doi.org/10.1007/s00213-019-05370-5 PMID: 31667531

[44] Navarrete, F.; García-Gutiérrez, M.S.; Gasparyan, A.; Navarro, D.; Manzanares, J. CB2 receptor involvement in the treatment of substance use disorders. *Biomolecules,* **2021**, *11*(11), 1556. http://dx.doi.org/10.3390/biom11111556 PMID: 34827554

[45] García-Gutiérrez, M.S.; García-Bueno, B.; Zoppi, S.; Leza, J.C.; Manzanares, J. Chronic blockade of cannabinoid CB2 receptors induces anxiolytic-like actions associated with alterations in GABAA receptors. *Br. J. Pharmacol.,* **2012**, *165*(4), 951-964. http://dx.doi.org/10.1111/j.1476-5381.2011.01625.x PMID: 21838753

[46] Navarrete, F.; Pérez-Ortiz, J.M.; Manzanares, J. Cannabinoid CB2 receptor-mediated regulation of impulsive-like behaviour in DBA/2 mice. *Br. J. Pharmacol.,* **2012**, *165*(1), 260-273. http://dx.doi.org/10.1111/j.1476-5381.2011.01542.x PMID: 21671903

[47] Youssef, D.A.; El-Fayoumi, H.M.; Mahmoud, M.F. Beta-caryophyllene alleviates diet-induced neurobehavioral changes in rats: The role of CB2 and PPAR-γ receptors. *Biomed. Pharmacother.,* **2019**, *110*, 145-154. http://dx.doi.org/10.1016/j.biopha.2018.11.039 PMID: 30469079

[48] Mascia, P.; Pistis, M.; Justinova, Z.; Panlilio, L.V.; Luchicchi, A.; Lecca, S.; Scherma, M.; Fratta, W.; Fadda, P.; Barnes, C.; Redhi, G.H.; Yasar, S.; Le Foll, B.; Tanda, G.; Piomelli, D.; Goldberg, S.R. Blockade of nicotine reward and reinstatement by activation of alpha-type peroxisome proliferator-activated receptors. *Biol. Psychiatry,* **2011**, *69*(7), 633-641. http://dx.doi.org/10.1016/j.biopsych.2010.07.009 PMID: 20801430

[49] Haile, C.N.; Kosten, T.A. The peroxisome proliferator-activated receptor alpha agonist fenofibrate attenuates alcohol self-administration in rats. *Neuropharmacology,* **2017**, *116*, 364-370. http://dx.doi.org/10.1016/j.neuropharm.2017.01.007 PMID: 28088358

[50] de Guglielmo, G.; Melis, M.; De Luca, M.A.; Kallupi, M.; Li, H.W.; Niswender, K.; Giordano, A.; Senzacqua, M.; Somaini, L.; Cippitelli, A.; Gaitanaris, G.; Demopulos, G.; Damadzic, R.; Tapocik, J.; Heilig, M.; Ciccocioppo, R. PPARγ activation attenuates opioid consumption and modulates mesolimbic dopamine transmission. *Neuropsychopharmacology,* **2015**, *40*(4), 927-937. http://dx.doi.org/10.1038/npp.2014.268 PMID: 25311134

[51] Melis, M.; Carta, G.; Pistis, M.; Banni, S. Physiological role of peroxisome proliferator-activated receptors type α on dopamine systems. *CNS Neurol. Disord. Drug Targets,* **2013**, *12*(1), 70-77. http://dx.doi.org/10.2174/18715273113120100012 PMID: 23394525

[52] Nutt, D.J.; Lingford-Hughes, A.; Erritzoe, D.; Stokes, P.R.A. The dopamine theory of addiction: 40 years of highs and lows. *Nat. Rev. Neurosci.,* **2015**, *16*(5), 305-312. http://dx.doi.org/10.1038/nrn3939 PMID: 25873042

[53] Solinas, M.; Belujon, P.; Fernagut, P.O.; Jaber, M.; Thiriet, N. Dopamine and addiction: what have we learned from 40 years of research. *J. Neural. Transm. (Vienna),* **2019**, *126*(4), 481-516. http://dx.doi.org/10.1007/s00702-018-1957-2 PMID: 30569209