School of Behavioral Neuroscience and Behavioral Disorders
IBRO - September 16 to 25th, 2024

Investigation of innovative strategies for the treatment of the use of psychostimulant drugs: smoked form of cocaine

September 23rd, 2024

Cecilia Scorza, PhD
Departamento de Neurofarmacología Experimental
Instituto de Investigaciones Biológicas Clemente Estable
Montevideo-Uruguay

Ministerio de Educación y Cultura

iibce
INSTITUTO DE INVESTIGACIONES BIOLÓGICAS CLEMENTE ESTABLE

Overview

- Cannabinoids background
- Evidence of CBD and SUD
- Smoked cocaine: chemical composition (adulterants)
- Main results: CBD and locomotor sensitization
- Neuroinflammation hypothesis related to SUD
- Conclusions

Substance use disorder*

- The global number of illicit drug users (over 296 million people) has increased by 23% whereas people living with SUD **have escalated compared to the last decade**.**

- **Cocaine** is one of the most globally abused drugs**.

- **SUD (including CUD), is a chronic, relapsing brain disorder** characterized by **(i)** a compulsion to **seek and take drugs**, **(ii) loss of control** over drug intake (craving), and **(iii)** emergence of a negative emotional state (e.g., dysphoria, anxiety, and irritability) that defines a motivational **withdrawal syndrome** when access to the drug is prevented (relapse).

- **Critical questions**: What determines whether a person undergoes **the transition** from drug use to drug abuse and SUD?; it is still unclear why not all persons exposed to drugs, develop SUD.

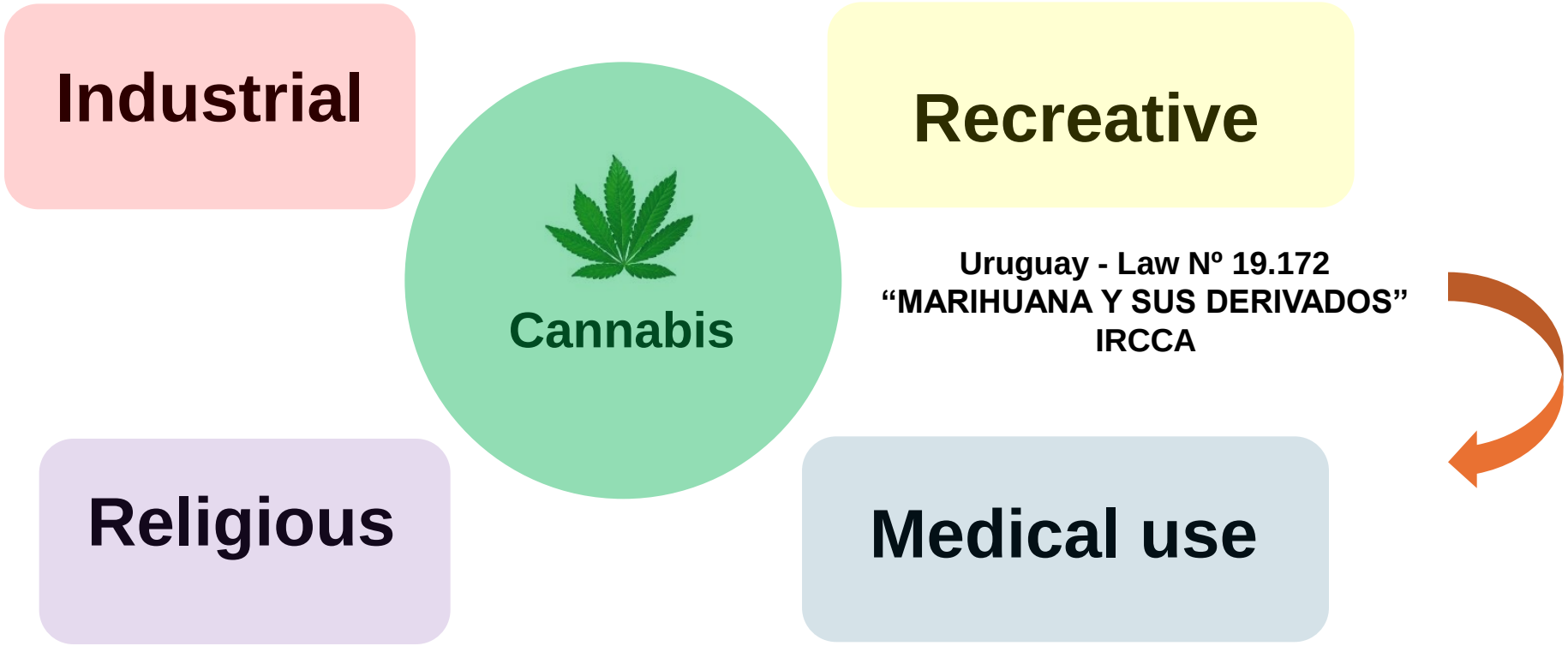
- There are no effective treatments for CUD; so, it is required to develop innovative therapeutic strategies.

- Further research is needed.

** Diagnostic and statistical maunal of mental disorders (DSM-V)*

***UNODC World Drug Report, 2024 https://www.unodc.org/*
Everitt B, Eur. J of Neurosci. 2014; Berlin et al. Genes Brain and Behavior, 2016; Olsen, Med Clin North Am. 2022.

Different uses of Cannabis

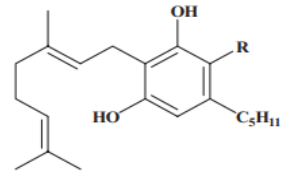


Clinical and preclínical research interest

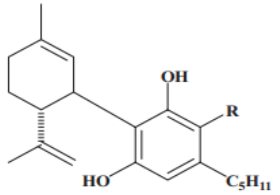
IRCCA: Instituto de Regulación y Control de Cannabis

The main cannabinoids in Cannabis sativa

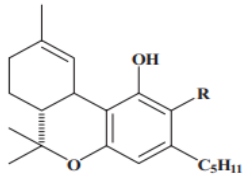
CBG: precursor



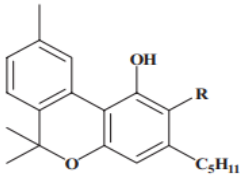
R = H, cannabigerol (CBG)
R = COOH, cannabigerolic acid (CBGA)



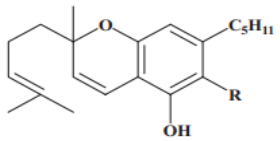
R = H, cannabidiol (CBD)
R = COOH, cannabidiolic acid (CBDA)



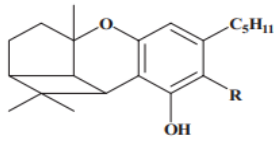
R = H, Δ^9-tetrahydrocannabinol (Δ^9-THC)
R = COOH, Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA)



R = H, cannabinol (CBN)
R = COOH, cannabinolic acid (CBNA)



R = H, cannabichromene (CBC)
R = COOH, cannabichromenic acid (CBCA)



R = H, cannabicyclol (CBL)
R = COOH cannabicyclolic acid (CBLA)

Fig. 3. Structure of cannabidiol (CBD) and delta–9–tetrahydrocannabinol (Δ^9–THC).

- THC is produced as an acid (Δ^9-Tetrahydrocannabinolic acid, Δ^9-THCA) in the **glandular trichomes** of the leaves and undergoes decarboxylation with age or heating to form Δ^9-THC.
- **THC is the most abundant**, and responsible for the cannabis effect (marijuana; CB1-Rs).
- Other constituents that might contribute in some way to the effects of cannabis include (terpens, flavonoids, and alkaloids)

- Medicinal use of cannabis/cannabinoids: **CANNABIDIOL (CBD),** a non-psychotomimetic compound of cannabis.

Fraguas-Sánchez and Torres-Suárez, Drugs 2018;
Majdi et al. Medical Hypotheses, 2019;
Echeverry et al. Adv Exp Med Biol. 2021.

Some therapeutic applications of cannabinoids or therapeutic potential

High quality evidence

- Pain (neuropathic pain): Δ^9-THC
- Multiple sclerosis (spasticity): Δ^9-THC
- Epilepsy: CBD
- Cancer (palliative treatments): Δ^9-THC
- Weight loss (AIDS): Δ^9-THC

CANNABINOIDS

Moderate-low quality evidence

- Parkinson´s disease
- Alzheimer´s disease
- Huntington's disease
- Addictions
- Glaucoma
- Post-traumatic stress syndrome
- Tourette syndrome: Δ^9-THC
- Anxiety: CBD
- Cancer: Δ^9-THC, CBD
- Antidepressant-like effects
- Antipsychotic-like effects

Fraguas-Sánchez and Torres-Suárez, Drugs 2018

Some therapeutic applications of cannabinoids or therapeutic potential

High quality evidence

- Pain (neuropathic pain): Δ^9-THC
- Multiple sclerosis (spasticity): Δ^9-THC
- Epilepsy: CBD
- Cancer (palliative treatments): Δ^9-THC
- Weight loss (AIDS): Δ^9-THC

CANNABINOIDS

Moderate-low quality evidence

- Parkinson´s disease
- Alzheimer´s disease
- Huntington's disease
- Addictions
- Glaucoma
- Post-traumatic stress syndrome
- Tourette syndrome: Δ^9-THC
- Anxiety: CBD
- Cancer: Δ^9-THC, CBD

* CBD as co-adjuvant treatment in ethanol and opioid abuse.

Fraguas-Sánchez and Torres-Suárez, Drugs 2018

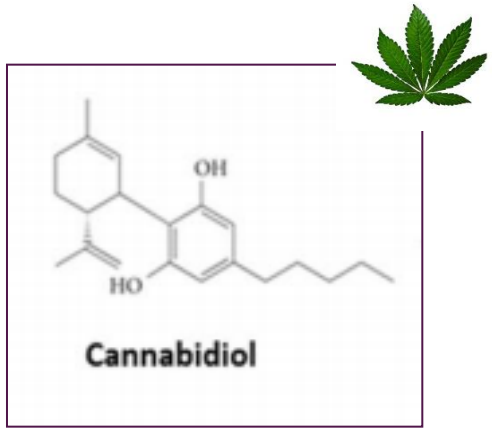
First preclinical reports were related to morphine and heroin, suggesting that CBD attenuates the rewarding effects of opioids (CPP) and can serve as a preventive therapeutic.

Galaj and Xi, Int J of Molecular Sciences, 2020

Advantages of CBD

Clinical and preclinical studies indicate that CBD can be useful for cocaine-use disorder.

- Non-psychotomimetic compound of cannabis.
- **No rewarding properties** (i.e., limited abuse liability)
- **High** degree of **safety**.
- Therapeutic actions as **anxiolytic, antipsychotic, antidepressant, anti-inflammatory, anti-convulsive effect**; some of them are domains affected by cocaine abuse (i.e., co-morbid disorders)



Fraguas-Sánchez and Torres-Suárez, Drugs 2018;
Majdi et al. Medical Hypotheses, 2019.

Some reviews of CBD and SUD...

frontiers in Psychiatry

MINI REVIEW
published: 19 February 2019
doi: 10.3389/fpsyt.2019.00063

The Endocannabinoid System and Cannabidiol's Promise for the Treatment of Substance Use Disorder

Yann Chye [1], Erynn Christensen [1], Nadia Solowij [2,3] and Murat Yücel [1]*

[1] Brain and Mental Health Research Hub, Monash Institute of Cognitive and Clinical Neurosciences, School of Psychological Sciences, Monash University, Melbourne, VIC, Australia, [2] School of Psychology and Illawarra Health and Medical Research f Wollongong, Wollongong, NSW, Australia, [3] The Australian Centre for Cannabinoid Clinical and , New Lambton Heights, NSW, Australia

molecules

Molecules 2019, 24, 2583

Review

Cannabidiol Treatment Might Promote Resilience to Cocaine and Methamphetamine Use Disorders: A Review of Possible Mechanisms

Claudia Calpe-López [1], M. Pilar García-Pardo [2] and Maria A. Aguilar [1,*]

[1] Unit of Research "Neurobehavioural mechanisms and endophenotypes of addictive behavior", Department of Psychobiology, University of Valencia, Avda. Blasco Ibañez 21, 46010 Valencia, Spain
[2] Department of Psychology and Sociology, University of Zaragoza, 44003 Teruel, Spain
* Correspondence: asuncion.aguilar@uv.es; Tel.: +34-963864611

Neuropharmacology 207 (2022) 108948

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

ELSEVIER

Cannabidiol and substance use disorder: Dream or reality

Saeideh Karimi-Haghighi [a,b], Yasaman Razavi [b], Daniela Iezzi [c,d], Andrew F. Scheyer [c,d], Olivier Manzoni [c,d], Abbas Haghparast [b,*]

[a] *Clinical Neurology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
[b] *Neuroscience Research Center, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[c] *INMED, INSERM U1249, Marseille, France*
[d] *Aix-Marseille University, Marseille, France*

frontiers in Pharmacology

REVIEW
published: 20 May 2021
doi: 10.3389/fphar.2021.626010

Role of Cannabidiol in the Therapeutic Intervention for Substance Use Disorders

https://www.frontiersin.org/articles/10.3389/fphar.2021.626010/full

Francisco Navarrete[1,2], *María Salud García-Gutiérrez*[1,2], *Ani Gasparyan*[1,2], *Amaya Austrich-Olivares*[1] *and Jorge Manzanares*[1,2]*

[1]*Instituto de Neurociencias, Universidad Miguel Hernández-CSIC, San Juan de Alicante, Spain,* [2]*Red Temática de Investigación Cooperativa en Salud (RETICS), Red de Trastornos Adictivos, Instituto de Salud Carlos III, MICINN and FEDER, Madrid, Spain*

Despite the range of the psychosocial and pharmacological therapeutic approaches for substance use treatment, relapse prevalence into drug consumption is estimated between 40 and 75% (Sinha, 2011; Pasareanu et al., 2016; Andersson et al., 2019). This high rate of recurrence is largely due to the ineffectiveness of the available drugs or the lack of specific treatments (e.g., cannabis, cocaine, or amphetamine-type use disorders). Thus, there is a growing need to significantly improve our knowledge about the underlying mechanisms involved in the development of drug dependence to finally design new pharmacological tools with higher efficacy and safety. In this sense, the manipulation of the endocannabinoid system (ECS) by administering cannabinoid compounds has raised much interest due to its close functional involvement in the regulation of emotion, cognition, and reward…

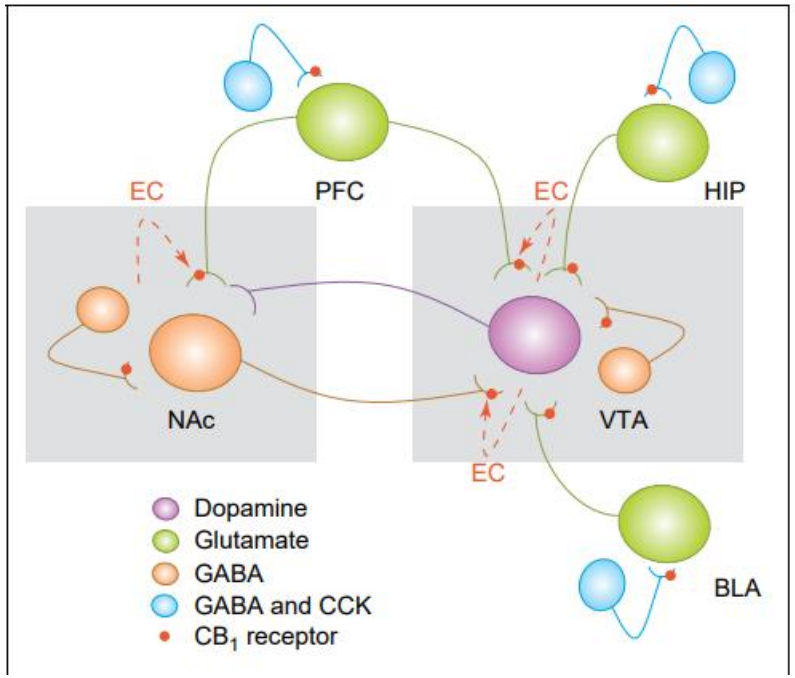
Drugs of abuse and the endocannabinoid system

Review TRENDS in Neurosciences Vol.29 No.4 April 2006

Involvement of the endocannabinoid system in drug addiction

Rafael Maldonado, Olga Valverde and Fernando Berrendero

Laboratori de Neurofarmacologia, Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, Carrer Dr. Aiguader 80, 08003 Barcelona, Spain

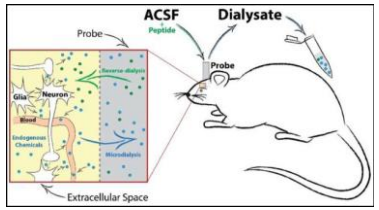


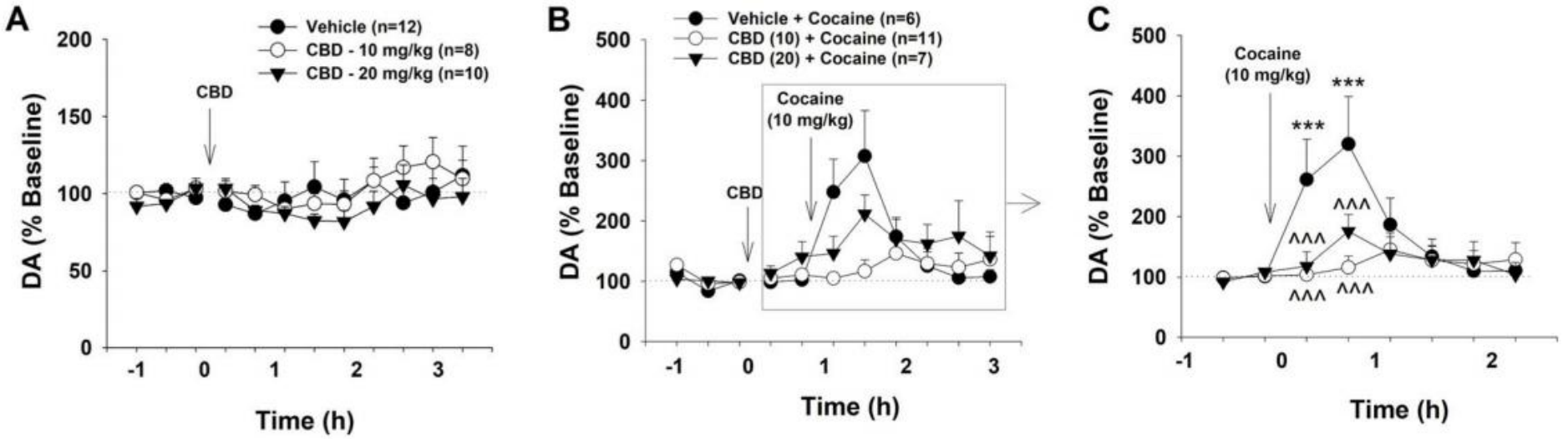
Motivational circuit: PFC-VTA-NAc-Hc-BLA

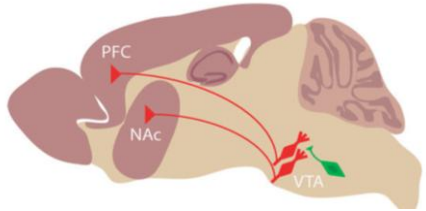
Drugs of abuse interact with these common brain circuits producing adaptive changes leading to a profound dysregulation of brain motivational and reward pathways and SEnC modulates it.

Preclinical data of CBD and psychostimulants (I)

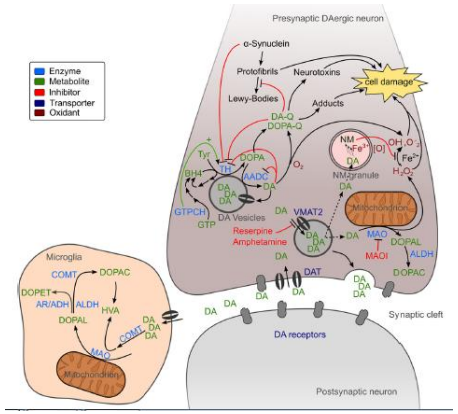
CBD attenuates cocaine-induced dopamine in the NAc







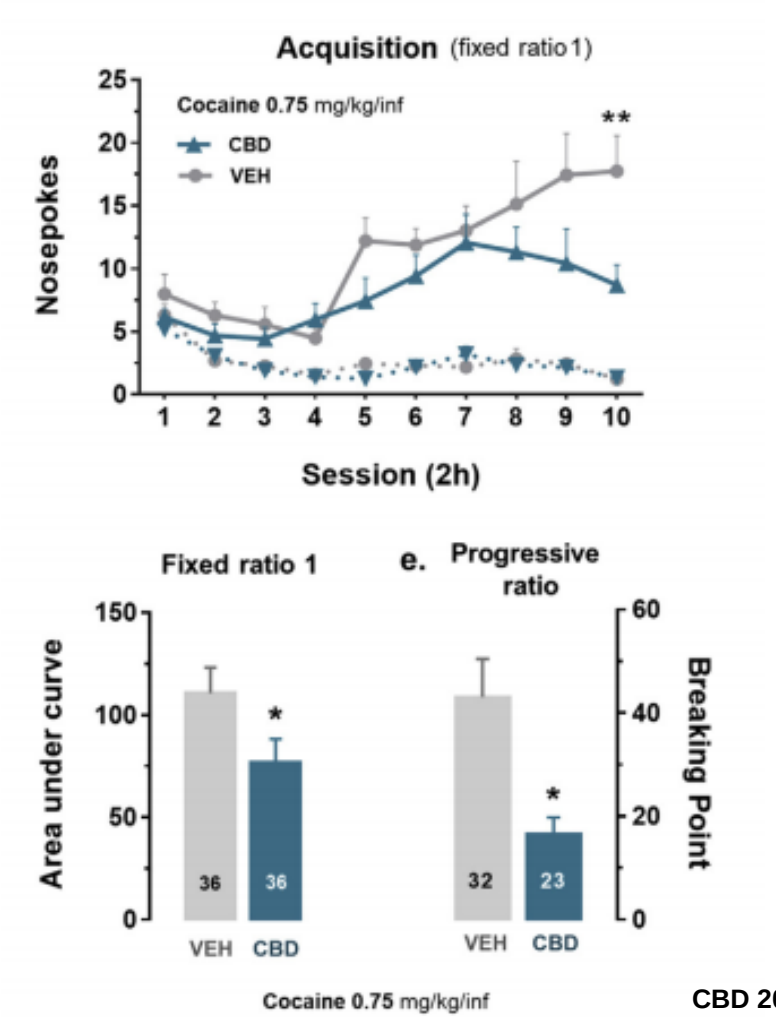
Mesolimbic and mesocortial systems and rewarding effect of drug of abuse



Luscher and Ungless, PLoS Med, 2006

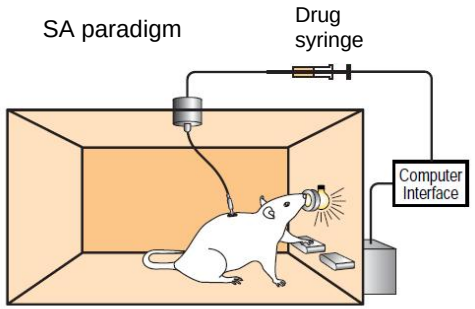
Galaj et al. Neuropharmacology, 2020

Preclinical data of CBD and psychostimulants (II)



CBD 20 mg/kg/10 days

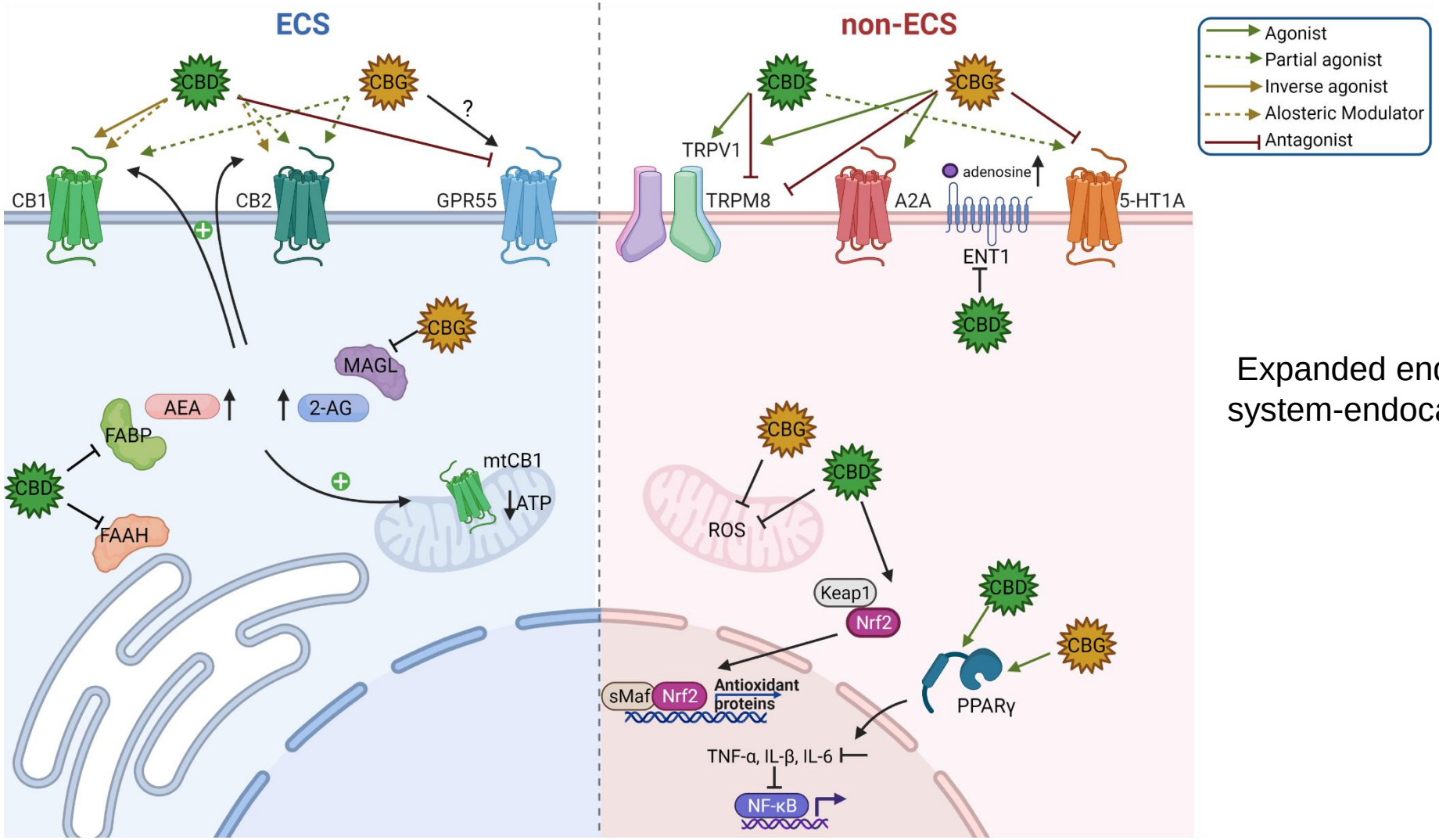
CBD reduces the cocaine reinforcing in self-administration paradigm



Parameter: number of lever press or nose pokes to obtain the drug

Luján et al. Neuropharmacology, 2018

Sites of action of CBD in the brain



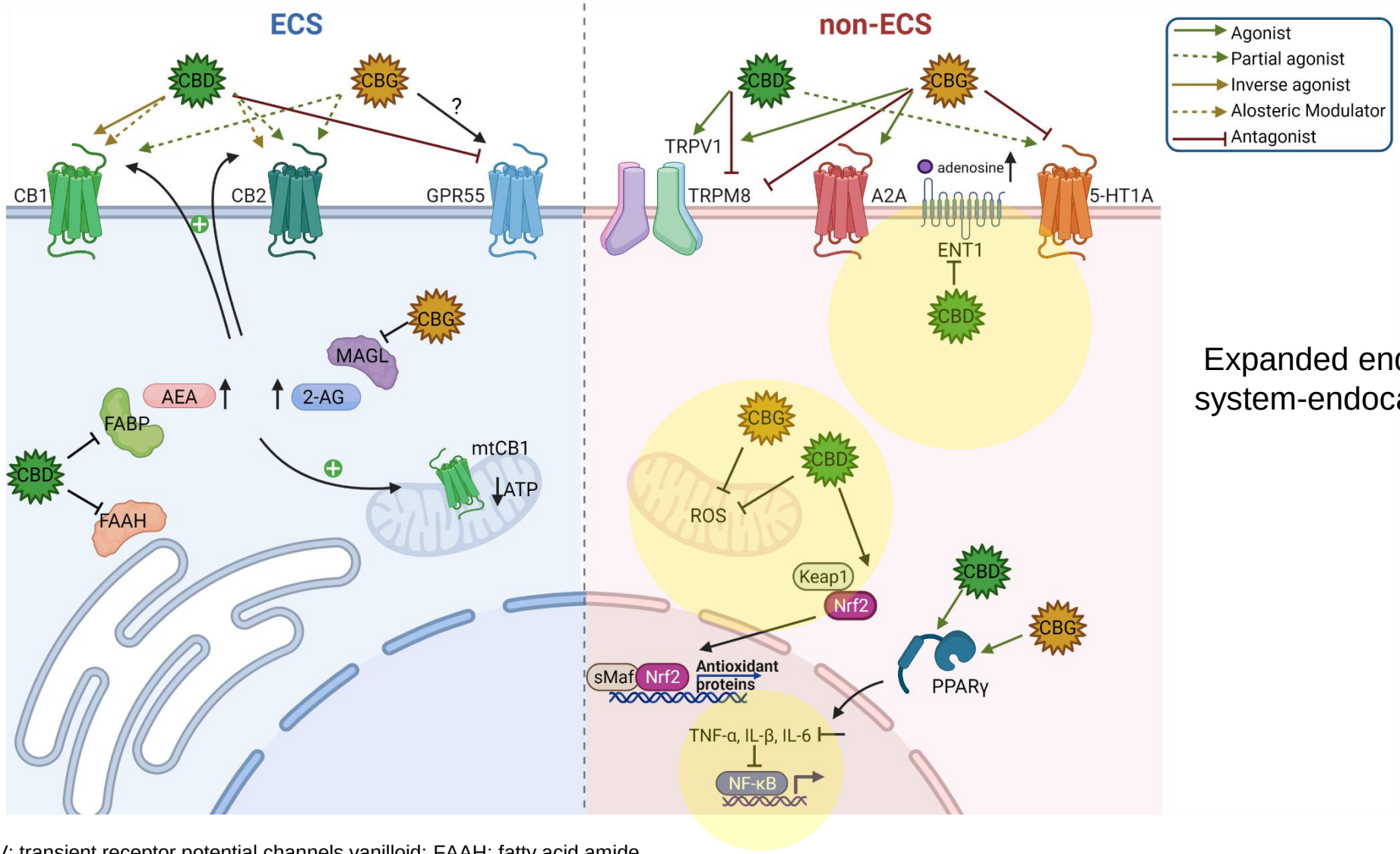
Expanded endocannabinoid system-endocannabinoidome.

Veilleux, Di Marzo, et al. Curr Diab Rep. 2019

TRPV: transient receptor potential channels vanilloid; FAAH: fatty acid amide hydrolase, FAAH, MAGL: monoacylglycerol lipase); AEA: anandamide; PPARs: peroxisome proliferator-activated receptors.

Echeverry et al., Medicinal Usage of Cannabis and Cannabinoids, Burlington: Academic Press, pp. 197-205, 2023

Sites of action of CBD in the brain



Expanded endocannabinoid system-endocannabinoidome.

Veilleux, Di Marzo, et al. Curr Diab Rep. 2019

TRPV: transient receptor potential channels vanilloid; FAAH: fatty acid amide hydrolase, FAAH, MAGL: monoacylglycerol lipase); AEA: anandamide; PPARs: peroxisome proliferator-activated receptors.

Echeverry et al., Medicinal Usage of Cannabis and Cannabinoids, Burlington: Academic Press, pp. 197-205, 2023

Our studies

Cocaine forms

- Different forms of cocaine: * hydrochloride salt (water-soluble), snorted or i.v. injection
  * cocaine base or freebase (water-insoluble), smoked

- CP is the earliest step in the purification process of cocaine hydrochloride. Cocaine, as a main alkaloid, is in its base form, **smokable**. Impurities and **adulterants**.

- Crack is prepared through heat evaporation of powdered cocaine with a base (sodium bicarbonate) to produce an alkaline cocaine product, which is typically inhaled (**smoked**).

CP and crack smoked forms of cocaine

Castagno Adicciones, 2000; Hatsukami and Fischman JAMA,1996; Lopez-Hill et al. Behav Brain Res. 2011.

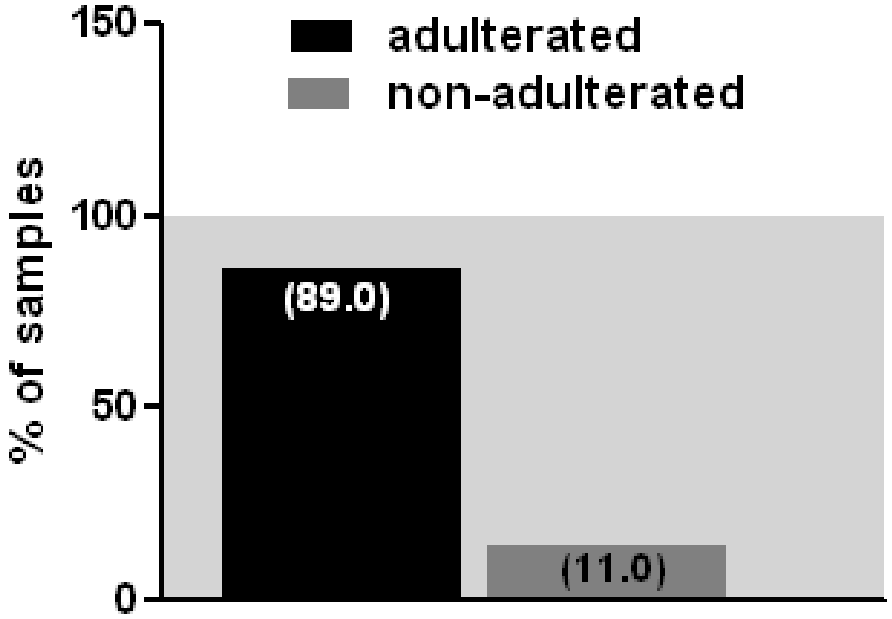
Smokable form of cocaine: crack or cocaine paste

- Coca-paste (CP) is mainly consumed in Latin American countries.
- There is a clinical consensus about smoked cocaine: induces a prototypical profile characterized by **fast and high dependence** and high-risk perception.
- The **route of administration** could explain that profile, but we proposed **chemical composition** as another relevant factor.
- No effective treatment is available for CP dependence.

- Official permits were obtained from Technical Forensic Institute (Montevideo-Uruguay) and Uruguayan Drugs Board (Junta Nacional de Drogas)
- **These are samples that could be potentially smoked by drug users.**

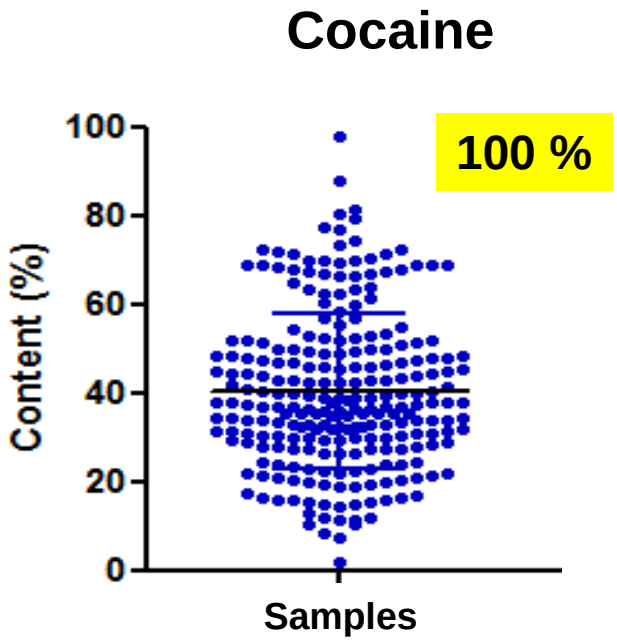
Chemical composition

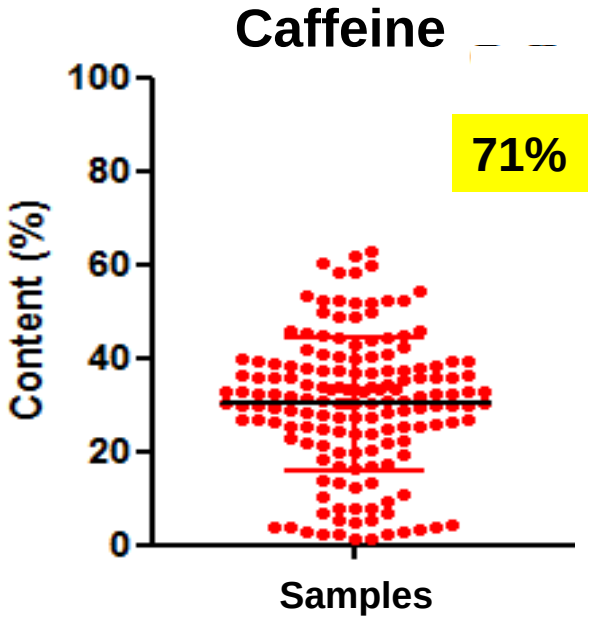
Frequency of cocaine ana caffeine in N = 306 seized-samples



HPLC-DAD analyses revealed that 89 % of CP samples were adulterated while 11% were not.

Caffeine and other adulterants were present.

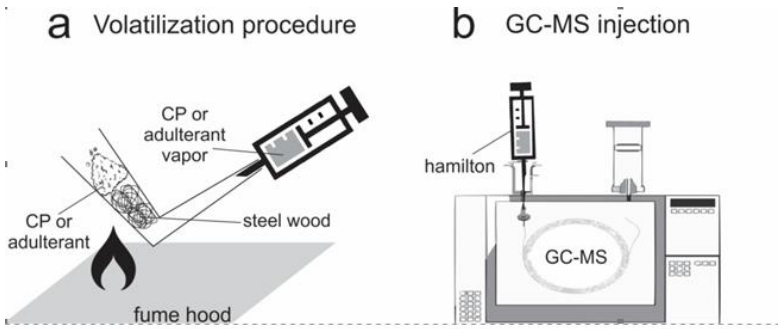




Abin Carriquiry et al. Neurotox. Res. 2018

Chemical composition

Caffeine:
- Active adulterant.
- Facilitates the volatilization of smoked drugs.
- Can be volatilized.



GC-MS analyses

After CP volatilization, adulterants are preserved in the fume

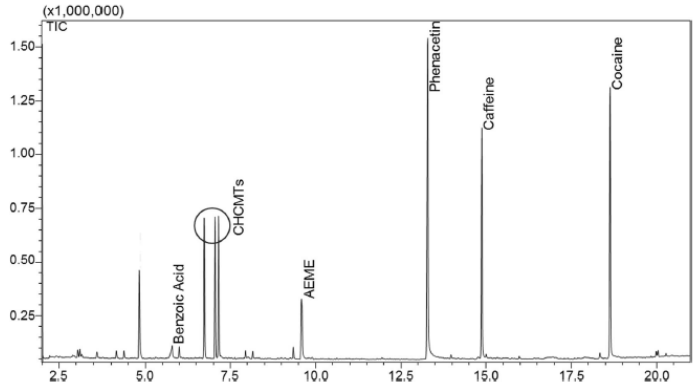


Fig. 4 Representative GC-MS chromatograms (TIC) of a CP volatilized seized sample adulterated with caffeine and phenacetin. To simplify, no mass spectrums were shown in this figure. Carbomethoxycycloheptatrienes (CMCHTs); anhydroecgonine methyl ester (AEME)

Non-pyrolysis compounds from caffeine volatilization were detected.

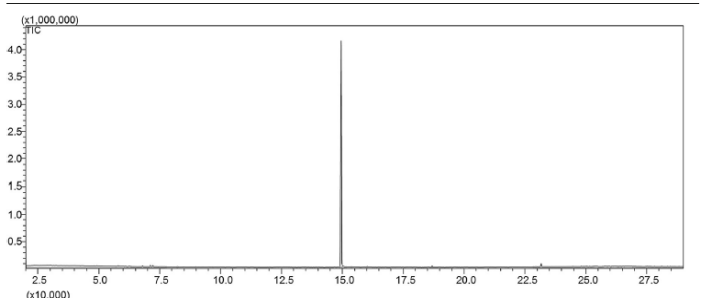


Fig. 6 Representative GC-MS chromatogram of caffeine isolated and volatilized. TIC chromatogram (upper), mass spectrum of the caffeine vapor sample (middle), and the reference mass spectrum of caffeine (lower)

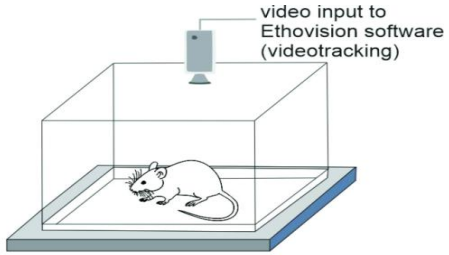
Galvalisi et al. Neurotox. Res. 2017; Abin Carriquiry et al. Neurotox. Res. 2018

Caffeine as an active adulterant in CP-seized samples

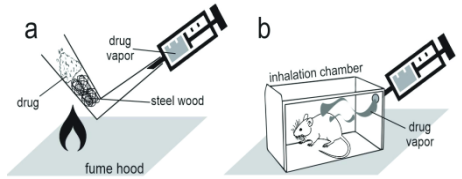
- Caffeine enhances the **acute stimulant effect** of cocaine in CP-seized samples after i.p. injection / pulmonary inhalation route of administration.

CP and subrogate CP

López-Hill et al. Behav Brain Res. 2011;
Prieto et al. Rev Psiquiat Uru. 2012;
Galvalisi et al. IBRO Congress, 2015;
Scorza et al. Neuromethods, 2023.



Open Field (OF)

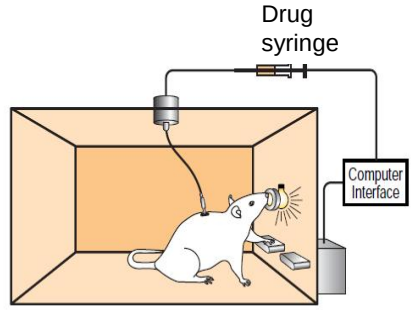


Passive pulmonary inhalation

- Caffeine enhances the **reinforcing** effect of cocaine and its **motivational** value. (significant increase in the breaking point) after i.v. injection.

Subrogate CP

Prieto et al. Psychopharmacology, 2016.

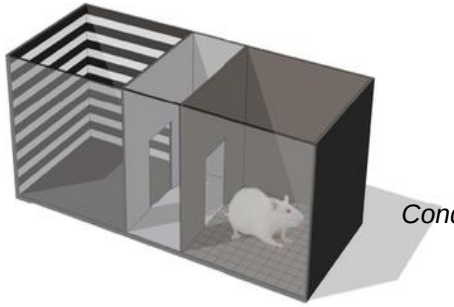


Self-administration paradigm (SA)

- Caffeine potenties **reward-associated memories** elicited by i.p. cocaine; associated with **changes in IEGs** expression in NAcc and mPFC.

Subrogate CP

Muñiz et al. Frontiers in Behav Neurosci. 2017.

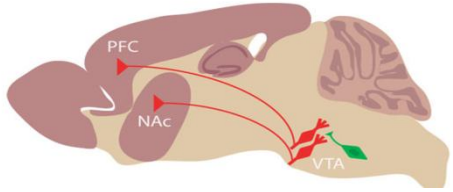


Conditioned Place Preference (CPP)

- Caffeine collaborates in the **acute rewarding effect** of CP samples determined by the DA release in NAc Shell.

CP and subrogate CP

Scorza et al. Neuromethods, 2023.



Mesolimbic system; microdialysis

A specific study…

Contents lists available at ScienceDirect

International Journal of Drug Policy

journal homepage: www.elsevier.com/locate/drugpo

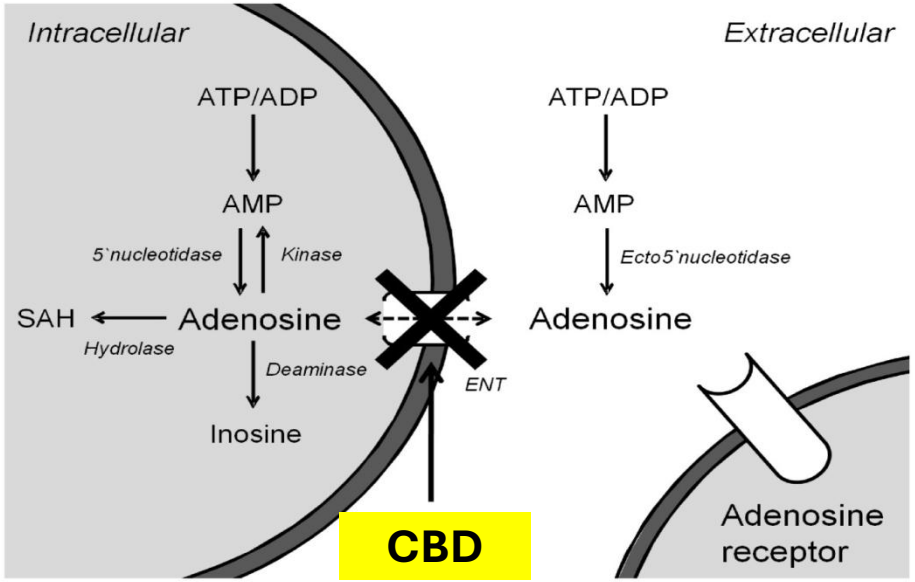
Addressing the stimulant treatment gap: A call to investigate the therapeutic benefits potential of cannabinoids for crack-cocaine use

Benedikt Fischer [a,b,c,1,*], Sharan Kuganesan [a], Andrea Gallassi [d], Renato Malcher-Lopes [e], Wim van den Brink [f,g], Evan Wood [h,i]

2015 - from Canada, The Netherlands, Brazil

Abstract: Crack-cocaine use is prevalent in numerous countries, yet concentrated primarily – largely within urban contexts – in the Northern and Southern regions of the Americas. It is associated with a variety of behavioral, physical and mental health and social problems which gravely affect users and their environments. Few evidence-based treatments for crack-cocaine use exist and are available to users in the reality of street drug use… **An important therapeutic potential for crack-cocaine use may rest in cannabinoids**, which have recently seen a general resurgence for varied possible therapeutic usages for different neurological diseases. Distinct potential therapeutic benefits for crack-cocaine use and common related adverse symptoms may come specifically from cannabidiol (CBD)-one of the numerous cannabinoid components found in cannabis – with **its demonstrated anxiolytic, antipsychotic, anti-convulsant effects and potential benefits for sleep and appetite problems**. The possible therapeutic prospects of cannabinoids are **corroborated by observational studies from different contexts documenting crack-cocaine users' 'self-medication' efforts towards coping with crack-cocaine-related problems, including withdrawal and craving, impulsivity and paranoia.** Cannabinoid therapeutics offer further benefits of being available in multiple formulations…

CBD increases adenosine levels by the blockage of ENT-1



Carrier et al. PNAS, 2006

- Caffeine is a psychostimulant that promotes wakefulness by non-selectively antagonizing the adenosine A1 receptors (A1R) and A2 receptors (A2AR) in the nucleus accumbens (NAcc).
- A1 and A2A-Rs are expressed in brain motor and reward circuits.
- Caffeine potentiates the effect of cocaine.

Carrier et al. PNAS, 2006

- **Our hypothesis:** a competitive action between the increased levels of adenosine (evoked by CBD) and caffeine for the adenosine receptors binding site may result in an attenuation of caffeine action by CBD.

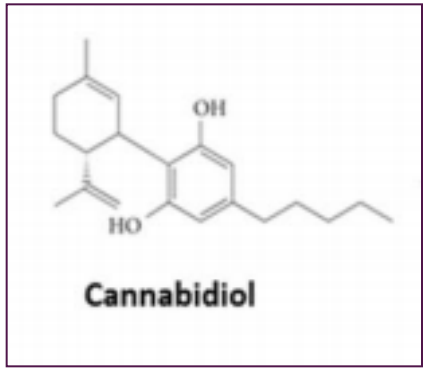
Can CBD attenuate the effects induced by cocaine + caffeine?

Behavioral sensitization induced by psychostimulants

- Repeated non-contingent exposure to psychostimulant drugs produces **locomotor sensitization**, a progressive and enduring augmentation of locomotor responses to the drug after a withdrawal period and the re-exposure to the drug.
- This phenomenon implies **neuroplastic changes** in the brain (meso-cortico-limbic circuit).
- LS can be useful to better understand the **initial phases of drug intake that influence**, but does not provide a complete picture of SUD.
- LS phenomenon has been proposed to be useful to **drug-induced neuroadaptations related to drug craving** in rodents.

Robinson and Berridge, Brain Res Brain Res Rev.1993;
Pierce and Kalivas Brain Res Rev. 1997;
Kuhn, Kalivas, et al. Front Behav Neurosci. 2019.

• Can **Cannabidiol** attenuate some effects induced by the combined cocaine plus caffeine?



Previous result:

Locomotor sensitization

CP and Coc+Caff

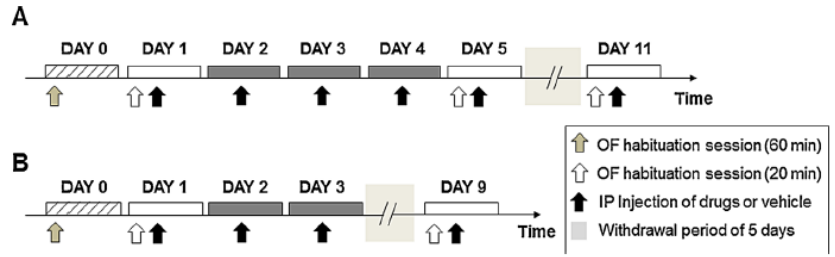


FIGURE 1. Experimental protocols for CP1- cocaine- and CP1 surrogate-induced sensitization. Rats were treated with the different drugs and corresponding vehicles during 5 (A; protocol I) or 3 days (B; protocol II) and the locomotor activity was recorded each day. Five days later, animals were challenged at days 11 or 9 (protocol I and II, respectively) with the corresponding treatments.

Sample	Cocaine	Caffeine
CP1	68.9	15.0

High content of cocaine and caffeine

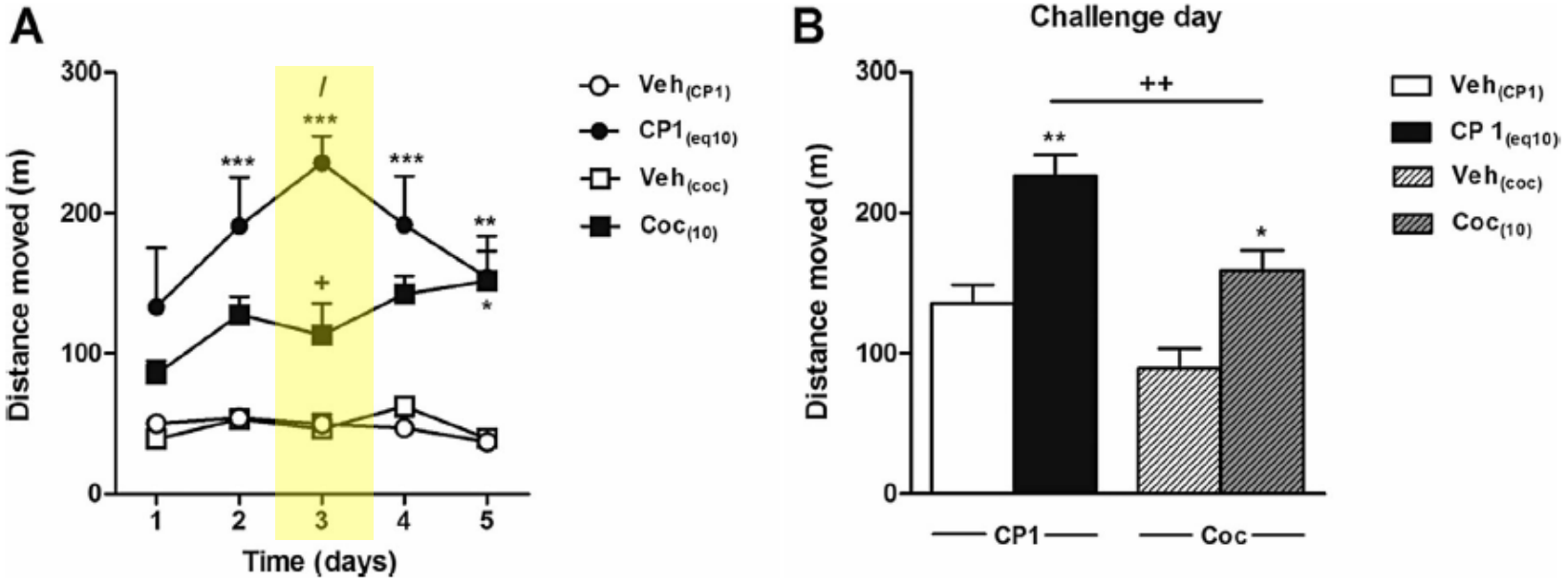


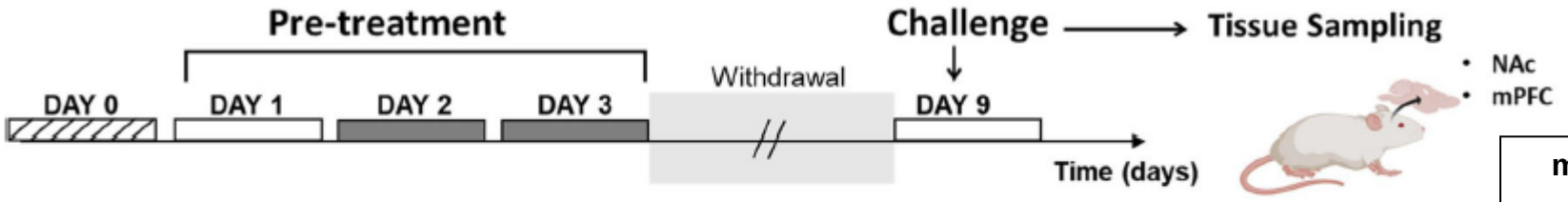
FIGURE 2. Initiation (A) and expression (B) of sensitization induced by CP1 and cocaine in pretreated rats with CP1 and cocaine or vehicle (protocol I). Doses expressed in mg/kg and the respective vehicles are shown in parentheses [CP1(eq10); Coc(10); Veh(CP1); Veh(coc)]. Data are expressed as mean ±–SEM. Two-way and one-way ANOVA followed by Newman-Keuls test. * = denotes statistical differences vs. each control group; + = denotes statistical differences between CP1(eq10) and Coc(10); / = denote statistical differences versus day 1. *** $p < 0.001$; **,++ $p < 0.01$; *, +, / $p < 0.05$. $N = 4–6$.

CP1 was injected at a equivalent dose to 10 mg/kg cocaine base
Other group of animals were injected with cocaine (10 mg/kg)

Prieto et al. Am J Addict. 2015

Locomotor sensitization + CBD

Behavioral assay



metabolic activity by CO-I

Pre-treatment			Challenge	Experimental group nomenclature
Vehicle (3% Tween 80)	30 min →	Saline	Coc (5 mg/kg) + Caf (2.5 mg/kg)	Veh-Sal
CBD (20 mg/kg)	30 min →	Saline	Coc (5 mg/kg) + Caf (2.5 mg/kg)	CBD-Sal
Vehicle (3% Tween 80)	30 min →	Coc (5 mg/kg) + Caf (2.5 mg/kg)	Coc (5 mg/kg) + Caf (2.5 mg/kg)	Veh-Coc+Caf
CBD (20 mg/kg)	30 min →	Coc (5 mg/kg) + Caf (2.5 mg/kg)	Coc (5 mg/kg) + Caf (2.5 mg/kg)	CBD-Coc+Caf

CBD purificado

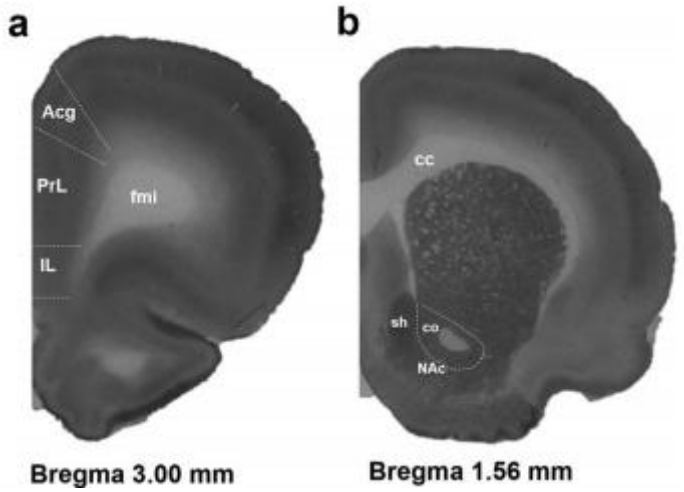
PHYTOPLANT RESEARCH

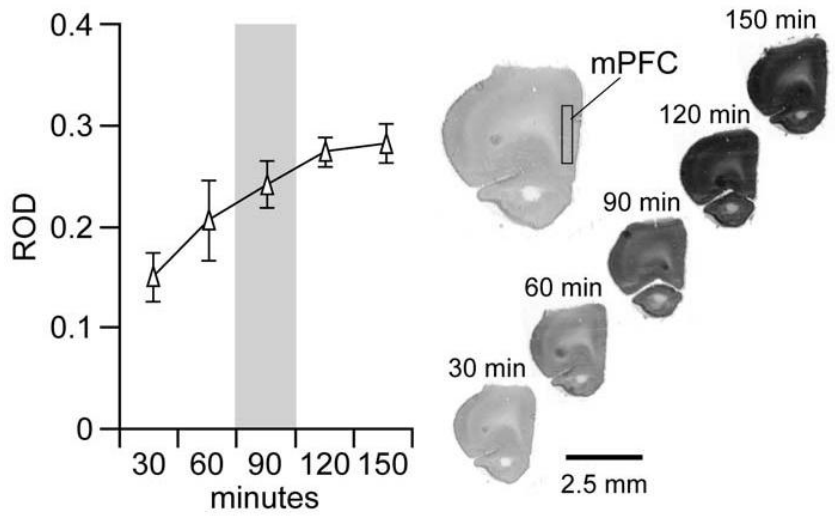
Cocaine (5) + Caffeine (2.5) = subrogate CP

CBD 20 mg/kg

Prieto et al. Neurotox. Research, 2020

Cytochrome oxidase I (CO-I) histochemistry





- Cytochrome oxidase c is a mitochondrial enzyme associated with the energetic cellular metabolism.
- CO-I activity is a **marker for the metabolic activity** of brain regions, and levels of cytochrome oxidase reaction are intimately associated with the neuron metabolic machinery, closely related to the levels of **neuronal activity.**
- Chronically, more active neuron would have greater energy demand and would be expected to have a more active cytochrome system**. Higher levels of functional activity demand oxidative metabolism.** A reduction in neuronal activity has presumably resulted in reduced energy demand.
- CO-I suggests the **functional state** of these brain regions involved in the behavioral sensitization

Wong-Riley, Brain Res. 1979.
Tseng et al. Biol Psychiatry, 2006

CBD attenuates the expression of the coc+caff locomotor sensitization but not the development

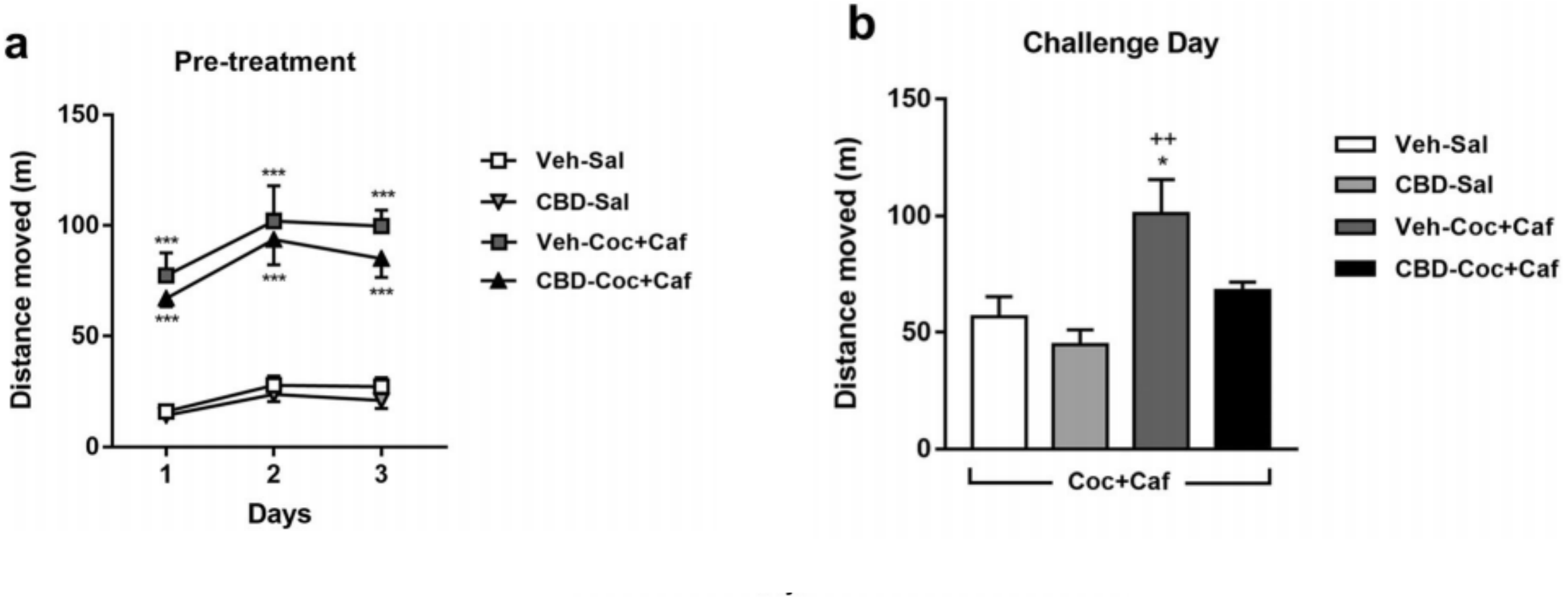
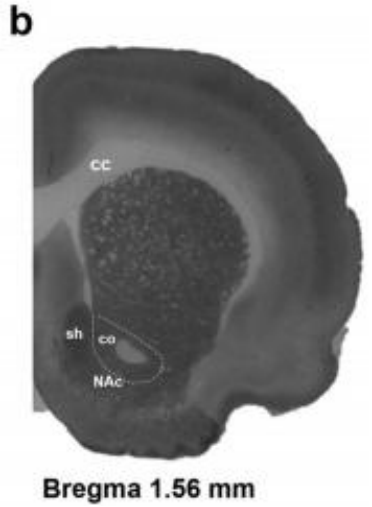
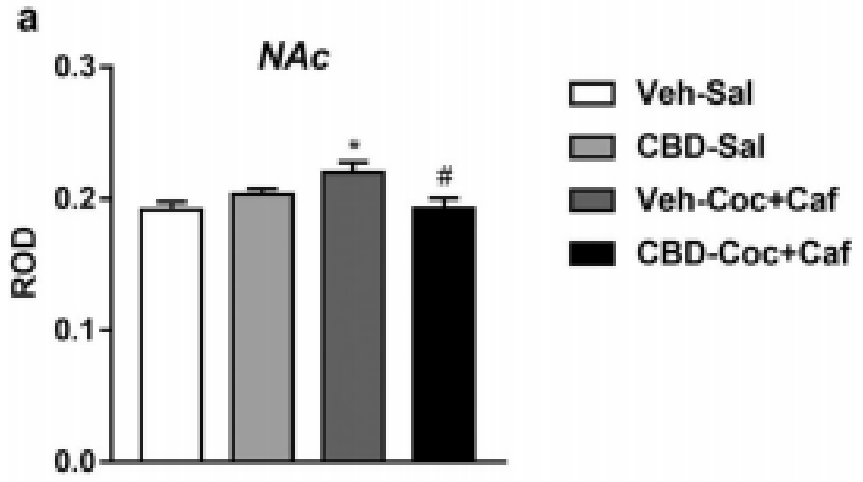


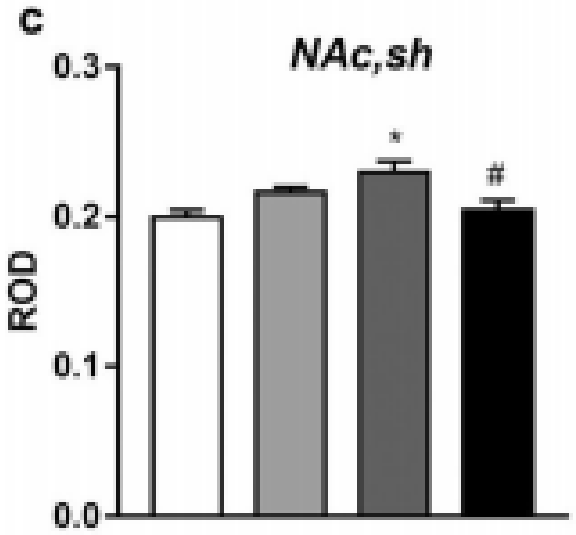
Fig. 3 Effect of CBD pretreatment on the pretreatment period (**a**), and the challenge day induced by Coc+Caf (**b**). Data are expressed as mean ± SEM. Two-way repeated measures ANOVA and one-way ANOVA followed by Tukey's post hoc test. $*p < 0.05$ and $***p < 0.001$ different from Veh-Sal; $++p < 0.01$ different from CBD-Sal. $N = 6–7$

CBD attenuates the metabolic change induced by coc+caff in the NAc



CO-I staining in NAc





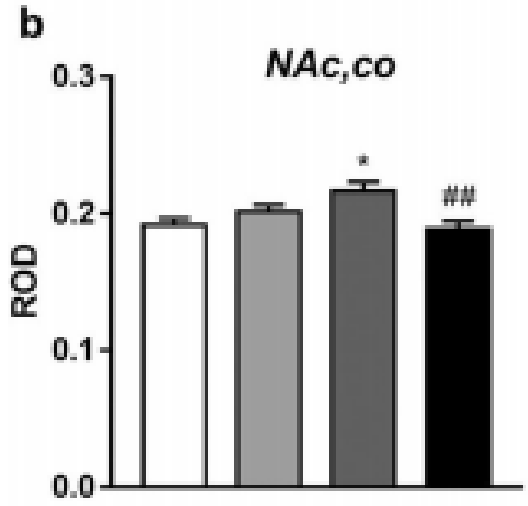
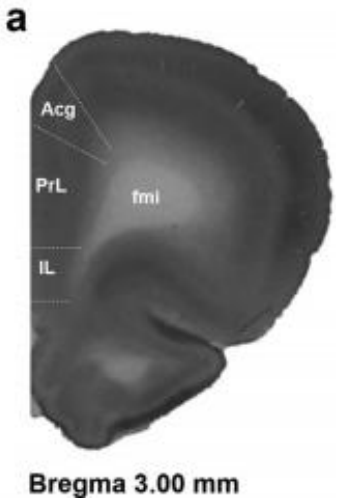


Fig. 5 CO-I activity after the Coc+Caf expression of locomotor sensitization and CBD pretreatment, in the total NAc (**a**), NAc core (NAc,co; **b**), and NAc shell (NAc,sh; **c**). Data of relative optical density (ROD) are expressed as mean ± SEM. One-way ANOVA followed by Tukey's post hoc test. *$p < 0.05$ different form Veh-Sal; #$p < 0.05$ and ##$p < 0.01$ different from Veh-Coc+Caf. $N = 6–7$

CBD attenuates the metabolic change induced by coc+caff in the mPFC



CO-I staining in mPFC

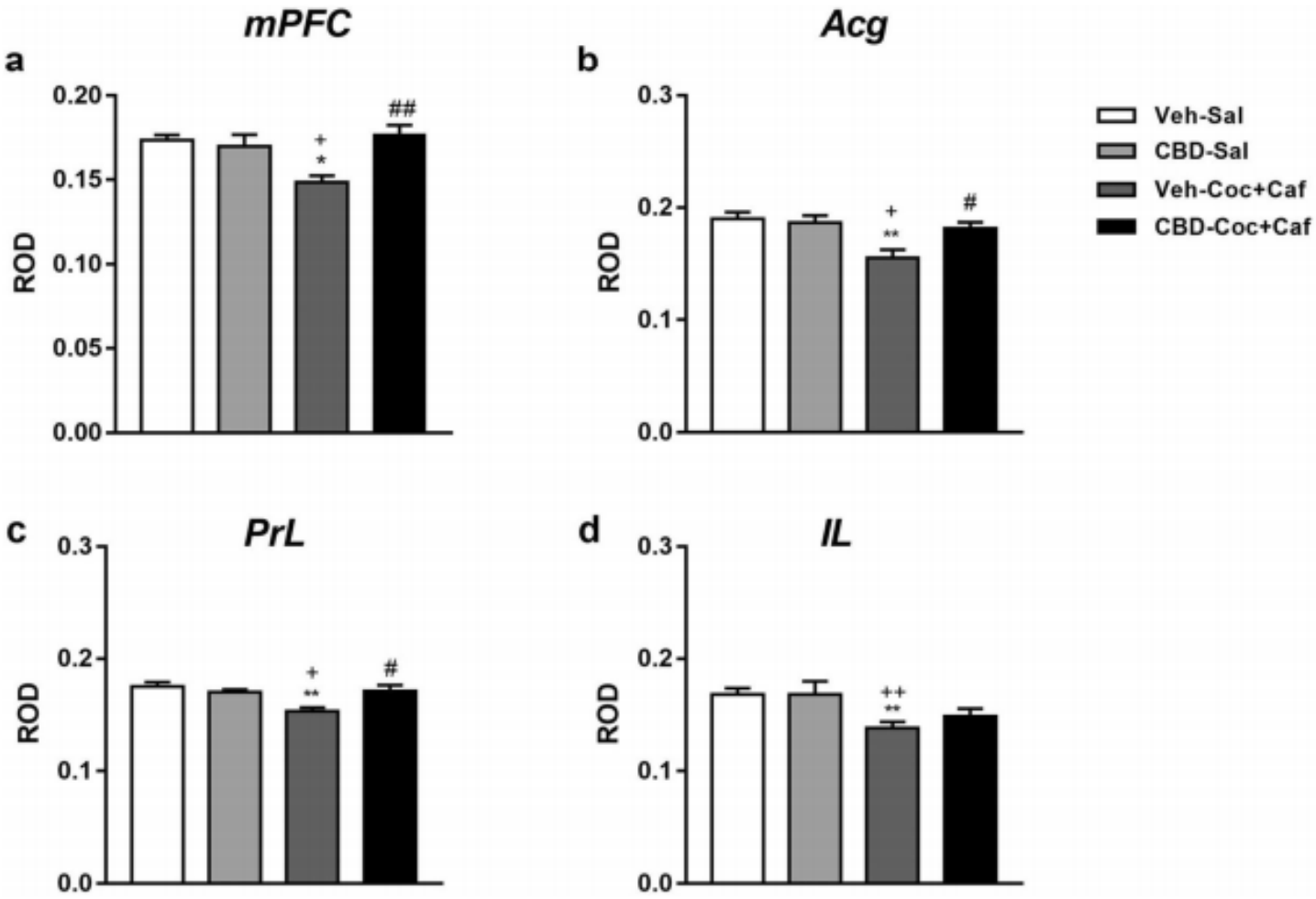


Fig. 4 CO-I activity after the Coc+Caf expression of locomotor sensitization and CBD pretreatment, in total mPFC (**a**), anterior cingular cortex (Acg; **b**), prelimbic cortex (PrL; **c**), and infralimbic cortex (IL; **d**). Data of relative optical density (ROD) are expressed as mean ± SEM. One-way ANOVA followed by Tukey's post hoc test. $*p < 0.05$ and $**p < 0.01$ different form Veh-Sal; $+p < 0.05$ and $++p < 0.01$ different from CBD-Sal; $\#p < 0.05$ and $\#\#p < 0.01$ different from Veh-Coc+Caf. $N = 6–7$

Putative sites of action of CBD…

How could CBD reduce cocaine/METH addiction?

Normalization of the drug-induced alterations in the DA mesolimbic system

↓ hyperactivity of reward system
↓ tyrosine hydroxylase (VTA)
↓ Oxidative stress (NAcc)
↓ DA neuronal firing frequency (VTA)

↑ DA and adenosine (NAcc)
↑ c-fos expression (NAcc)

Partial agonist of DA D2R

Modulation of cannabinoid system

↓ CB1R (NAcc)

↑ CB1R (Hipp)
↑ CB2R (NAcc)

Indirect activation of CB1R (vmPFC)

Inverse agonist or antagonist of CB1R

Modulation of other neurotransmitter systems

↓ μ-OR (NAcc)
Allosteric modulation of μ- and δ-OR

↓ glutamate (Hipp)
↓ GluA1/2AMPAR (striatum)

↑ 5-HT/glutamate (cortex)

↑ 5-HT
Agonist of 5-HT1AR

Inhibition of 7-nAChR

Modulation of signal transduction pathways

↑ Wnt (GSK-3, Akt, -catenin) (NAcc)
↑ mTORC1 (mTOR, p70S6K) (NAcc)

↑ MAPK, ERK1/2 and CREB (Hipp)

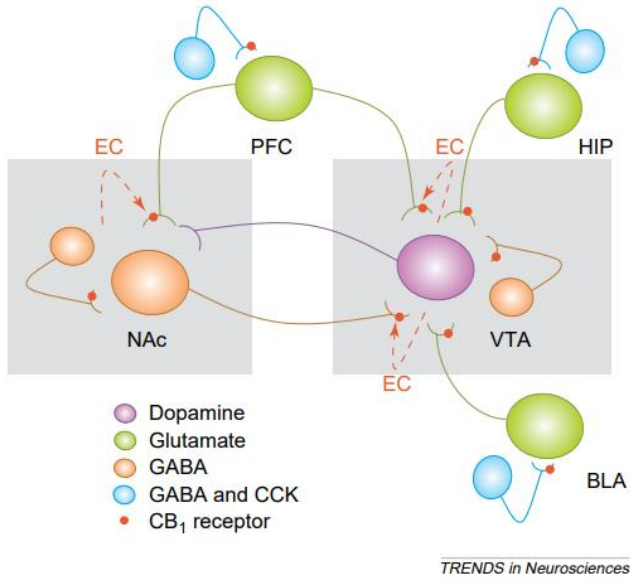
↑ BDNF (Hipp)
↑ BDNF-TrkB-mTOR (Amyg, mPFC and Hipp)
↑ neural progenitor proliferation (Hipp)

Reversion of the drug-induced neuroinflammation

↓ proinflammatory effects
↓ glial reactivity
↓ IL-1β,IL-6 and IL-10 (PFC)
↓ TNF-α,IL-1β and IL-6 (Hipp)

Agonist of PPARγ

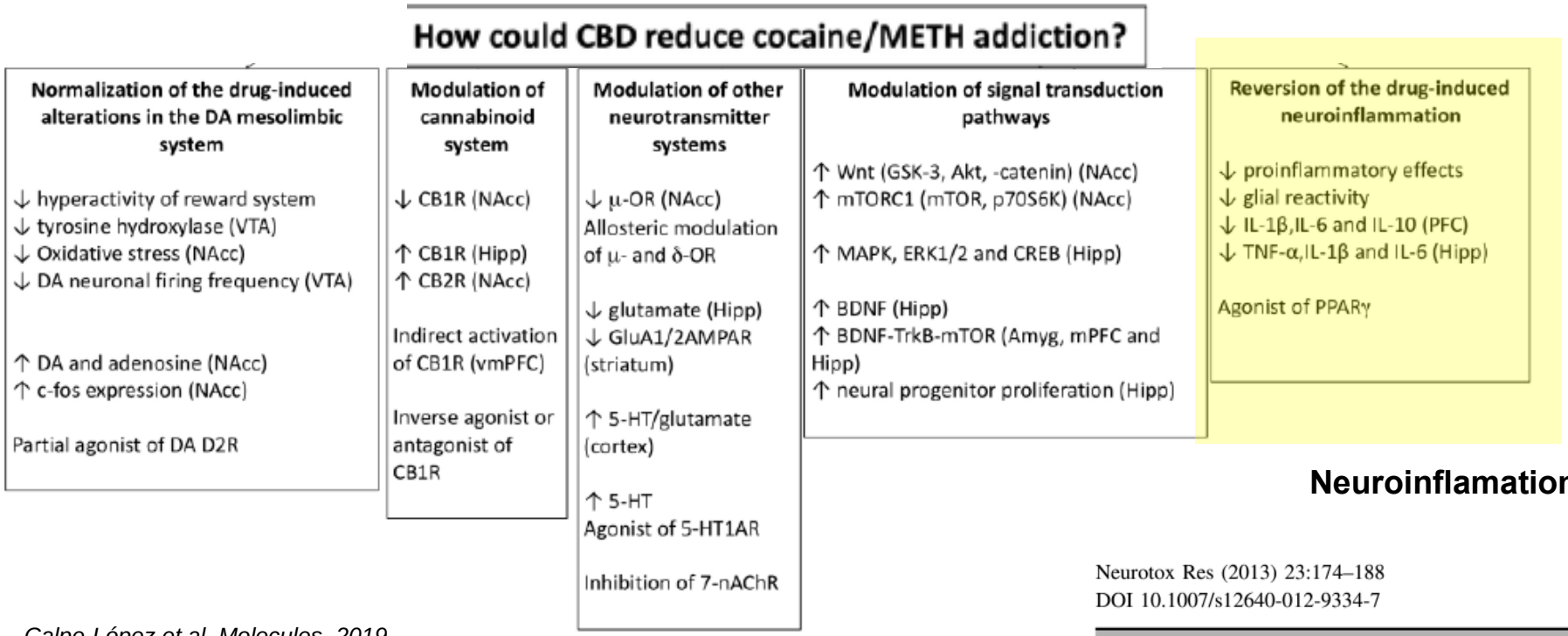
Calpe-López et al. Molecules, 2019



CB1 receptors are abundant in the brain reward circuitry and participate in the addictive properties induced by different drugs of abuse. The DAergic neurons of the mesocorticolimbic pathway are controlled by excitatory and inhibitory inputs that are modulated by CB1 receptors. Thus, endocannabinoids can be released following depolarization in the NAc and from DAergic neurons in the VTA, and they modulate GLUergic and GABAergic afferents by acting as retrograde messengers on CB1 receptors. The presence of CB1 receptors in other structures related to motivation and reward, such as the basolateral amygdala and the hippocampus, also contributes to this function of the endocannabinoid system.

Maldonado et al. Trends in Neurosciences, 2006

Putative sites of action of CBD…



Calpe-López et al. Molecules, 2019

Neuroinflamation hypothesis…

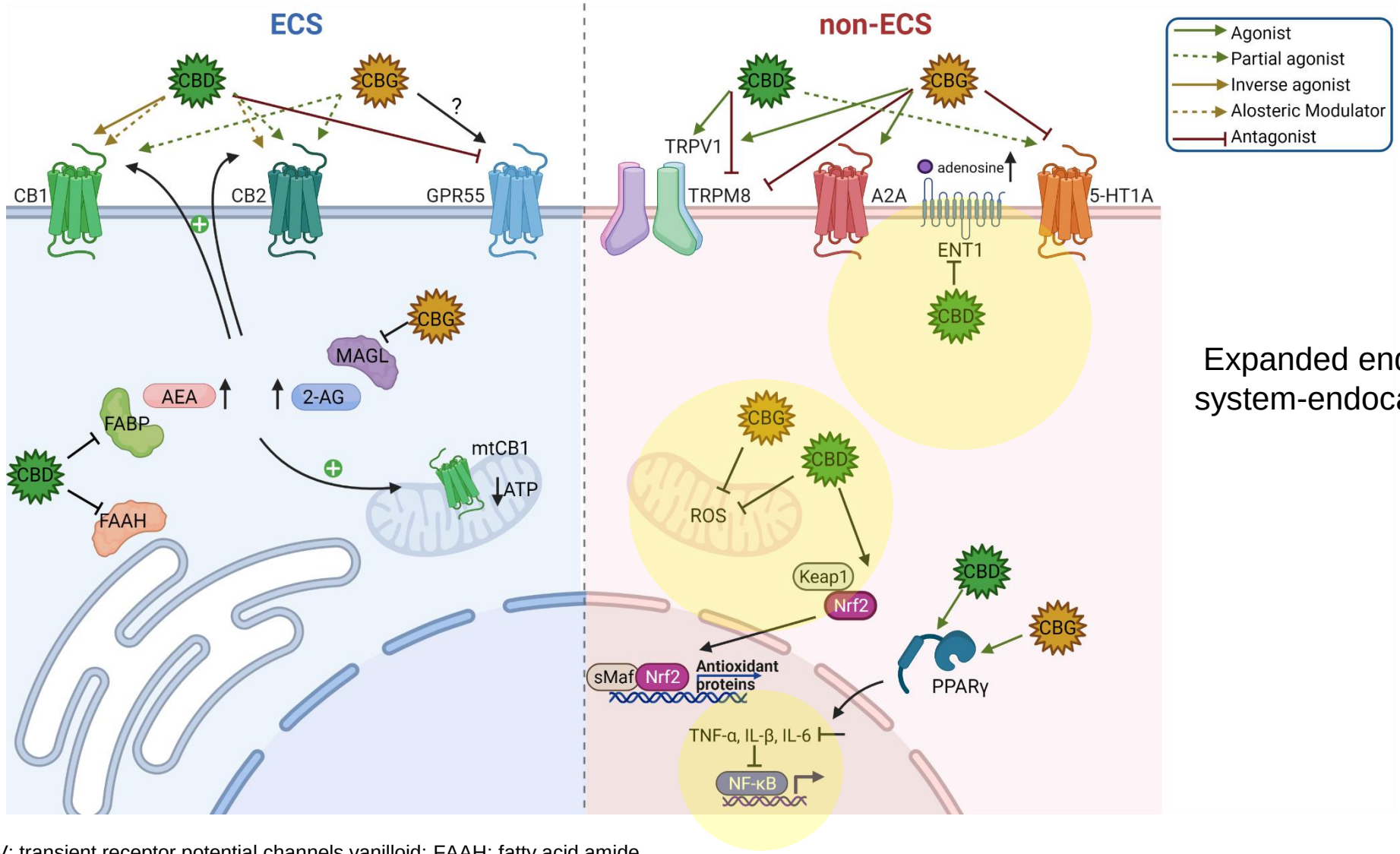
Neurotox Res (2013) 23:174–188
DOI 10.1007/s12640-012-9334-7

REVIEW ARTICLE

Psychostimulant Abuse and Neuroinflammation: Emerging Evidence of Their Interconnection

Kenneth H. Clark · Clayton A. Wiley · Charles W. Bradberry

Sites of action of CBD in the brain



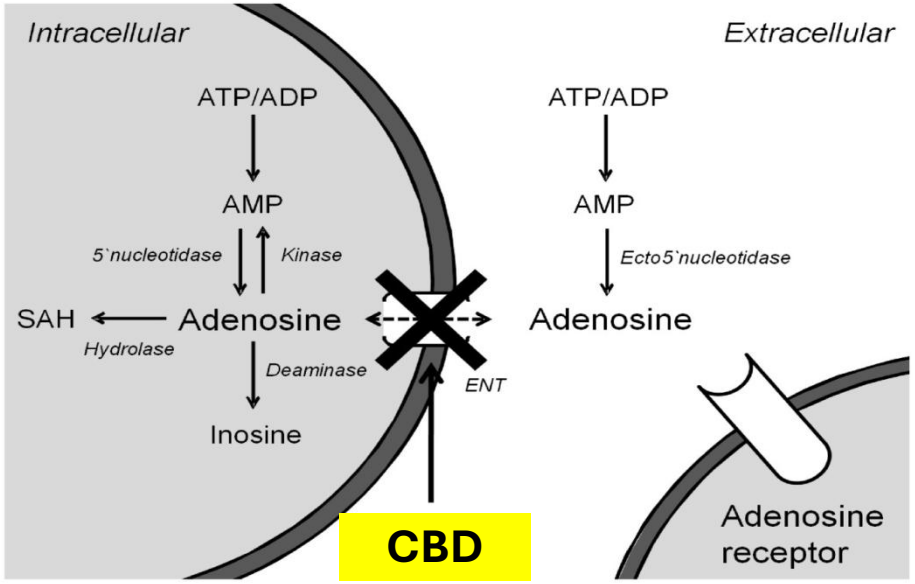
Expanded endocannabinoid system-endocannabinoidome.

Veilleux, Di Marzo, et al. Curr Diab Rep. 2019

TRPV: transient receptor potential channels vanilloid; FAAH: fatty acid amide hydrolase, FAAH, MAGL: monoacylglycerol lipase); AEA: anandamide; PPARs: peroxisome proliferator-activated receptors.

Echeverry et al., Medicinal Usage of Cannabis and Cannabinoids. Burlington: Academic Press, pp. 197-205, 2023.

CBD increases adenosine levels by the blockage of ENT-1



Carrier et al. PNAS, 2006

The equilibrative nucleotide transporter 1 (ENT-1; adenosine uptake protein) is the primary mechanism of adenosine reuptake. Adenosine is a purine nucleoside neurotransmitter. Release of adenosine is an endogenous mechanism to attenuate cellular stress and inflammation.

ENT1 was proposed to mediate the anti-inflammatory effect of CBD.

Carrier et al. PNAS, 2006

- **Can cocaine and caffeine induce neuroinflammatory processes?**
- Can CBD diminish the development of increased locomotor sensitivity caused by the combination of cocaine and caffeine through its anti-inflammatory properties?

2024; doi: 10.1038/s41380-024-02443-6.

Molecular Psychiatry

www.nature.com/mp

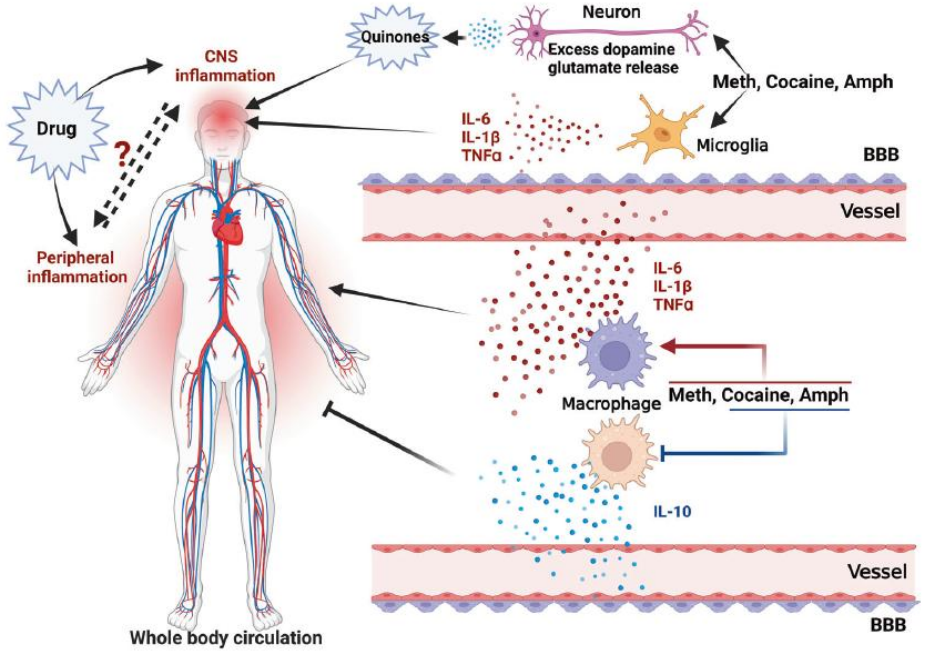
PERSPECTIVE

Microglia in neuroimmunopharmacology and drug addiction

Hongyuan Li[1], Linda R. Watkins[2] and Xiaohui Wang[1,3,4]✉

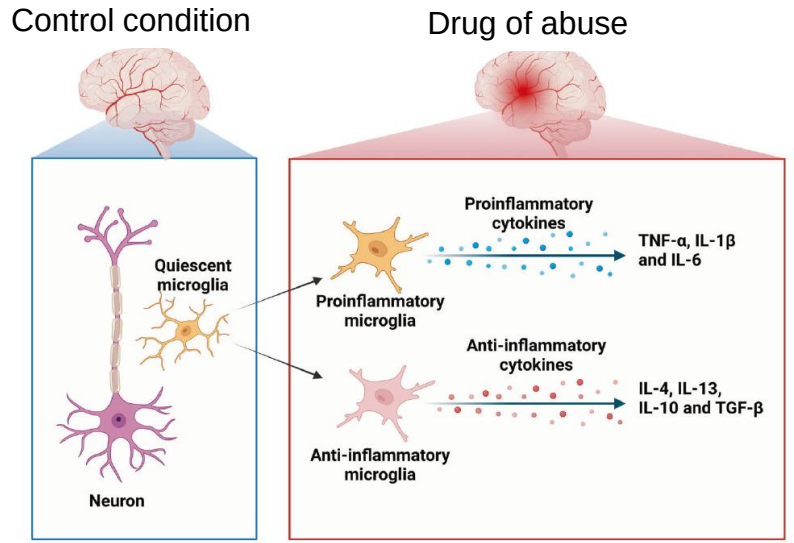
Drug addiction is a chronic and debilitating disease that is considered a global health problem. Various cell types in the brain are involved in the progression of drug addiction. Recently, the xenobiotic hypothesis has been proposed, which frames substances of abuse as exogenous molecules that are responded to by the immune system as foreign "invaders", thus triggering protective inflammatory responses. An emerging body of literature reveals that microglia, the primary resident immune cells in the brain, play an important role in the progression of addiction. Repeated cycles of drug administration cause a progressive, persistent induction of neuroinflammation by releasing microglial proinflammatory cytokines and their metabolic products. This contributes to drug addiction via modulation of neuronal function. In this review, we focus on the role of microglia in the etiology of drug addiction. Then, we discuss the dynamic states of microglia and the correlative and causal evidence linking microglia to drug addiction. Finally, possible mechanisms of how microglia sense drug-related stimuli and modulate the addiction state and how microglia-targeted anti-inflammation therapies affect addiction are reviewed. Understanding the role of microglia in drug addiction may help develop new treatment strategies to fight this devastating societal challenge.

Molecular Psychiatry; https://doi.org/10.1038/s41380-024-02443-6



- These cytokines may contribute to changes in synaptic plasticity, which can impact the availability and reuptake of neurotransmitters.

- Microglia undergo drastic morphological changes characterized by swollen soma and shortened processes and releases pro-inflammatory cytokines.



Neuropsychopharmacology REVIEWS (2017) 42, 156–177

REVIEW

Glial and Neuroimmune Mechanisms as Critical Modulators of Drug Use and Abuse

Michael J Lacagnina[1], Phillip D Rivera[1] and Staci D Bilbo*,[1]
[1]Department of Psychology & Neuroscience, Duke University, Durham, NC, USA

Drugs of abuse cause persistent alterations in synaptic plasticity that may underlie addiction behaviors. Evidence suggests glial cells have an essential and underappreciated role in the development and maintenance of drug abuse by influencing neuronal and synaptic functions in multifaceted ways. Microglia and astrocytes perform critical functions in synapse formation and refinement in the developing brain, and there is growing evidence that disruptions in glial function may be implicated in numerous neurological disorders throughout the lifespan. Linking evidence of function in health and under pathological conditions, this review will outline the glial and neuroimmune mechanisms that may contribute to drug-abuse liability, exploring evidence from opioids, alcohol, and psychostimulants. Drugs of abuse can activate microglia and astrocytes through signaling at innate immune receptors, which in turn influence neuronal function not only through secretion of soluble factors (eg, cytokines and chemokines) but also potentially through direct remodeling of the synapses. In sum, this review will argue that neural–glial interactions represent an important avenue for advancing our understanding of substance abuse disorders.
Neuropsychopharmacology Reviews (2017) **42,** 156–177; doi:10.1038/npp.2016.121; published online 31 August 2016

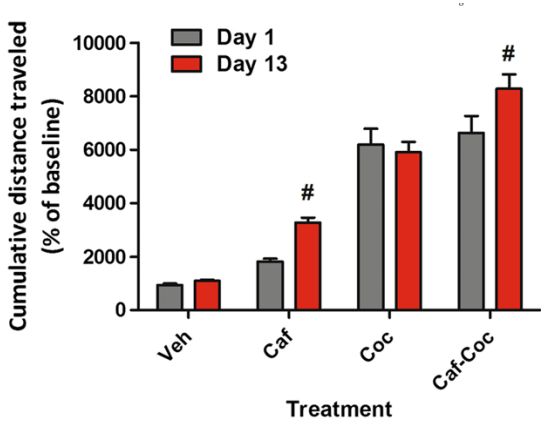
Published in final edited form as:
Neurotox Res. 2016 May ; 29(4): 525–538. doi:10.1007/s12640-016-9601-0.

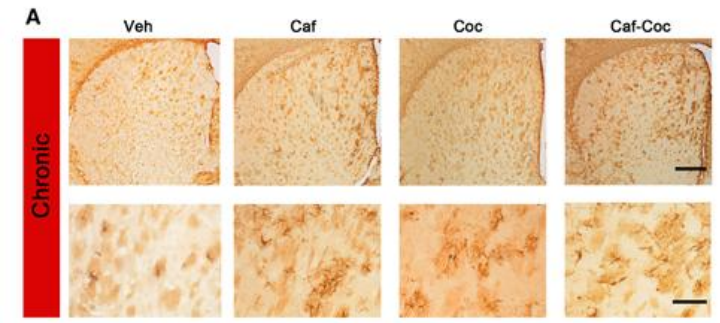
Combined Effects of Simultaneous Exposure to Caffeine and Cocaine in the Mouse Striatum

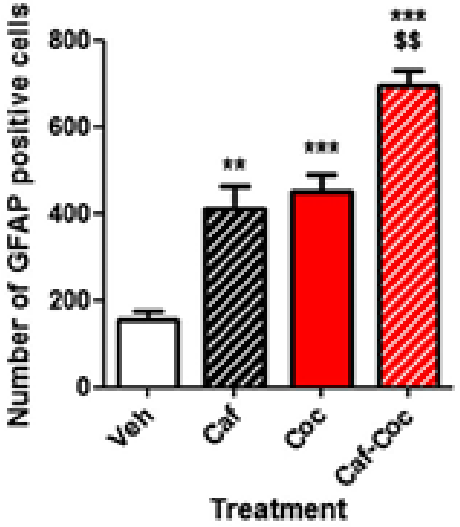
Javier A. Muñiz[1], Gimena Gomez[1], Betina González[1], María Celeste Rivero-Echeto[2], Jean Lud Cadet[3], Edgar García-Rill[4], Francisco J. Urbano[2], and Veronica Bisagno[1]

Aim: effect of caffeine, cocaine, and their combination on sensitization and striatal GFAP immunoreactivity after chronic treatment

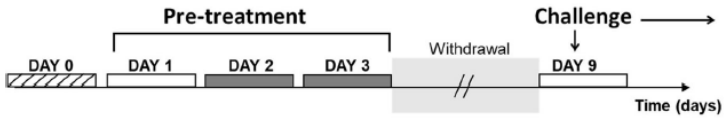
Parameter: glial Fibrillary Acidic Protein (GFAP+)
Astroglyosis marker associated with glia inflammation process



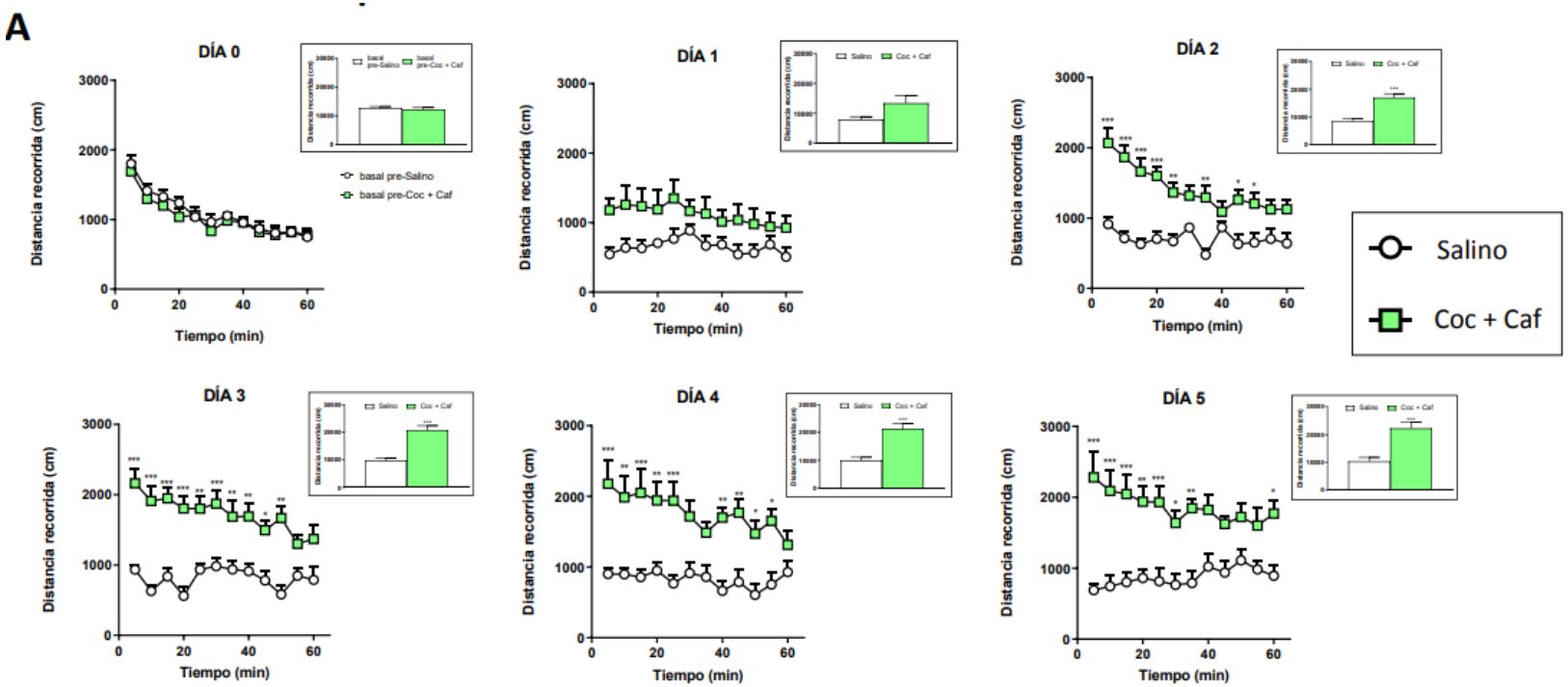




Locomotor sensitization induced by Coc+Caff is associated with microglia activation



Microglial reactivity evaluated by reactividad microglial anti-Iba1 immunofluorescence (Nacc)



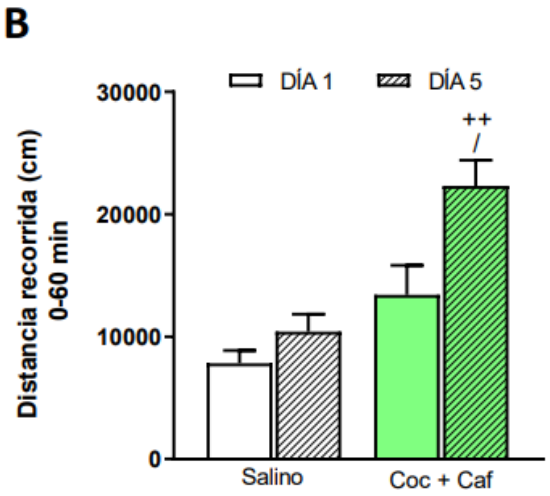
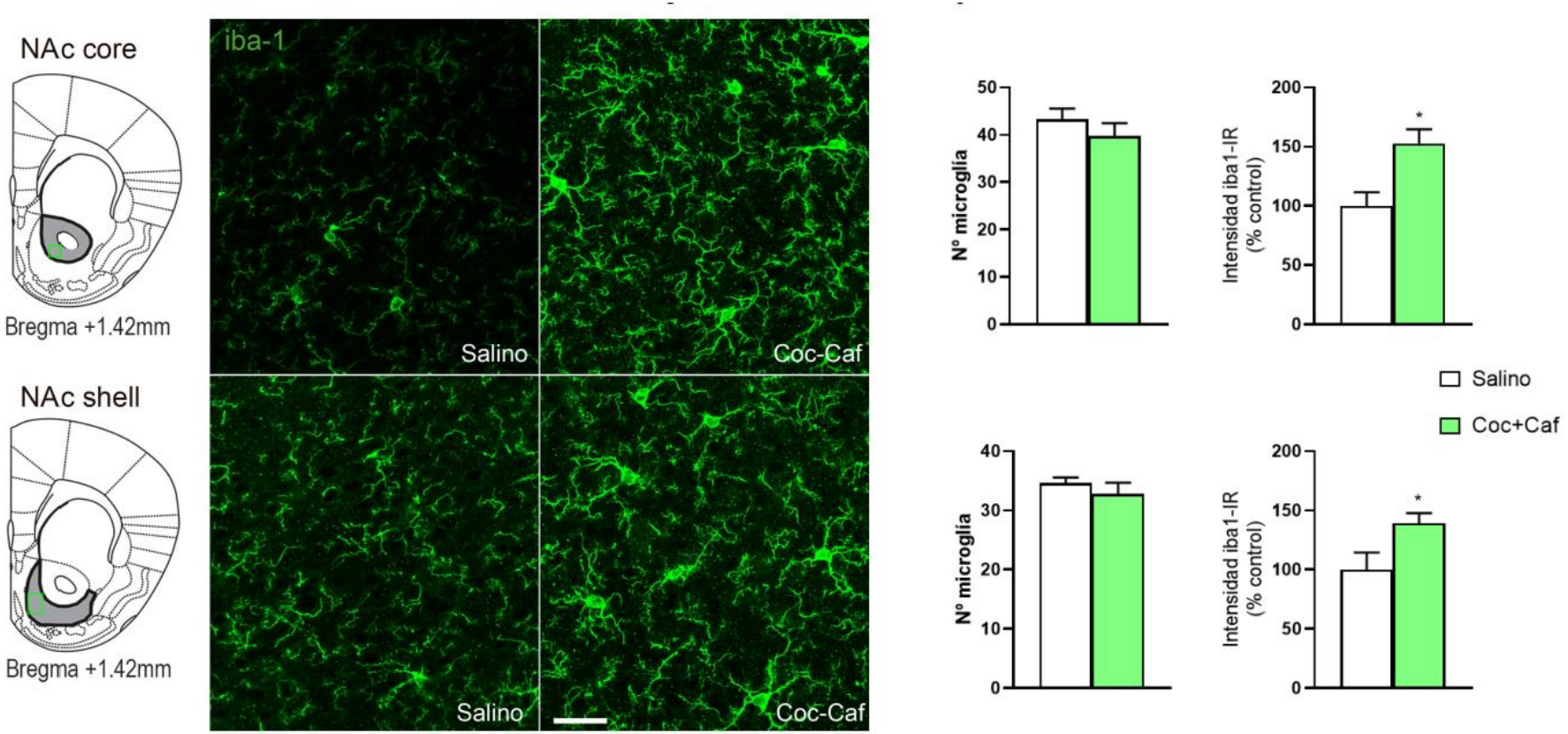


Figura 1. Efecto de Coc (5 mg/kg) + Caf (2.5 mg/kg) o salino sobre la actividad locomotora durante el desarrollo de la sensibilización (A) y durante el primer y último día del tratamiento (B). Datos expresados como promedio ± SEM. One-way ANOVA seguido de Newman-Keuls o Student t-test. * = vs Salino; / = día 1 vs día 2; + = día 5 Salino vs Coc + Caf. *;/= $p \leq 0.05$; **; ++ = $p \leq 0.01$; *** = $p \leq 0.001$. N=8 por grupo experimental.

Immunoreactivity for Iba-1 increased by Coc+Caff repeated treatment

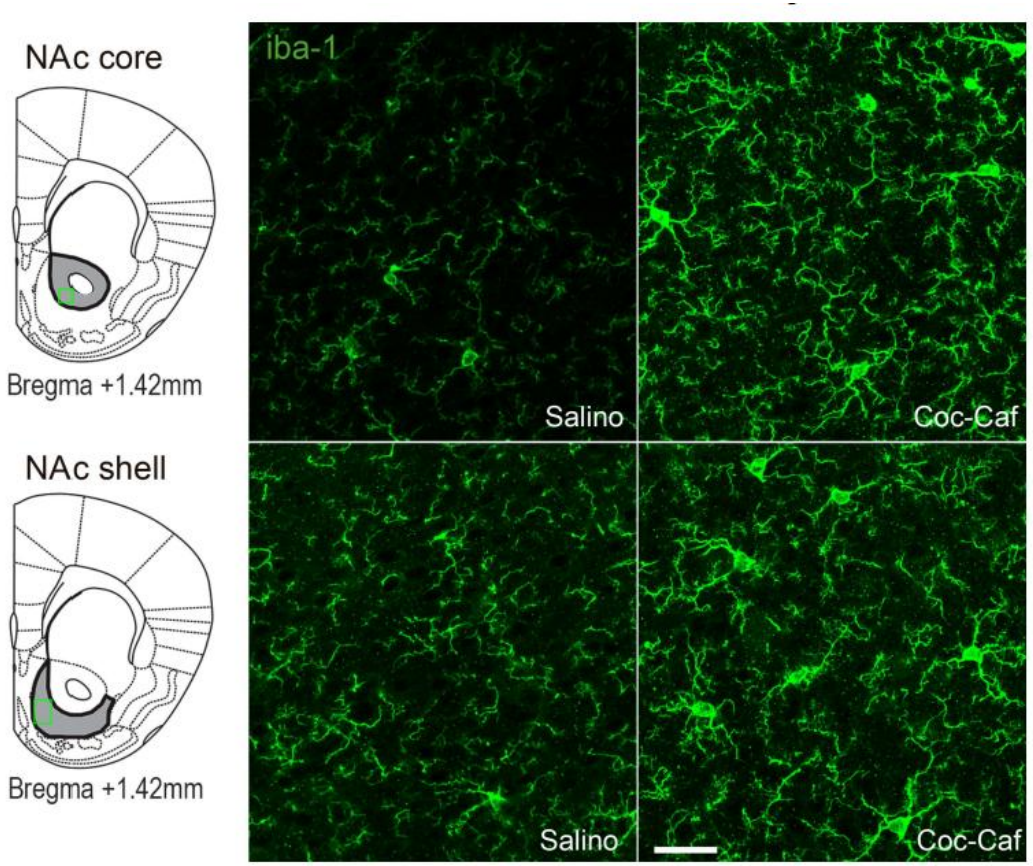
Microglia, which have been estimated to comprise 10-15 % of all CNS cells, orchestrates the primary brain innate immune response. Cell-surface **markers of microglia activation include Iba1 (ionized calcium-binding protein).** Microglial activation is linked to the production of pro-inflammatory cytokines (IL-1β), IL-6, and TNFα

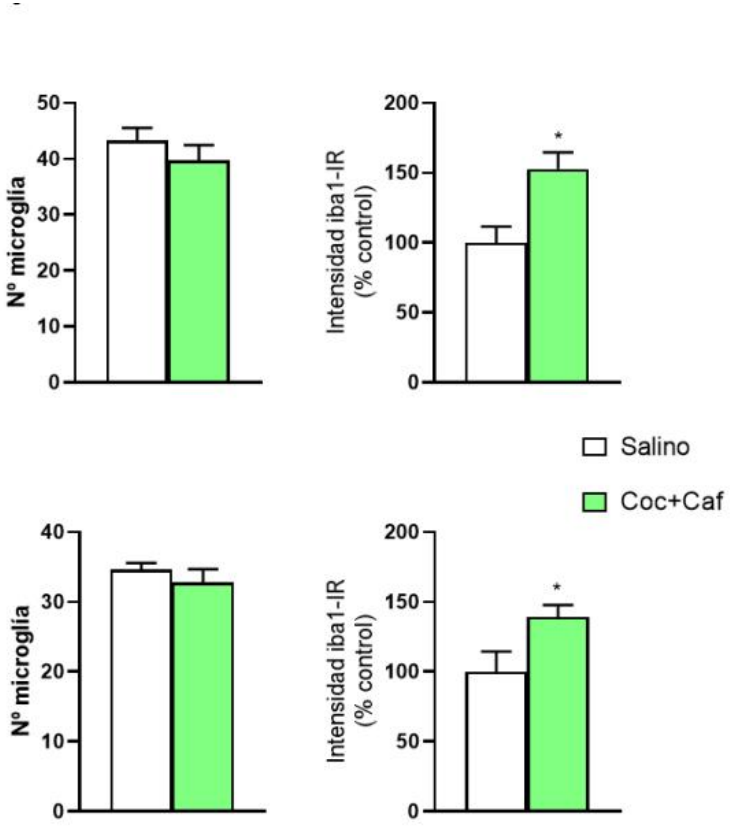
Immunofluorescence: Sections containing the nucleus accumbens core (NAc/c) and shell (NAc/sh) were used. Microglia was identified using rabbit **anti-iba-1** (iba-1: ionized calcium-binding adapter molecule-1, Cat #019-19741, Wako, US, 1:2000) followed by corresponding secondary antibody (Alexa Fluor 488, Invitrogen, US, 1:400). Cellular nuclei were visualized with Hoechst 33258 (1ng/mL).



- An increase in Iba-1 fluorescence intensity (expressed as % with respect to the mean of control group) was observed. No change was observed in the number of Iba-1 positive cells.

Immunoreactivity for Iba-1 increased by Coc+Caff repeated treatment





CBD?

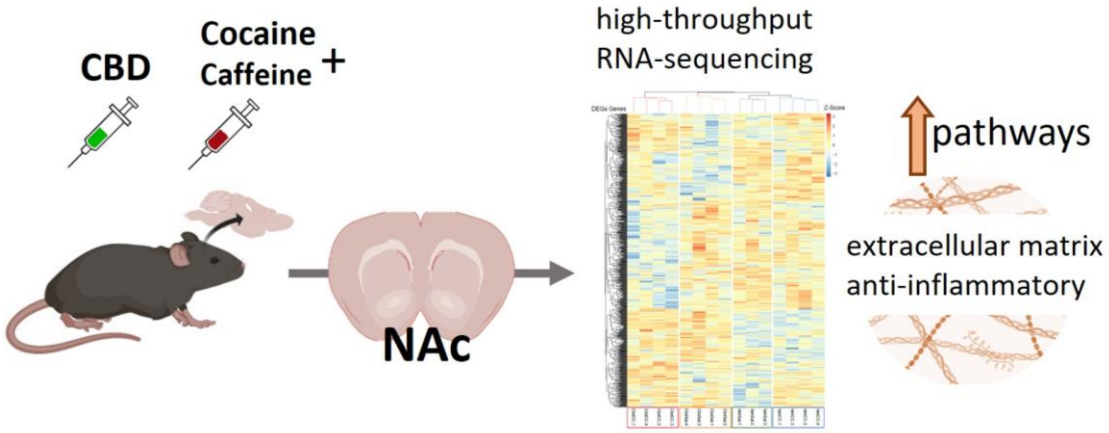
Ongoing experiments Iba-1
Plasmatic and regional cytokines

Immunofluorescence: Sections containing the nucleus accumbens core (NAc/c) and shell (NAc/sh) were used. Microglia was identified using rabbit **anti-iba-1** (iba-1: ionized calcium-binding adapter molecule-1, Cat #019-19741, Wako, US, 1:2000) followed by corresponding secondary antibody (Alexa Fluor 488, Invitrogen, US, 1:400). Cellular nuclei were visualized with Hoechst 33258 (1ng/mL).

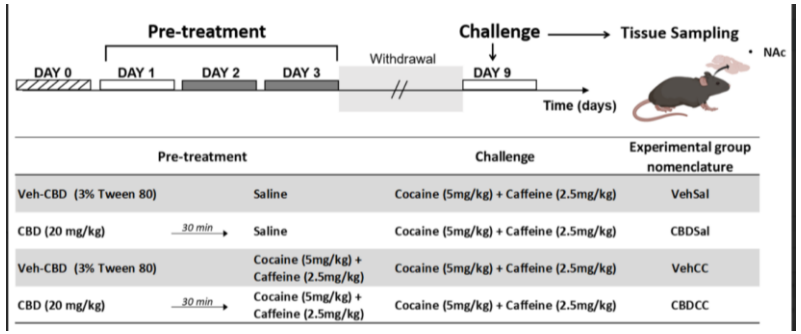
CBD prevents the locomotor sensitization induced by Coc+Caff and upregulates genes of extracellular matrix and anti-inflammatory pathways

Collaboration:

José Sotelo Silveira (IIBCE)
José Prieto (Fac. Ciencias, UdelaR
Rafael Fort (IIBCE)



A transcriptome-wide analysis in the NAcc



Pre-treatment		Challenge	Experimental group nomenclature
Veh-CBD (3% Tween 80)	Saline	Cocaine (5mg/kg) + Caffeine (2.5mg/kg)	VehSal
CBD (20 mg/kg) 30 min	Saline	Cocaine (5mg/kg) + Caffeine (2.5mg/kg)	CBDSal
Veh-CBD (3% Tween 80)	Cocaine (5mg/kg) + Caffeine (2.5mg/kg)	Cocaine (5mg/kg) + Caffeine (2.5mg/kg)	VehCC
CBD (20 mg/kg) 30 min	Cocaine (5mg/kg) + Caffeine (2.5mg/kg)	Cocaine (5mg/kg) + Caffeine (2.5mg/kg)	CBDCC

Cannabidiol prevents cocaine and caffeine sensitization modulating expression of extracellular matrix and anti-inflammatory genes in Nucleus Accumbens

José Pedro Prieto†, Rafael Fort†, Guillermo Eastman, Oliver Kaminski, Carlos Ferreiro-Vera, Verónica Sánchez de Medina, Cecilia Scorza, José Roberto Sotelo-Silveira**

Preprint-ResearchGate DOI 10.1101/2023.09.28.560030
Submitted

Conclusions

- Our preclinical results contribute to identify the therapeutic potential of CBD in SUD field, specially in psychostimulants abuse (CUD).

- Remains to be confirmed if CBD prevents both neuroinflammation and sensitization processes induced by Coc+caff. Some positive results were obtained in the RNA transcriptional analysis.

- More preclinical and clinical studies are necessary to further evaluate the role of CBD as a new therapeutic intervention for SUD.

Acknowledgements

- **Depto. de Neurofarmacología, IIBCE**

  Analía Richeri, PhD
  Jessika Urbanavicius, PhD
  Ximena López Hill, PhD
  Sara Fabius, MSc. student
  Ma. José Ferreiro, PhD
  Gaby Martínez, PhD
  Diego Flores Luna, MSc. student
  Jimena Faggeti, MSc. student
  Valentina Perini, MSc. student
  Florencia Bai
  Cristina Parada, PhD student
  José Pedro Prieto, PhD
  Oliver Kaminsy, Bach
  Martín Galvalisi, MSc
  Ma. Paula Latorre

- **Depto. de Neuroquímica, IIBCE**

  Carolina Echeverry, PhD
  Giselle Prunell, PhD

- **Depto. de Microbiología, IIBCE**

  Sofía Fernández, PhD
  Claudia Piccini, PhD
  Pablo Zunino, PhD
  Joaquín Lozano, MSc

- **Depto. de Genómica, IIBCE**

  José Sotelo Silveira, PhD
  Rafael Fort, PhD

- **Plataforma de Servicios Analíticos, IIBCE**

  Juan Andrés Abin Carriquiry, PhD
  Marcela Martínez, PhD student
  Sandra Pérez, Technician

- **Facultad de Medicina, Universidad de la República**

  Patricia Lagos, PhD

- **Universidad de Cagliari, Italia**

  Valentina Valentini, PhD

- **Universidad de Buenos Aires**

  Verónica Bisagno, PhD
  Betina González, PhD
  Javier Muñiz, PhD, Máximo Sosa

- **University of California at Los Angeles**, USA; Department of Psychiatry and Biobehavioral Sciences.

  McGregor Ronald, PhD

- **Dra. Raquel Peyraube** Pharma-Origin

- **CBD is donated by**

  Xavier Nadal, PhD
  Carlos Ferreira Vera
  Verónica Sánchez de Medina

This work was funded by

Premio Concursable

Thank you for your attention

cscorza@iibce.edu.uy / scorzacecilia@gmail.com

@CeciliaScorza