IBOGAINE FOR THE TREATMENT OF OPIOID DEPENDENCE: FROM MECHANISMS OF ACTION TO CLINICAL EFFICACY

Genís Oña Esteve

UNIVERSITAT ROVIRA I VIRGILI
IBOGAINE FOR THE TREATMENT OF OPIOID DEPENDENCE: FROM MECHANISMS OF ACTION TO CLINICAL EFFICACY
Genís Oña Esteve

Genís Oña Esteve

Ibogaine for the Treatment of Opioid Dependence: From Mechanisms of Action to Clinical Efficacy

Doctoral thesis

Thesis supervised by

Dr. Maria Teresa Colomina Fosch

Dr. José Carlos Bouso

UNIVERSITAT ROVIRA i VIRGILI

UNIVERSITAT ROVIRA I VIRGILI
IBOGAINE FOR THE TREATMENT OF OPIOID DEPENDENCE: FROM MECHANISMS OF ACTION TO CLINICAL EFFICACY
Genís Oña Esteve

I STATE that the present study, entitled "Ibogaine for the treatment of opioid dependence: from mechanisms of action to clinical efficacy," presented by Genís Oña Esteve, was conducted under my supervision at Universitat Rovira i Virgili and the *International Center for Ethnobotanical Education Research & Service* (ICEERS), in fulfilment of the requirements for the degree of Doctor, and meets the requirements to qualify for International Mention.

Reus, 15 July 2024

Doctoral Thesis Supervisors

Maria Teresa Colomina Fosch **José Carlos Bouso**

UNIVERSITAT ROVIRA I VIRGILI
IBOGAINE FOR THE TREATMENT OF OPIOID DEPENDENCE: FROM MECHANISMS OF ACTION TO CLINICAL EFFICACY
Genís Oña Esteve

All the experimental phases of this thesis were carried out at the ICEERS Research Department, the Hospital Sant Joan Reus-Baix Camp, and the following groups and Departments at the Universitat Rovira i Virgili: the Neurobehavior and Health (NEUROLAB) research group, the Research Center for Behavior Assessment (CRAMC), the Department of Psychology, the Biochemistry and Biotechnology Department, and the Centre for Environmental, Food and Toxicological Technology (TecnATox). This work was funded by:

The Doctorats industrials program – AGAUR – Generalitat de Catalunya
Dr. Bronner's
The Riverstyx Foundation
The Etheridge Foundation
The Multidisciplinary Association for Psychedelic Studies
The Nikean Foundation

UNIVERSITAT ROVIRA I VIRGILI
IBOGAINE FOR THE TREATMENT OF OPIOID DEPENDENCE: FROM MECHANISMS OF ACTION TO CLINICAL EFFICACY
Genís Oña Esteve

La simplicidad lógica característica de las ciencias
nunca es alcanzada por la naturaleza sin alguna mezcla de ficción

F.P. Ramsey

UNIVERSITAT ROVIRA I VIRGILI
IBOGAINE FOR THE TREATMENT OF OPIOID DEPENDENCE: FROM MECHANISMS OF ACTION TO CLINICAL EFFICACY
Genís Oña Esteve

ACKNOWLEDGEMENTS

Vaig començar la tesi amb una llarga melena i una vista excel·lent, i l'acabo amb una extensa calva i amb ulleres per veure-hi de lluny, signes dels potencials efectes perjudicials d'embarcar-se en una tesi doctoral. O això és el que dirien alguns. En el transcurs d'aquests anys he anat adquirint les habilitats explícites i implícites, formals i informals, que configuren el saber i procedir científic i que m'impedeixen establir una relació de causalitat entre aquests trets físics actuals i el treball en la meva tesi i, per tant, em permeten donar les gràcies a moltes coses d'aquests anys en aquest famós apartat introductori d'agraïments.

Estic molt d'acord amb l'afirmació que diu que la investigació científica és més aviat un art, molt similar al treball de l'artesà que és capaç de perfeccionar les seves habilitats amb el pas dels anys. Així ho sentenciava Beveridge en un famós manual d'introducció a la matèria, que porta per títol, precisament, *The art of scientific investigation*. Si la recerca és un art, el procés de tesi doctoral és un dels tallers on s'aprèn. I per arribar en aquest taller, en el meu cas, han passat moltes coses. Moltíssimes. He pres males decisions moltes vegades, n'he pres algunes d'encertades només en algunes ocasions. Però agraeixo tot el que ha passat perquè avui en dia em trobi aquí, en una casa enmig de les muntanyes boscoses del sistema prelitoral central, escrivint aquestes línies.

Òbviament, hi ha hagut elements que han jugat un paper més important que altres a l'hora de conduir-me cap al camí actual i els quals voldria fer explícits aquí, de la forma més exhaustiva que la meva consciència i memòria em permetin.

Primer que tot gràcies als meus pares per sempre estar allà, de forma incondicional, i educar-me de la millor manera possible.

Gràcies a les plantes i productes que la terra ens dóna de forma incondicional; per la pluja, el Sol, la lluna i el vent, que sostenen en silenci la vida.

Gràcies a tots els autors i autores que m'han inspirat i en els quals he trobat racons impagables d'afinitat i comprensió. A Hermann Hesse, Aldous Huxley, Jack London o Goethe; i també gràcies a Judit Neddermann pel seu art, pel consol de les seves lletres, per les experiències meravelloses dels seus concerts i per la inspiració personal. Gràcies a Jim Morrison per obrir-me a un altre món, i a tants altres artistes (sobretot els clàssics, dels 70 cap enrere!).

Gràcies a molts dels meus professors, que durant l'etapa d'educació secundària i batxillerat m'insistien en continuar estudiant, mentre jo insistia en seguir fent campana.

Gràcies a totes les persones que s'han preocupat per mi en algun moment i m'han donat un cop de mà. I gràcies també a aquelles que no ho han fet, doncs m'han obligat a persistir i trobar la manera.

Gràcies a totes les persones que han treballat incansablement pels psicodèlics, que han estat molt de temps lluitant contra murs inquebrantables, convençuts de que tothom

hauria de tenir l'experiència profunda que aquestes eines poden induir. He d'afegir unes línies més sobre això. Encara que l'esforç d'aquestes persones sigui legítim i es faci des d'un desig bondadós, i que, efectivament, si aquesta experiència profunda apareix et pot canviar la vida, no podem esperar que tothom tingui les mateixes experiències. *No tothom* té experiències espirituals, transformadores, ni tant sols remotament positives. També hi ha persones que ho poden passar molt malament i patir les conseqüències durant molt de temps. Espero que ara que aquests murs han caigut i que tothom està entusiasmat amb aquestes substàncies es tingui això present.

Spuch, segueixes molt present en els nostres cors, sé que n'ets conscient. Vas ser més llest que ningú i t'has estalviat les responsabilitats de la vida adulta, una pandèmia global i altres coses que segur vindran. Però vas estar temps suficient en aquest món com per ensenyar-nos moltes coses. Gràcies per tot.

Gràcies a Jordi Riba, amb qui vaig aprendre molt, no només sobre farmacologia. T'hauria d'haver escoltat més, Jordi.

I ja entrant en el context de la meva tesi, gràcies a tantes persones que l'han fet possible. Tècnics, administratius, analistes, telefonistes, secretaris, avaluadors, infermers, metges, caps de servei, coordinadors, etc.

Gràcies al meravellós equip de l'Hospital de Reus (que cada dos per tres canvia de nom i de gerent, així que poso el nom popular) que hem format per dur a terme l'estudi. Al super equip de infermeria (Edu, Neus i Andrés), que ha estat sempre al peu del canó, a l'Eulàlia i la Lourdes, la Carme Ligero, el Josep Mª Alegret, en Toni Llort, i sobretot gràcies a la Tre Borràs, capitana incansable del vaixell, per la valentia, la paciència i la mà esquerra.

Muito obrigado também a Rafael Guimarães e a Jaime EC Hallak por facilitar em todos os aspectos possíveis minha estadia de doutorado em seu laboratório no Brasil, por me receber de forma tão calorosa e amável e permitir que eu conhecesse não apenas seu trabalho pioneiro com substâncias psicoativas, mas também a complexa e rica cultura de um país que sempre levarei no coração.

Moltes gràcies a la Teresa Colomina, qui des d'un principi va confiar en mi, i en moments delicats sempre m'ha brindat el seu suport i consell. Per mi no hi pot haver millor exemple de investigadora, mentora i professora. Com en el meu cas, també hi ha moltes altres persones que han sentit la seva presència i professionalitat com una benedicció, i no ho podem agrair prou. Donades les circumstàncies del món universitari i especialment els escandols que surten de tant en tant sobre el món de les tesis doctorals, són les persones així les que marquen una diferència. Així que gràcies Teresa!

Gràcies també a Judit Biosca per tota la feina i suport durant la redacció dels articles en que ha col·laborat. Si m'enduc algun mèrit en relació a les òmiques aquest serà ilegítim, tot el mèrit és seu.

Moltes gràcies a José Carlos Bouso per oferir-me la possibilitat de fer aquesta tesi. Tu vas moure fils, vas muntar l'estructura i les relacions, deixant-me entrar a casa teva i fer

la feina que s'havia de fer. Considero els anys que he treballat amb tu com un llegat que sempre m'acompanyarà i del qual he après molt.

I com no? Gràcies infinites a Pinotxo, Kenya i Freeda per tot el seu amor, i a la Eva, per ser, per estar, per florir, per tota ella. Tamos juntos mor.

Cal Junts (Ranxos de Bonany), 2024.

UNIVERSITAT ROVIRA I VIRGILI
IBOGAINE FOR THE TREATMENT OF OPIOID DEPENDENCE: FROM MECHANISMS OF ACTION TO CLINICAL EFFICACY
Genís Oña Esteve

GENERAL SUMMARY

Substance use disorders remain one of the most challenging health problems to address. Specifically, opioid dependence has caused serious public health issues in countries such as the United States and Canada over the last decade, underscoring the need for innovative and effective treatments.

Recently, mental health researchers have shown a renewed interest in psychedelic drugs. Substances such as lysergic acid diethylamide (LSD), psilocybin mushrooms, and ayahuasca have shown promising results in treating conditions including major depression and anxiety disorders. Among these, ibogaine, an alkaloid found naturally in the West African plant *Tabernanthe iboga*, appears particularly effective in treating substance use disorders. However, despite its widespread underground and unsupervised use, controlled trials evaluating the safety and efficacy of ibogaine are lacking, and its mechanisms of action remain largely unknown.

In this thesis, we conducted both clinical and preclinical studies on ibogaine to provide more evidence about this molecule and to expand our understanding of it. Clinically, we performed a systematic review of adverse events in humans associated with ibogaine to collect updated safety data. Subsequently, we designed a Phase II, randomized, double-blind clinical trial. In this trial, low, single doses of ibogaine (100 mg) were administered in the context of methadone detoxification. Plasma samples from the trial were analyzed using a metabolomic approach. The systematic review and clinical trial data were complemented with a narrative review, which identified all potential ibogaine targets associated with its anti-addictive effect and provided updated mechanistic literature. Preclinically, we designed a study with mice to elucidate further mechanisms of action. Following acute administration of ibogaine, brain tissue was analyzed using transcriptomic analysis to determine the expression levels of a wide array of genes.

The clinical results were highly promising. The systematic review highlighted the need for medical supervision during ibogaine treatments due to its potential to prolong the QT interval and its complex metabolism. In the clinical trial, which included 20 patients, we observed a significant decrease in both tolerance to methadone and opioid withdrawal syndrome (OWS). As a result, 17 out of 20 patients were able to halve their methadone dose over seven days without experiencing OWS symptoms and discontinue their daily methadone use for an average of 18.03 hours. No serious adverse events were reported. Results from the metabolomic analysis suggest that ibogaine can potentially reverse the effects of chronic opioid use on energy metabolism. These findings align with the multi-target profile of ibogaine identified in the narrative review. The preclinical study revealed new potential pathways associated with ibogaine's anti-addictive effects. Specifically, genes related to hormonal pathways and synaptogenesis showed increased expression after acute ibogaine administration. Additionally, gender differences were observed, with females exhibiting changes in 28 genes compared to eight in males.

This thesis provides the first evidence of ibogaine's safety and efficacy in a Phase II study and delves deeper into its mechanisms of action through a review, a preclinical study, and an analysis of human plasma samples using innovative techniques. We conclude that ibogaine represents a promising candidate for the treatment of opioid use disorders, warranting further research.

UNIVERSITAT ROVIRA I VIRGILI
IBOGAINE FOR THE TREATMENT OF OPIOID DEPENDENCE: FROM MECHANISMS OF ACTION TO CLINICAL EFFICACY
Genís Oña Esteve

ABBREVIATIONS

ABC = ATP-binding cassette

APA = American Psychiatric Association

ATP = Adenosine triphosphate

BCRP = Breast cancer resistance protein

BDNF = Brain-derived neurotrophic factor

cAMP = Cyclic adenosine monophosphate

CNS = Central Nervous System

CPP = Conditioned place preference

CRF = Corticotropin-releasing factor

CYP2D6 = Cytochrome P450 2D6

CYP3A4 = Cytochrome P450 3A4

DAT = Dopamine transporter

DOR = Delta opioid receptor

DSM-V-TR = Diagnostic and statistical manual of mental disorders V – Text revision

eCRF = Electronic case report form

EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine

GABA = Gamma-aminobutyric acid

GDNF = Glial cell line-derived neurotrophic factor

hERG = Human ether-a-go-go-related gene

IBO = Ibogaine

KOR = Kappa opioid receptor

LC = Locus coeruleus

MOR = Mu opioid receptor

NAC = Nucleus accumbens

nAChR = Nicotinic acetylcholine receptor

NIDA = National Institute on Drug Abuse

NMDA = N-Methyl-d-aspartate

NMR= Nuclear magnetic resonance

NOR = Noribogaine

NTFs = Neurotrophic factors

OUD = Opioid use disorder

OWS = Opioid withdrawal syndrome

PAG = Periaqueductal gray

P-gp = P-glycoprotein

PNSD = Plan Nacional Sobre Drogas

RCT = Randomized and controlled clinical trial

RT-qPCR = Real-time quantitative polymerase chain reaction

SERT = Serotonin transporter

SUD = Substance use disorder

US = United States

VTA = Ventral tegmental area

Table of contents

List of figures and tables

Figures

Tables

- Publication 4

INTRODUCTION

1. Introduction

Drug use has been highly prevalent throughout human cultures and historical periods, serving various purposes such as medicinal, recreational, and religious (Crocq, 2007). Often, drugs have been mixed and combined for these purposes. Drug use is also a common behavior among other mammals and is thus considered a trait shared by certain animals, including humans (Siegel, 2005). However, while drug use may be, to some extent, *normal* across different species, the development of drug dependence is more characteristic of humans since there is little evidence to suggest that animals become addicted to substances in nature (Siegel, 2005).

Opioids, which include extracts or substances isolated from the opium poppy (*Papaver somniferum*) as well as semi- or fully synthesized molecules that bind to opioid receptors, are one of the drug classes most strongly associated with the development of dependence. Due to their unique analgesic properties, opioids have been highly valued and widely consumed.

The problems associated with opioid dependence, or opioid use disorder (OUD), began to escalate on a larger scale after the 1980s. During this period, the attitude towards the use of opioids shifted from "opiophobia" to increased demands from both pain specialists and patients for the expanded use of these drugs in appropriate medical care (Lyden & Binswanger, 2019; Morgan, 1985). A publication (Porter & Jick, 1980) consisting of a single paragraph was often cited as evidence of the low dependence potential of opioids. This situation was exacerbated by misleading techniques and fraudulent practices used by pharmaceutical companies, leading to a nationwide public health crisis in certain countries, especially the United States (Humphreys et al., 2022; Keefe, 2021).

Unfortunately, effective treatments for tackling substance use disorders (SUDs), particularly OUD, are lacking. Currently, only substitution therapies are available, and there is no commercialized drug specifically indicated for treating symptoms of OWS, craving, or tolerance.

Interestingly, recent interest in the potential applications of psychedelic drugs has highlighted the “anti-addictive” effects of ibogaine, a naturally-occurring alkaloid found in the plant *Tabernanthe iboga*. Case reports and observational studies suggest that this molecule could diminish OWS, craving, and tolerance to various drugs, including opioids. In the context of this thesis, the mechanisms of ibogaine will be explored through a narrative review and a preclinical study involving transcriptomic analysis. In addition, the adverse event profile of ibogaine in humans and its preliminary safety and efficacy will be assessed through a systematic review and the first Phase-II, randomized and double-blind trial with this substance.

1.1. Substance use disorders

The use of drugs by non-human animals tends to be seasonal and does not affect their survival or reproduction (Calvey, 2019). In contrast, humans are at risk of becoming dependent on drugs and can potentially suffer lethal consequences. Some authors have adopted evolutionary perspectives, suggesting that this difference between humans and other animals may be due to the self-domestication process that humans underwent during the last 2000-5000 years (Calvey, 2019). This process could have altered the function of the dopaminergic system, leading to specific vulnerabilities to SUDs. A similar process has been observed in domesticated versus wild

monkeys; the former show interest in psychedelic mushrooms, while the latter are generally afraid of them (Siegel, 2005).

The phenomenon of drug dependence should be framed within the bio-psycho-social model, as various factors, including the environment, stress, socioeconomic status, and epigenetic changes, modulate this behavior in a complex manner (Pedrero-Pérez, 2015). However, we can also describe the establishment of drug dependence from a biochemical perspective.

Most people who use drugs of abuse do not develop SUDs (Cruz, 2015; Nicholson et al., 2002). Therefore, the main interest in the field of drug dependence is to elucidate the brain mechanisms responsible for the transition from hedonic, non-problematic drug use to the development of SUDs. This transition involves multiple reinforcing cycles that lead to the establishment of pathologic behavioral patterns. This process can be divided into three different phases: the binge/intoxication phase, the withdrawal/negative stage, and the preoccupation/anticipation (craving) stage (Koob & Volkow, 2010) (See Figure 1).

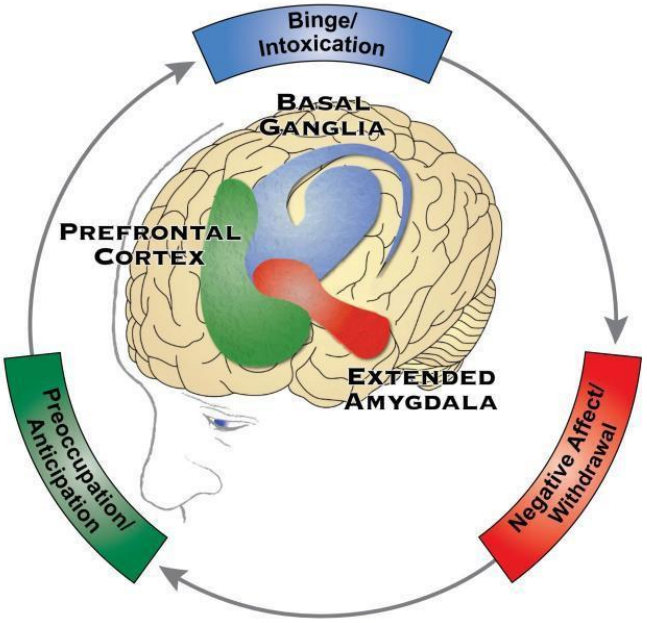


Figure 1. Phases of drug use with the main associated brain areas. Source: National Institute on Alcohol Abuse and Alcoholism (National Institute of Health, US). Available online at: https://www.niaaa.nih.gov/publications/cycle-alcohol-addiction

The first phase of binge/intoxication is related to the "reward system," which was initially described in the brain through electrical stimulation and intracranial self-stimulation (Olds & Milner, 1954). Although this system involves extended neurocircuitry, the most sensitive areas, defined by the lowest thresholds, are localized in the trajectory of the medial forebrain bundle that connects the ventral tegmental area (VTA) to the basal forebrain (Olds & Milner, 1954). Initially, it was thought that dopamine released in these areas, also known as the mesolimbic dopamine system, played the primary role in creating the feeling of reward and thus

perpetuating drug use. However, it was later discovered that other neurotransmitters also play a significant role in creating feelings of reward (Lewis et al., 2021). Dopamine function is currently understood more in terms of generating salience to stimuli (Robinson & Berridge, 1993). Additionally, the speed at which dopamine is released is essential in determining whether something is potentially rewarding and susceptible to impulsive or pleasurable behaviors (Schultz, 2007).

Concerning the withdrawal/negative stage, the extended amygdala plays a central role as a substrate that integrates different brain systems for stress and arousal. The extended amygdala receives numerous afferents from limbic structures such as the basolateral amygdala and hippocampus and sends efferences to the medial part of the ventral pallidum and a large projection to the lateral hypothalamus (Volkow et al., 2019). This substrate contributes to the emergence of negative emotional states in the absence of acute drug use, thus reinforcing drug dependence.

Dopamine is also strongly associated with the withdrawal stage, as chronic drug use leads to neuroadaptations that compromise dopamine systems during withdrawal. This results in decreased dopamine release in response to non-drug rewards, diminishing motivation for other activities (Melis et al., 2005; Volkow & Morales, 2015). In addition, the activity of the hypothalamic-pituitary-adrenal axis and the corticotropin-releasing factor (CRF) is enhanced during withdrawal, leading to anxiety-like responses (Koob & Volkow, 2010).

The preoccupation/anticipation (craving) stage appears to be a key element for relapse, and as a result, SUDs are sometimes categorized as chronic disorders. In preclinical research, two dimensions of craving have been defined: drug-induced reinstatement (McFarland & Kalivas, 2001) and cue-induced reinstatement (when elements or contexts related to drug use are present and trigger craving) (Everitt & Wolf, 2002). The former is more associated with the medial prefrontal cortex/nucleus accumbens/ventral pallidum circuit, primarily mediated by glutamate, while the latter involves the basolateral amygdala, which may interact with the prefrontal cortex in drug-induced reinstatement (Kalivas & O'Brien, 2008; Shaham et al., 2003). Animal studies on this stage have shown deficits in tasks involving the orbitofrontal, the prefrontal cortex, and the hippocampus (Jentsch et al., 2002; Schoenbaum et al., 2004). Similar results have been found in studies involving humans. For instance, individuals with cocaine use disorder exhibit impaired cognitive functions mediated by the medial and orbital prefrontal cortices, as well as memory deficits mediated by the hippocampus, which can predict treatment outcomes (Aharonovich et al., 2006).

1.2. Opioid use disorder

Opioid use disorder specifically refers to the problematic use of opioid drugs. Products derived from the plant *Papaver somniferum* (opium poppy) are known as opiates, with morphine and codeine being among them (See Figure 2). Both drugs are extensively used to treat certain types of pain or dry cough. In addition to substances naturally found in the *P. somniferum*, semi-synthetic (heroin) or synthetic opioids (fentanyl, methadone; see Figure 2) have been developed to create highly efficacious pain treatments.

Natural opiates, and more recently, synthetic opioid drugs, have been utilized for countless years in European and Asian territories. Aside from their analgesic properties, these substances

also induce psychoactive/euphoric effects. Their potential to generate dependence has also been recognized since antiquity. For instance, the Persian physician Imad al-Din Mahmud Shirazi wrote a treatise on opium in the 16th century (Shirazi, 2011), dedicating several chapters to its harmful aspects. Concerning dependence, he noted the difficulties faced by several individuals who were unable to cease their use of opium during Ramadan due to withdrawal symptoms. He suggested using rectal and slow-release oral opium products at night to prevent these symptoms.

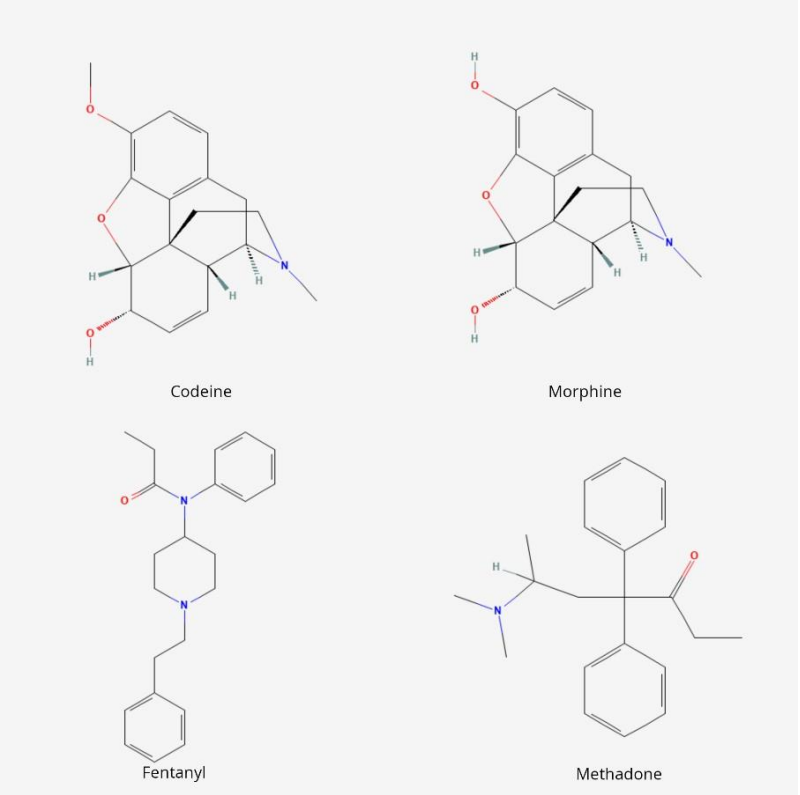


Figure 2. Chemical structures of different opioids. Available online at https://pubchem.ncbi.nlm.nih.gov/compound/5284371 (codeine); https://pubchem.ncbi.nlm.nih.gov/compound/5288826 (morphine); https://pubchem.ncbi.nlm.nih.gov/compound/3345 (fentanyl); https://pubchem.ncbi.nlm.nih.gov/compound/4095 (methadone).

The latest edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V-TR) (APA, 2022) states that OUD can be diagnosed when at least two of the following criteria are met:

1. Opioids are often taken in larger amounts or over a longer period than was intended.

2. There is a persistent desire or unsuccessful efforts to cut down or control opioid use.

3. A great deal of time is spent on activities necessary to obtain the opioid, use the opioid, or recover from its effects.

4. Craving or a strong desire or urge to use opioids.

5. Recurrent opioid use resulting in a failure to fulfill major role obligations at work, school, or home.

6. Continued opioid use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of opioids.

7. Important social, occupational, or recreational activities are given up or reduced because of opioid use.

8. Recurrent opioid use in situations in which it is physically hazardous.

9. Continued opioid use despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance.

10. Tolerance, as defined by either of the following:

- A need for markedly increased amounts of opioids to achieve intoxication or desired effect.
- A markedly diminished effect with continued use of the same amount of an opioid.

11. Withdrawal, as manifested by either:

- The characteristic opioid withdrawal syndrome.
- Taking opioids (or a closely related substance) to relieve or avoid withdrawal symptoms.

In terms of severity, 2-3 criteria are considered mild, 4-5 moderate, and 6 or more indicates severe OUD on the spectrum (APA, 2022). Three key aspects of these criteria are particularly relevant in clinical terms. First, the overwhelming desire to obtain and use opioids despite personal, social, and professional consequences. Second, the development of opioid tolerance, where increasing doses are needed to achieve the same effect. Third, the onset of OWS upon cessation of opioid use. The following section will briefly introduce the pharmacological mechanisms underlying tolerance and OWS.

1.2.1. *Pharmacological and clinical aspects of the opioid use disorder*

Opiates are characterized by their agonistic activity on opioid receptors, which are G-protein coupled receptors with seven transmembrane subunits. In the human body, there are three main opioid receptors: mu (μ; MOR), delta (δ; DOR), and kappa (k; KOR). These receptors derive their names from their prototypical agonists (morphine, N-allylnormetacine, and ketocyclazocine, respectively; Martin, 1979). Endogenous opioid peptides (endorphins, enkephalins, and dynorphins) modulate distinct functions by interacting with central or peripheral MOR, DOR, and KOR receptors. These functions include nociception, appetite, respiration, reward processing, and gastrointestinal motility. These peptides generally show low selectivity for specific receptor types, except for endomorphins (Zadina et al., 1997). In contrast, synthetic peptides and alkaloids can demonstrate high selectivity for KOR, MOR, or DOR

receptors, which is why these compounds have been used to define the distinct pharmacological properties of each receptor (Feng et al., 2012). Synthetic opioids can be broadly categorized into four chemical groups: morphinan derivatives, diphenylheptane derivatives, benzomorphan derivatives, and phenylpiperidine derivatives (Pathan & Williams, 2012).

Each of the three receptor subtypes has different characteristics, with the MOR being the most extensively studied (Taylor & Manzella, 2016). MOR is predominantly distributed in the Central Nervous System (CNS), particularly in the spinal cord, brainstem, thalamus, and cerebral cortex (Trescot et al., 2008). Activation of MOR primarily produces desired effects such as analgesia and euphoria (Bodnar, 2013), but it also leads to undesired effects, including dependence, tolerance development, respiratory depression, nausea and vomiting, and constipation (Akbarali et al., 2014; Brownstein, 1993; Christie, 2009; Mutolo et al., 2007).

The prototypical MOR agonist is morphine, which was isolated in 1805 (Sertürner, 1817). With the invention of the hypodermic needle in 1853, its clinical use expanded significantly, primarily for pain management. Analgesia induced by morphine and other MOR agonists involves the activation of MOR receptors located on the presynaptic terminals of nociceptive C-fibers and A-delta fibers. This activation indirectly inhibits voltage-dependent calcium channels, thereby reducing cyclic adenosine monophosphate (cAMP) levels and inhibiting the release of neurotransmitters, including glutamate, substance P, and calcitonin gene-related peptide from the nociceptive fibers (Trescot et al., 2008). Key brain regions involved in these processes include the periaqueductal grey (PAG) and the nucleus reticularis paragigantocellularis (NRPG), which are modulated through the inhibitory pathways stimulated by MOR agonists (Pathan & Williams, 2012).

The analgesic and other desired effects of MOR agonists are unfortunately accompanied by certain undesired effects, most notably the induction of drug dependence and abuse potential (Zhang et al., 2022). MOR agonists likely induce drug dependence by suppressing a potent GABAergic input originating from the rostromedial tegmental nucleus. Additionally, evidence suggests that inhibiting GABAergic input from D2-expressing neurons in the nucleus accumbens (NAC) may contribute, albeit to a lesser extent, to these rewarding effects (Matsui et al., 2014). Moreover, the activation of MOR suppresses GABA interneurons in the hippocampus, enhancing the activity of pyramidal cells and potentially supporting learning and memory processes, particularly those concerning drug-related experiences (Madison & Nicoll, 1988; Zieglgansberger et al., 1979).

In the context of OUD, opioid tolerance develops quickly, necessitating higher doses over time to achieve the same effect. The phenomenon of drug tolerance is highly complex and not yet fully understood. However, it is at least partially mediated through MOR desensitization and internalization. The regulation of receptor density on the cellular membrane is managed through a process known as endocytosis. This involves enclosing the receptor within a vesicle of cell membrane material, effectively creating a localized membrane "bubble" encapsulating the receptor and facilitating its internalization into the cellular body. Once hidden within the intracellular environment, the receptor undergoes a functional cessation, resulting in what is known as down-regulation. In experimental studies involving rodents, those lacking β-arrestin2 (a specific down-regulatory factor) exhibited sustained morphine-induced analgesia, whereas those possessing this regulatory factor developed morphine tolerance (Bohn et al., 2002).

Another potential mechanism involved in opioid tolerance is mediated through ATP-binding cassette transporters, mainly P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP). Both P-gp and BCRP are highly involved in effluxing the drug from the cell. Notably, animals that have developed tolerance to opioids show elevated levels of P-gp and BCRP (Mercer & Coop, 2011). In the specific case of methadone, *in vivo* studies with P-gp KO mice or rats exposed to P-gp inhibitors revealed higher brain concentrations of methadone and enhanced analgesic effects when P-gp was absent or inhibited (Rodriguez et al., 2004; Wang et al., 2004).

OWS is another significant phenomenon associated with OUD. OWS manifests with symptoms such as pain, muscle spasms, tremors, abdominal cramps, nausea, diarrhea, anxiety, insomnia, and sweating (Kosten & Baxter, 2019). While the cessation of short-acting opioids (heroin, oxycodone) is associated with severe OWS that can persist up to 7 days, OWS associated with the cessation of long-acting opioids (methadone, buprenorphine) is characterized by milder symptoms that can last for two weeks or longer (Kosten & Baxter, 2019; Kosten & O'Connor, 2003).

OWS arises from the complex adaptations that occur during the sustained use of opioid drugs, affecting most organs and tissues rich in opioid receptors. For instance, the gastrointestinal tract, which has a high density of opioid receptors, is often affected during OWS, leading to diarrhea, nausea, or vomiting (Kosten & Baxter, 2019). Insomnia is also a common symptom and may result from disturbances in the ascending reticular activating system originating in the brainstem, thalamus, and hypothalamus. The locus coreuleus (LC) is also a key brain region associated with OWS. The LC is crucial in regulating attention, vigilance, and autonomic nervous system functions. The binding of an opioid drug to μ-opioid receptors on LC neurons inhibits enzymes in the cyclic adenosine monophosphate (cAMP) pathway, reducing LC neuron firing rates and noradrenaline release. This results in acute opioid effects, including drowsiness and reduced blood pressure, respiration, and muscle tone. With the repeated use of opioids, LC neurons adapt to this opioid inhibition by increasing the supply and activity of enzymes, upregulating the cAMP pathway to restore "normal" levels of cAMP production. Therefore, the firing rate of LC and noradrenaline release returns to normal levels. However, the development of tolerance to opioids leads to LC hyperactivity upon abrupt cessation of use. This causes excessive cAMP production and noradrenaline release, lasting for days or weeks until the LC neurons readapt to the absence of opioids. This noradrenergic hyperactivity is the primary neurobiological mechanism underpinning acute OWS (Cao et al., 2010; Kosten & Baxter, 2019).

Activation of the DOR is associated with neuroprotection (Feng et al., 2012), antidepressant effects (Torregrossa et al., 2006), and peripheral analgesia (Fristad et al., 2006), but it can also induce seizures (Jutkiewicz et al., 2006). Similarly, activation of the k opioid receptor induces analgesia (Brownstein, 1993), antidepressant effects (Harrison, 2013), and neuroprotection (Chunhua et al., 2014) but is also linked to psychotomimetic effects (Ona et al., 2022; Pfeiffer et al., 1986) and dysphoria (Walsh et al., 2001). Several selective DOR and KOR agonists have been developed to avoid agonist activity at the MOR and, therefore, achieve opioid analgesia without the side effects of drug dependence (Isbell, 1977). TAN-67 was the first selective DOR agonist developed (Nagase et al., 1992), while SNC80 derivatives have been among the most frequently studied compounds in this class (Calderón, 2011). However, despite their lack of addictive properties, many of these compounds have shown side effects such as seizures or catalepsy, which have hindered their further clinical development (Nagase & Saitoh, 2020). Selective KOR agonists have demonstrated analgesic effects and promising anti-addictive properties (Kivell et

al., 2014). Nonetheless, severe aversive psychotomimetic effects and dysphoria have limited their clinical application (Piercey et al., 1982).

In conclusion, opioid receptor agonists, despite their clinical utility, present significant challenges that limit their broader application in medical practice. Foremost among these challenges is the propensity of these agents to induce drug dependence, accompanied by OWS upon cessation. Furthermore, the rapid onset of tolerance to these agents poses a substantial barrier to sustained use. Despite the extensive historical utilization of opioid agonists and the longstanding recognition of these issues, there remains a pressing need for the development of effective strategies to mitigate these adverse effects, ensuring safer and more sustainable clinical use.

1.2.2. The current opioid use disorder epidemic

During the 19th century, morphine and heroin, along with whole extracts or refined opium from *P. somniferum*, were commercially promoted to physicians and patients as reliable and effective means of relieving pain and other conditions (Lyden & Binswanger, 2019). Most of these remedies remained in use by both physicians and the lay public until the 20th century. By 1914, the problems associated with opioid dependence had become evident, leading to the introduction of the first United States law regulating the production, importation, and distribution of opiates.

In the late 20th century, the synthetic opioid oxycodone was marketed as a non-addictive medication as a result of commercial and illegal practices of the company selling it, which was later convicted on criminal charges (DOJ, 2020). This led to three major “waves” of opioid overdoses. The first wave occurred in the 1990s when opioid prescriptions significantly increased, resulting in overdose deaths involving prescription opioids (including natural and semi-synthetic opioids and methadone). The second wave began in 2010 with a sharp rise in overdose deaths involving heroin, and the third wave started in 2013, marked by significant increases in overdose deaths involving synthetic opioids, particularly illicitly manufactured fentanyl (CDC, 2023). Notably, between 2005 and 2014, opioid-related hospitalizations in the United States increased by 64%, and opioid overdose death rates rose by 27% between 2015 and 2019 (Jones et al., 2015; Martins et al., 2015; Weiss et al., 2018). Similar trends have been observed in Canada (Belzak & Halverson, 2018).

In Spain, the most severe opioid crisis occurred during the 1980s and part of the 1990s, when intravenous heroin use led to a significant increase in morbidity and mortality, criminality, and cases of HIV and HCV infections associated with its route of administration, causing major social alarm (Gamella, 1994). The response to this crisis did not emerge until 1985 when the first National Drug Plan (PNSD) was created. In addition, harm reduction strategies were progressively introduced to mitigate the damage caused by illicit heroin use (Martínez-Oró & Pallarés, 2013).

The problems associated with the use of oxycodone and fentanyl have been mostly restricted to the United States and Canada. While these drugs are also clinically used in Spain, they have not caused comparable public health harms. This may be attributed to better harm reduction strategies, a higher quality public health system, and the absence of strong and widespread informal opioid markets, among other reasons (Martínez-Oró, 2019). Conversely, the opioid

crisis that occurred in Spain decades ago continues to have consequences related to iatrogenic dependence on treatments offered to people with OUD, as described in the following sections.

1.2.3. Available Treatments for Opioid Use Disorder

The first approach to treating OUD often involves detoxification (medically supervised withdrawal) to interrupt opioid use. However, these interventions rarely yield results over the mid- or long-term and may increase the risk of overdose as the patient's tolerance diminishes and usual doses become potentially fatal (Strang et al., 2003). Following detoxification, rehabilitation programs are necessary to offer psychological support, which can further enhance the benefits of initial treatment (Schuckit, 2016).

As previously stated, abrupt discontinuation of opioids leads to OWS, a group of symptoms largely opposed to the central actions of opioids (Farrell, 1994; Torres-Lockhart et al., 2022). Patients fear OWS because they perceive it as severe subjective suffering. This fear of withdrawal symptoms is one of the main reasons why clinical detoxification is so challenging.

To achieve detoxification without the onset of OWS, two main treatments are used: methadone and buprenorphine. These treatments are based on opioid replacement therapy, which involves administering other opioids that are not associated with euphoric effects and have much longer half-lives. This approach maintains consistent drug levels in the body, helps alleviate craving and OWS, and enables individuals to better engage in daily activities (Schuckit, 2016).

Buprenorphine, a semi-synthetic opioid and partial μ agonist, is widely used in detoxification. It is also an antagonist at the k opioid receptor, which prevents hyperalgesia, an effect commonly induced by classic opioids due to the binding of the up-regulated dynorphin at the k receptor (Silverman, 2009). Buprenorphine does not recruit β-arrestin to the receptor, which is associated with adverse effects, such as respiratory depression, constipation, and tolerance. Therefore, it is considered a safer option than traditional opioids and is often prescribed to patients with other risk factors that limit their use (e.g., comorbid respiratory disease or co-prescribed benzodiazepines) (Spreen et al., 2022).

Methadone maintenance programs are more widely implemented in Spain and serve as the primary treatment for OUD. Thus, the following section will delve deeper into this treatment approach.

1.2.3.1. Methadone

Methadone is a synthetic opioid and diphenylheptane derivative that acts as a full agonist at the μ opioid receptor and a noncompetitive antagonist at the *N*-methyl-d-aspartate (NMDA) receptor. Methadone has two enantiomeric forms, d and l isomers. The l isomer is responsible for analgesic effects and has been found to have twice the analgesic potency of morphine (Mercadante & Bruera, 2018; Sim, 1973). Although once thought to be completely inactive, recent studies have shown that the d isomer has NMDA receptor antagonist activity and may play a part in morphine tolerance (Inturrisi, 2005).

Methadone was first researched as a treatment for drug dependence by H. Isbell in 1947. He administered up to 800 mg/day to both animals and humans and reported the phenomena of

tolerance and OWS (Isbell, 1947). Ironically, Isbell later collaborated with the CIA's MK-ULTRA project, which involved testing hallucinogens, including ibogaine, on both the general and incarcerated populations, sometimes without prior consent.

In 1965, the pharmacokinetic properties of methadone were described, revealing that its long-lasting effects are achieved more effectively through oral administration (Dávila & Navarro, 1998). Methadone has a half-life of 8-59 hours, which is longer in opioid naïve individuals and shorter in opioid-dependent subjects (Grissinger, 2011). Studies have shown that after thirty days of daily doses of methadone (40-80 mg) (Verebely et al., 1975) or after 5–12 months of treatment with 60-80 mg/day (Holmstrand et al., 1978), plasma levels of methadone substantially decrease due to the self-induction of its metabolism. This necessitates dose adjustments for some patients in methadone maintenance programs (Horns et al., 1975; Victorri-Vigneau et al., 2019).

Methadone is mostly metabolized through the CYP3A4 and CYP2D6 enzymes (Fonseca, 2010; Foster et al., 1999). Its main metabolite is EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine), an inactive molecule.

In 1965, methadone was first proposed as a substitute for heroin (Dole & Nyswander, 1965). Although various hospitals in the United States used methadone as substitution therapy during the 1960s and 1970s, it was not until the 1980s and 1990s that methadone maintenance programs were established in different countries under a harm reduction approach.

However, despite the advances and benefits brought by methadone programs, missing a daily dose can lead to severe OWS, making it challenging to discontinue its use (Amato et al., 2013; Mattick et al., 2014). Consequently, a significant portion of patients in methadone maintenance programs are unable to stop using it, resulting in iatrogenic drug dependence. Additionally, methadone has long-term adverse effects that raise concerns, including cognitive decline (Baldacchino et al., 2017), oral health issues (Brondani & Park, 2011), and the potential risk of respiratory depression and subsequent death, as methadone is a full opioid agonist (Drummer, 1992).

Methadone maintenance treatments have proven effective in keeping patients engaged in their treatment programs and reducing the use of illegal opioids (Farré et al., 2002). Furthermore, these programs reduce the behaviors that increase the risk of HIV and other sexually transmitted infections such as hepatitis C (Gibson et al., 1999). They also contribute to a reduction in criminal activities associated with drug use and decrease the likelihood of overdose deaths (Brugal et al., 2005). However, there is a high number of non-responders (30-80%) (Bell et al., 2006), and long-term treatment is associated with iatrogenic dependence.

In Spain, methadone maintenance programs could not be implemented until 1990 due to political issues. The existing legislation was modified with the enactment of a Royal Decree that facilitated the distribution of responsibilities to autonomous communities (Fuente et al., 2006). Currently, 49,014 individuals are enrolled in methadone programs in Spain (OEDA, 2022). Even when participants in these programs are well-adapted and not actively using drugs of abuse, they still need to take a daily dose of methadone due to OWS (Gutiérrez-Cáceres et al., 2019). Unfortunately, we lack effective treatments for the detoxification of methadone. The usual procedure involves a very slow dose tapering process that can take years. However, many people experience OWS symptoms even when reducing the dose by 1 or 2 mg, causing them to stagnate at a certain dose. Additionally, individuals with comorbid mental health disorders or

pain conditions benefit more from methadone, making its discontinuation even more challenging.

1.3. Ibogaine as a Potential Solution

Ibogaine (see Figure 3) is one of the alkaloids found in the *Tabernanthe iboga* shrub. Known for its powerful psychoactive effects, it is often described as an "atypical psychedelic" due to its distinct binding profile. Ibogaine exhibits affinity for various receptors, including opioid and glutamate receptors, among others. Unlike other psychedelic drugs, ibogaine also carries cardiovascular risks that are not typically associated with these drugs.

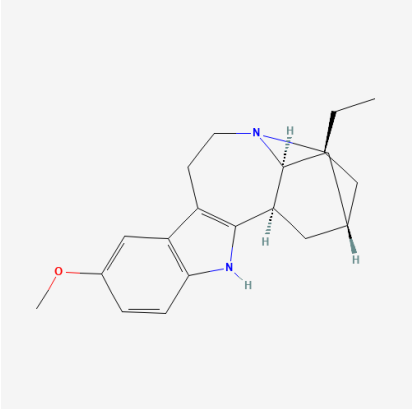


Figure 3. Chemical structure of ibogaine. Available online at: https://pubchem.ncbi.nlm.nih.gov/compound/197060

T. iboga was introduced to Western countries in 1864 when botanical samples from Gabon were classified in France. In 1900, ibogaine was isolated from the root bark simultaneously by two groups: Dybowski and Landrin and Haller and Heckel. Its pharmacodynamic properties were explored during the first decade of the 20th century. At that time, ibogaine was recommended as a treatment for asthenia and fatigue, with doses ranging from 10 to 30 mg/day. From 1939 to 1970, ibogaine was commercially available in France under the name "Lambarène" as a neuromuscular stimulant. It was sold in 8 mg tablets and used to help individuals overcome fatigue, depression, and infectious diseases (Goutarel et al., 1993; Wasko et al., 2018). Other products containing ibogaine or extracts of ibogaine-containing *Tabernanthe* species were also commercialized. A manuscript uncovering the early history of ibogaine, written by the author of this thesis, is currently being processed for publication. This article discovered that these products were used in Brazil and Mexico in the early 20th century.

The serendipitous discovery of ibogaine's potential anti-addictive properties is credited to Howard Lotsof (Alper & Lotsof, 2007). Lotsof, a regular heroin user, participated in informal meetings in New York between 1962 and 1963, where the subjective and therapeutic effects of various drugs were explored. During one of these meetings, ibogaine was used by 20 individuals, including seven heroin users. They took at least 19 mg/kg of ibogaine and observed that

symptoms of OWS disappeared in five out of seven people the following day. These individuals remained abstinent for over six months (Alper & Lotsof, 2007).

In 1982, Howard Lotsof established the non-profit Dora Weiner Foundation to legitimize the use of ibogaine for treating drug dependence (Brown, 2013). In 1986, Lotsof founded the private company NDA International, which provided funding and support for preclinical research on the anti-addictive effects of ibogaine (Alper et al., 2001). Research conducted in Rotterdam resulted in the first scientific publication demonstrating the efficacy of ibogaine in attenuating OWS (Dzoljic et al., 1988).

Following Lotsof's discovery, preclinical studies were conducted to investigate the effects of ibogaine on drug self-administration. These studies demonstrated a reduction in the self-administration of cocaine, opioids, and alcohol (Cappendijk & Dzoljic, 1993; Glick et al., 1991, 1994; Sershen & Lajtha, 1994; Dworkin et al., 1995; Rezvani, 1995). Notably, a dose-dependent relationship was established for this effect (Glick et al., 1991, 1994). Similar findings were observed in studies involving food reinforcement programs (Dworkin et al., 1995). These results suggest that the effects of ibogaine on drug self-administration are not specific to particular substances but rather affect a general mechanism involved in the brain's reward circuitry (Dworkin et al., 1995).

A study conducted with rats reported a reduction in alcohol self-administration. Interestingly, this effect was observed when ibogaine was administered through intraperitoneal and intragastric routes but not when administered subcutaneously. This suggests that noribogaine, the primary metabolite of ibogaine, may play a crucial role in its anti-addictive effects (Mash, 2023; Rezvani et al., 1995).

However, a meta-analysis conducted by Belgers et al. (2016) concluded that ibogaine does not modify conditioned place preference (CPP) in rats dependent on morphine or amphetamine. It is important to note that CPP is a paradigm used to assess Pavlovian conditioning, which involves automatic and involuntary responses. On the other hand, drug self-administration includes both Pavlovian conditioning and operant conditioning, the latter being related to voluntary behavior. Therefore, studies utilizing the self-administration paradigm have greater translational relevance in understanding the effects of ibogaine on SUDs.

Overall, preclinical studies have demonstrated the potential of ibogaine to reduce drug self-administration, indicating its anti-addictive effects. However, further research is needed to elucidate the underlying mechanisms and to determine the clinical efficacy and safety of ibogaine for treating substance use disorders.

Following promising preliminary evidence, in 1993, the FDA advisory group on drug abuse granted Dr. Deborah Mash's team at Miami University approval for a Phase I pharmacokinetic study of ibogaine. In 1995, the FDA authorized a protocol for a study involving individuals dependent on cocaine and opioids (Mash, 2010). Around the same time, the National Institute on Drug Abuse (NIDA) developed Phase I and Phase II protocols for the use of fixed doses of 150 mg and 300 mg of ibogaine versus placebo in the treatment of cocaine dependence (Alper, 2001). Simultaneously, the Erasmus University in Rotterdam was planning a clinical trial involving human subjects (Alper et al., 2001).

Unfortunately, these projects faced a setback in 1993 when a fatality occurred in the Netherlands. A woman participating in a clinical trial with ibogaine died in a non-medical

context. Although no causal relationship was established between ibogaine use and the patient's death, this incident led to the discontinuation of treatments supported by NIDA and made it challenging to secure funding for further studies (Alper et al., 2001; Brown, 2013).

As a result, the use of ibogaine has become increasingly limited to alternative and informal settings. An international network of ibogaine providers has emerged, forming a "medical subculture" around the substance (Alper et al., 2008). Many individuals seeking alternative treatment for drug dependence turn to this medical subculture in their quest for help.

1.3.1. Ibogaine as a Treatment for Opioid Use Disorder: Preclinical and Clinical Evidence

Ibogaine appears to be particularly effective in treating OUD (Brown & Alper, 2018; Davis, 2017). As early as 1957, before its potential for treating drug dependence was fully recognized, Ciba Pharmaceuticals (now Novartis) filed a patent for ibogaine with the US Patent Office, specifically for its application in reducing opioid tolerance (US Patent Office, 1957). This early interest can be attributed to the experiments by Isbell with ibogaine, which were mentioned in the previous section. Thus, unpublished literature has already identified some of its anti-addictive effects.

Preclinical studies have demonstrated that ibogaine, at doses ranging from 20 to 80 mg/kg, effectively eliminates the withdrawal syndrome induced by naloxone or naltrexone in morphine-dependent rats (Dzoljic et al., 1988; Glick et al., 1992). However, another study (Sharpe & Jaffe, 1990) that administered ibogaine subcutaneously at doses of 5-40 mg/kg to morphine-dependent rats did not observe a reduction in withdrawal syndrome. This finding further underscores the importance of ibogaine metabolism in inducing its anti-addictive effects.

Various studies have reported divergent findings regarding the dosage and timing of ibogaine administration, particularly concerning the induction of withdrawal syndrome using naloxone. Administration of doses ranging from 40 to 80 mg/kg before naloxone has demonstrated a reduction in OWS symptoms (Popik et al., 1995). Conversely, a dose of 30 mg/kg administered after naloxone did not exhibit the same symptom-reducing effects (Francés et al., 1992).

Regarding morphine, a study conducted on rats indicated that a reduction in self-administration persisted for 72 hours in some animals, suggesting potential positive long-term effects. However, other animals required repeated doses of ibogaine to achieve a similar outcome (Glick et al., 1991).

Human studies have also provided data on the effectiveness of ibogaine in treating drug dependence, as indicated by case reports. Alper et al. (1999) reported on 25 cases (from an initial sample of 33) involving opiate and cocaine-dependent patients who experienced elimination of withdrawal symptoms for up to 72 hours following ibogaine treatment. The administered doses of ibogaine ranged from 6 to 29 mg/kg. Unfortunately, one death occurred within the sample, which was attributed to concurrent heroin use.

In an observational study, complete abstinence from opiates was reported in 12 out of 14 opiate-dependent patients, with this abstinence maintained for up to 12 months (Noller et al., 2018). The patients received an initial dose of 200 mg of ibogaine, followed by a subsequent dose ranging between 400 and 600 mg, administered 1 to 4 hours

later. Multiple low doses of 200 mg were then administered, with an average treatment duration of 60 hours.

A case report documented a successful treatment using ibogaine with a similar protocol but over an extended duration. The treatment involved administering ascending doses of ibogaine weekly for six weeks (Wilkins et al., 2017). Another study examined 191 cases of patients dependent on cocaine and opiates who underwent ibogaine treatment (8-12 mg/kg). The findings revealed significant reductions in craving for both substances and a favorable safety profile (Mash et al., 2018). Additionally, an observational study (Brown & Alper, 2018) included a long-term follow-up of 30 opiate-dependent patients who received high doses of ibogaine (1500 mg ± 900 mg) as a treatment. One month after the treatment, 50% of the patients remained abstinent from opiates. After three months, 33% maintained abstinence, followed by 23% at six months and 20% at twelve months. In certain case reports and open-label studies, medium to high doses of ibogaine were administered to individuals with methadone dependence (dos Santos et al., 2017).

Presently, three randomized controlled trials (RCTs) are underway to assess the safety and efficacy of ibogaine in treating substance use disorders. One industry-funded trial is focused on opioid use disorder [ClinicalTrials.gov ID NCT05029401]. Another independent study investigates its application for alcohol use disorder [ClinicalTrials.gov ID NCT03380728], while the third independent study, which is also the primary project of the current thesis, concentrates on treating opioid use disorder, specifically methadone dependency [ClinicalTrials.gov ID NCT04003948]. Consequently, it is anticipated that in the forthcoming years, the availability of data regarding the safety and efficacy profiles of ibogaine will significantly improve. The inclusion of clinical data obtained from randomized and controlled designs is essential for a comprehensive understanding, as it can provide clearer insights into the potential mechanisms of action of ibogaine, elucidating both its presumed anti-addictive effects and associated risks.

1.3.2. Overall pharmacology of ibogaine

Ibogaine undergoes extensive first-pass metabolism in the liver, primarily by cytochrome P4502D6 (CYP2D6) enzymes, converting it to noribogaine, the active metabolite. The pharmacokinetic properties of ibogaine and noribogaine differ significantly. In a non-randomized clinical setting, administering ibogaine at doses ranging from 500 to 1,200 mg showed rapid absorption from the gastrointestinal tract. The median time to reach peak plasma concentration (t_{max}) for ibogaine ranged from 1.75 to 4 hours, while for noribogaine, it ranged from 4 to 10 hours (Mash et al., 2018). The elimination half-life (t½) of ibogaine varied from 2.4 to 7.6 hours, whereas the t½ of noribogaine could not be determined but is believed to be much longer (Mash et al., 2018). Both ibogaine and noribogaine exhibit a large volume of distribution (Vd), with central values of 185 L and 47.5 L and peripheral values of 789 L and 1.022 L, respectively (Henstra et al., 2017). The slow elimination of ibogaine and noribogaine can be attributed to their lipophilic nature and enterohepatic circulation (Glue et al., 2015a).

Glue et al. (2015b) conducted a study on the pharmacokinetic properties of a low dose (20 mg) of ibogaine in healthy volunteers. The participants were divided into two groups: one group received pre-treatment with paroxetine, a potent CYP2D6 inhibitor, and the other received a placebo as a pre-treatment. This allowed the researchers to observe the influence of CYP2D6 on the pharmacokinetic parameters of ibogaine. The participants who received the placebo pre-

treatment showed very low plasma concentrations of ibogaine (C_{max} = 1.1) with a half-life of 2 to 5 hours. In contrast, the concentrations of noribogaine were significantly higher (C_{max} = 18.7) with a half-life of 13 hours. In the group that received paroxetine pre-treatment, higher plasma concentrations of ibogaine were observed (C_{max} = 29.5) and lower noribogaine plasma concentrations (C_{max} = 12.7), with respective half-lives of 10.2 and 20 hours. These results differ from those observed by Mash et al. (2001), where significant first-pass metabolism mediated by CYP2D6 was observed. However, no differences were found in the C_{max} of ibogaine between fast and slow metabolizers. It should be noted that comparing the results of these two studies is complex, as the data from Mash et al. (2001) was obtained in an open-label clinical context with drug-dependent patients who received therapeutic doses of ibogaine (10 mg/kg) using whole blood, while the data from Glue et al. (2015b) was obtained from a study on healthy volunteers using sub-therapeutic doses (20 mg) and plasma samples.

In another RCT, Glue et al. (2015a) investigated the pharmacokinetics, pharmacodynamics, and safety of four doses of noribogaine (3, 10, 30, and 60 mg) in healthy volunteers. Interestingly, they found that the half-life of noribogaine was greater at low doses (3 and 10 mg), reaching 49 hours, while the half-life for larger doses (30 and 60 mg) did not exceed 29 hours. The area under the curve (AUC) and peak plasma concentration (C_{max}) showed dose-dependent behavior. A manuscript providing pharmacokinetic data on ibogaine was recently published (Knuijver et al., 2024). However, data should be interpreted cautiously because subjects were administered with metoclopramide, a CYP2D6 inhibitor, before ibogaine administration.

The mechanisms of action of ibogaine and noribogaine are still not fully understood. It is known that they interact with multiple receptor systems, including N-Methyl-D-aspartate (NMDA) receptors, opioid receptors (K1, K2, μ, and δ2), serotonin receptors (5-HT2 and 5-HT3), muscarinic receptors (M1 and M2), nicotinic receptors (α3β4), and various targets of monoamine reuptake (Maciulaitis et al., 2008). Notably, glycine, which inhibits the binding of non-competitive NMDA receptor antagonists, can block the effect of ibogaine on withdrawal syndrome (Popik et al., 1995). This suggests that the antagonist action of ibogaine on NMDA receptors contributes to its effects on withdrawal syndrome.

The binding profile of noribogaine differs from that of ibogaine. Ibogaine exhibits a higher affinity for NMDA receptors in brain tissue and strongly stimulates the hypothalamic-pituitary-adrenal axis. In contrast, noribogaine leads to a greater extracellular concentration of serotonin in the brain due to its higher affinity for the serotonin transporter. Both ibogaine and noribogaine display features of MOR antagonism, with equilibrium dissociation constant (Ki) values of 3 μM (ibogaine) and 13 μM (noribogaine) (Antonio et al., 2013). Functional studies have shown that ibogaine exerts a non-competitive, antagonist effect on nicotinic receptors (Mash et al., 1998).

Koenig and Hilber (2015) proposed a mechanism by which ibogaine may lead to cardiac arrhythmias. They found that ibogaine inhibits *hERG* (human Ether-a-go-go-Related Gene) channels, repolarizing the action potential. This inhibition results in a delay in repolarization, leading to prolongation of the QT interval and potentially causing arrhythmias and sudden death. The authors also demonstrated that ibogaine inhibits sodium and calcium flow in ventricular cardiomyocytes.

Noribogaine has been shown to increase the hydrolysis of phospholipase C and activate protein kinase C, mediating various long-term changes that may be involved in the effects of ibogaine (Zubaran et al., 1999). Additionally, it has been suggested that the anti-addictive effect of ibogaine could be partially mediated by its influence on energy metabolism (Paškulin et al.,

2006). One proposed mechanism of action focuses on the activation of glial cell-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) (He et al., 2005; Marton et al., 2019). Preclinical ethanol research has shown that ibogaine can increase GDNF RNA expression when rats reduce their ethanol intake without causing neurotoxicity or cytotoxicity. Short-term exposure to ibogaine increases GDNF expression and its messenger RNA, promoting the expression of proteins associated with activating the GDNF signaling pathway (He & Ron, 2006).

The relationship between neurotrophic factors and SUDs is not yet fully understood. A recent meta-analysis indicated that peripheral levels of BDNF are lower in active drug users, particularly those using crack/cocaine and alcohol (Ornell et al., 2018). However, the levels of BDNF can vary at different stages of drug use, with acute intake of alcohol or cocaine leading to increased BDNF levels. In contrast, chronic use is associated with lower levels (Graham et al., 2007). Overexpression or administration of neurotrophic factors has been linked to the suppression of drug-seeking behavior in various animal models (Carnicella et al., 2009; Koskela et al., 2021). Therefore, the mechanism involving the promotion of neurotrophic factors could potentially contribute to the overall anti-addictive effects of ibogaine.

Both ibogaine and noribogaine have been observed to stimulate corticosterone secretion in the adrenal cortex and prolactin secretion in the pituitary (Baumann et al., 2001). Additionally, both substances can increase plasma prolactin levels. However, the significance of these physiological modifications in relation to the pharmacological effects of these substances has not been thoroughly described in the literature.

Regarding the hypothesis of ibogaine's inhibitory effect on P-glycoprotein (P-gp), evidence suggests that P-gp plays a role in tolerance or drug resistance phenomena. Animal studies have shown that subjects developing opioid tolerance after chronic administration have up-regulated P-gp, leading to lower levels of opioids in circulation (Mercer & Coop, 2011). This has led to the proposal that efflux transporters in the blood-brain barrier (BBB), particularly P-gp, may contribute to the development of opioid tolerance. Inhibition of P-gp could potentially reduce tolerance and gradually decrease the required opioid dose to avoid the symptoms of OWS, which is an essential step in combating opioid addiction.

Ibogaine has been shown to have a potent inhibitory effect on P-gp, as described by Tournier et al. (2010). However, this property is only mentioned briefly in the literature. Other compounds with inhibitory effects on P-gp, such as curcumin, have shown promising effects in treating opioid dependence (Hu et al., 2015). Given this evidence, the effect of ibogaine on P-gp warrants further consideration.

In summary, ibogaine has emerged as a potential therapeutic agent for the treatment of SUDs, particularly OUD, as it presents relevant mechanisms for addressing various aspects of OUD, such as tolerance and withdrawal. However, most evidence is of low quality and was obtained decades ago. New mechanistic investigations using modern techniques and clinical data from controlled contexts are necessary to further assess the potential role of ibogaine as a therapeutic option.

Considering the previous sections, the objective of this thesis is to present clinical data regarding the efficacy of ibogaine as a therapeutic agent for the treatment of opioid use disorder, compile existing literature on its adverse events, and explore potential targets and mechanisms of action through both a literature review and an experimental model employing a transcriptomic approach.

HYPOTHESES AND OBJECTIVES

2. Hypotheses and Objectives

Ibogaine has been utilized for the treatment of substance use disorders (SUDs) in informal settings and private clinics in certain countries for several decades. However, its efficacy and safety profiles have not been adequately described in clinical trials. Moreover, its mechanisms of action remain unclear, potentially limiting further research. Considering the significant proportion of individuals affected by SUDs, there is a pressing need to investigate new treatment options. The purpose of this thesis is to shed some light on the potential role of ibogaine as an anti-addictive agent, as well as its safety profile and its associated mechanisms of action.

2.1. Hypotheses

- Low doses of ibogaine administered in a controlled clinical setting are safe and efficacious in reducing drug tolerance and OWS severity and produce short-term metabolic changes.

- Multiple targets are involved in the anti-addictive effects produced by ibogaine and these targets are affected in a sex dependent manner.

2.2. Objectives

Following these hypotheses, two main objectives are set out in this research, with the corresponding secondary objectives.

Objective 1: To assess the efficacy of adverse effects of low doses of ibogaine for the treatment of opioid withdrawal syndrome associated with a process of opioid detoxification.

Secondary objectives:

- To review the existing literature analyzing the adverse events of ibogaine in humans.

- To assess the adverse events and cardiac effects associated with a low dose of ibogaine (100 mg) administered during methadone detoxification within a Phase-II clinical trial.

- To assess the acute subjective effects of a low dose of ibogaine (100 mg).

- To identify metabolic changes associated to an acute low dose of Ibogaine (100 mg).

Objective 2: To identify the potential mechanisms of action and molecular targets of ibogaine.

Secondary objectives:

- To review the existing literature to provide a comprehensive and updated overview of the mechanisms of action of ibogaine.

- To identify potential targets and suggest mechanisms of action of ibogaine by conducting a transcriptomic analysis in brain tissue of mice previously exposed to ibogaine.

- To describe gender differences in the potential targets identified for ibogaine.

GENERAL METHODOLOGY

3. General Methodology

This thesis is organized into two review articles and two experimental studies. Each attached manuscript presents the detailed methodology used. Here, we briefly describe the characteristics of the studies included in the results section.

The adverse events of ibogaine in humans were assessed through a systematic review, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

The first experimental study of this doctoral thesis (an open label trial) was done as part of a randomized and double-blind clinical trial that was performed under the "Ley de Investigación Biomédica 14/2007", Spanish regulation. As part of the clinical study, we conducted a metabolomic analysis on plasma samples from 13 subjects. This analysis revealed potential changes in the metabolome caused by ibogaine and suggested previously undescribed mechanisms of action.

We conducted a narrative review to explore further the potential mechanisms of action of ibogaine and its metabolite, noribogaine.

Finally, the second experimental study involves a preclinical investigation conducted in mice to identify potential targets and mechanisms of action for ibogaine. This study examines the acute effects of oral ibogaine administration in both male and female mice. Specifically, changes in gene expression in the frontal cortex were analyzed using transcriptomic techniques, complemented by real-time quantitative PCR (RT-qPCR). This study is the first to apply omics techniques in ibogaine research.

RESULTS

4. Results

This thesis is composed of four articles addressing the objectives previously described. Table 1 describes these objectives along with the corresponding references for the articles, whether already published or currently in the process of being published.

Table 1. Summary of studies included in this thesis.

<table>
<tr><td colspan="2">Objective 1: To assess the efficacy of adverse effects of low doses of ibogaine for the treatment of opioid withdrawal syndrome associated with a process of opioid detoxification.</td></tr>
<tr><td>To review the existing literature analyzing the adverse events of ibogaine in humans.</td><td>Ona, G., Rocha, J. M., Bouso, J. C., Hallak, J. E. C., Borràs, T., Colomina, M. T., & Dos Santos, R. G. (2022). The adverse events of ibogaine in humans: an updated systematic review of the literature (2015-2020). Psychopharmacology, 239(6), 1977–1987. https://doi.org/10.1007/s00213-021-05964-y</td></tr>
<tr><td>To assess the efficacy of a low dose of ibogaine (100 mg) for the treatment of opioid withdrawal syndrome.

To assess the adverse events and cardiac effects associated with a low dose of ibogaine (100 mg) administered in a process of methadone detoxification within a Phase-II clinical trial.

To assess the acute subjective effects of a low dose of ibogaine (100 mg).

To identify metabolic changes associated to an acute low dose of Ibogaine (100 mg)</td><td>Reversing tolerance in methadone detoxification with a low dose of ibogaine
(Submitted)</td></tr>
<tr><td colspan="2">Objective 2: To identify the potential mechanisms of action and molecular targets of ibogaine</td></tr>
<tr><td>To review the existing literature providing a comprehensive and updated picture of the mechanisms of action of ibogaine.</td><td>Ona, G., Reverte, I., Rossi, G. N., Dos Santos, R. G., Hallak, J. E., Colomina, M. T., & Bouso, J. C. (2023). Main targets of ibogaine and noribogaine associated with its putative anti-addictive effects: A mechanistic overview. Journal of psychopharmacology (Oxford, England), 2698811231200882. https://doi.org/10.1177/02698811231200882</td></tr>
<tr><td>To identify potential targets and suggest mechanisms of action of ibogaine by performing a transcriptomic analysis in brain tissue of mice previously exposed to ibogaine.

To describe gender differences on the potential targets identified for ibogaine.</td><td>Biosca-Brull, J., Ona, G., Alarcón-Franco, L., & Colomina, M. T. (2024). A transcriptomic analysis in mice following a single dose of ibogaine identifies new potential therapeutic targets. Translational psychiatry, 14(1), 41. https://doi.org/10.1038/s41398-024-02773-7</td></tr>
</table>

Publication 1

The adverse events of ibogaine in humans: an updated systematic review of the literature (2015-2020)

Genís Ona, Juliana Mendes Rocha, José Carlos Bouso, Jaime EC Hallak, Tre Borràs, Maria Teresa Colomina, Rafael G. dos Santos

Psychopharmacology, 2022; 239(6), 1977-1987.

https://doi.org/10.1007/s00213-021-05964-y

Study I overview:

What do we already know?

Ibogaine has been associated with certain health risks, particularly those affecting the cardiovascular system, as it prolongs the QT interval on the electrocardiogram. This increases the risk of suffering arrhythmias and sudden death. The last systematic review regarding the adverse events produced by ibogaine was published in 2015.

What does this study add?

This systematic review provides an update on the literature on adverse events reported with ibogaine use. Additionally, and unlike previous reviews, we classified the physical/psychological signs into acute (those occurring within 24 hours of ingestion) and prolonged (occurring at least 24 hours following ingestion).

Highlights

The most common adverse events, both acute and prolonged, were cardiovascular alterations.

Acute adverse events frequently reported included gastrointestinal symptoms (nausea, vomiting), and neurological alterations (seizures, dysmetria, anoxic brain injury, and unconsciousness).

Prolonged adverse events included QT prolongation, insomnia, speech alterations, delusions, aggressiveness, irritability, dissociation, and hallucinations (lasting more than 24 hours post-ingestion).

There was significant heterogeneity in the types of products (which included more or less purified forms of ibogaine or extracts of the *T. iboga* plant) and doses used. Most reports involved ibogaine use in informal settings.

Future research should analyze potential risk biomarkers to detect vulnerable populations and suggest suitable clinical profiles for ibogaine treatment.

The Adverse Events of Ibogaine in Humans: An Updated Systematic Review of the Literature (2015-2020)

Running Head: Adverse Events of Ibogaine in Humans

Genís Ona, MSc[a,b,c], Juliana Mendes Rocha, Msc[d], José Carlos Bouso, PhD[a,c], Jaime E. C. Hallak, MD, PhD[d,e], Tre Borràs, MD[f], Maria Teresa Colomina, MD, PhD[b,g], Rafael G. dos Santos, PhD[a,d,e]

[a] International Center for Ethnobotanical Education, Research, and Service (ICEERS), Barcelona, Spain.

[b] Universitat Rovira i Virgili, Department of Psychology and Research Center for Behavior Assessment (CRAMC), Tarragona, Spain.

[c] Universitat Rovira i Virgili, Medical Anthropology Research Center (MARC), Tarragona, Spain.

[d] Department of Neuroscience and Behavior, Ribeirão Preto Medical School, University of São Paulo, SP, Brazil.

[e] National Institute for Translational Medicine (INCT-TM), CNPq, Brazil.

[f] Hospital Universitari Sant Joan de Reus. Servei de Drogodependències i Salut Mental. Pla d'Accions sobre drogues de Reus. Reus, Spain.

[g] Universitat Rovira i Virgili, Research in Neurobehavior and Health (NEUROLAB), Tarragona, Spain.

GO: genisona@iceers.org; JMR: mendesrjuliana@gmail.com; JCB: jcbouso@iceers.org; JECH: jechallak@gmail.com; TB: Tre Borràs; MTC: mariateresa.colomina@urv.cat. RGDS: banisteria@gmail.com

***Correspondence:** Rafael G. dos Santos. Departamento de Neurociências e Ciências do Comportamento, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Hospital das Clínicas, Terceiro Andar, Av. Bandeirantes, 3900, Ribeirão Preto, São Paulo, Brazil, Tel/Fax: + 55 16 3602 2703, E-mail: banisteria@gmail.com.

Conflicts of interest and Source of Funding: JMR received funding from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil). JECH is recipient of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) 1A productivity fellowship. Research was supported in part by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). RGS is Fellow of the Programa Nacional de Pós-Doutorado, Brazil (PNPD/CAPES). The authors declare no conflict of interest.

Abstract

Context: Ibogaine is the main alkaloid of the African shrub *Tabernanthe iboga*. It produces hallucinogenic and psychostimulant effects, but it is currently known for the anti-addictive properties. Despite the potential therapeutic effects, several cases of fatalities and serious adverse events related to ibogaine/noribogaine use can be found in the literature. Most studies consist in case reports or were conducted under non-controlled settings, so causation cannot be clearly established. **Objectives:** To update (2015-2020) the literature on the adverse events and fatalities associated with ibogaine/noribogaine administration. **Methods:** Systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). **Results:** Eighteen studies were included in the final selection. Highly heterogeneous results were found in terms of kind of product used or the known dosages. The adverse events were classified in acute effects (<24 hours), mainly cardiac (the most common was QTc prolongation), gastrointestinal, neurological, and clinical alterations, and long-lasting effects (>24 hours), mainly persistent cardiac alterations, psychiatric, and neurological signs. **Conclusions:** There is a high need of Phase-I clinical trials that can describe the safety of different dosages of ibogaine with standardized products. Further research should perform clinical profiling of vulnerable populations, and design effective screening methods and clinical procedures.

Keywords: ibogaine; noribogaine; adverse events; safety; drug interactions.

Introduction

Ibogaine is the main alkaloid of the African shrub *Tabernanthe iboga*. Different indigenous cultures of West Central Africa have been using *T. iboga* for centuries for medicinal and religious purposes. Due to its hallucinogenic properties, it plays a central role in rites of passage of Bwiti and other traditions (Fernández 1982). In 1901, ibogaine was isolated from the root bark of *T. iboga*, and its pharmacodynamic properties were further explored during the first decade of the 20th century. Instead of characterizing the effects of wide dosing regimens, only low doses were tested. Since low doses of ibogaine seem to have psychostimulant effects, ibogaine was recommended as a treatment for asthenia. In fact, from 1939 until 1970 ibogaine was commercialized in France under the name of "Lambarène" as a neuromuscular stimulant Goutarel et al. 1993). Ibogaine is currently known for its anti-addictive effects, which were serendipitously discovered by H. Lotsoff, a heroin user, between 1962 and 1963 (Alper and Lotsoff, 2007; Alper 2001). Lotsoff and other heroin users tried ibogaine for its hallucinogenic effects but then discovered that many of them no longer experienced opioid withdrawal symptoms. Since then, many preclinical studies were conducted assessing the potential effects of ibogaine in animal models of substance use disorders (Belgers et al. 2016; Brown 2013).

Regarding the treatment of opioid dependence, it has been observed that the acute administration of ibogaine dose-dependently reduces the self-administration of morphine (Glick et al. 1994) and heroin (Dworkin et al. 1995) in rats. Additionally, in morphine-dependent rats, ibogaine doses ranging from 4 to 16 micrograms and administered intracerebroventricularly eliminate the withdrawal syndrome induced by naloxone or naltrexone (Dzoljic et al. 1988). Similar results were obtained in non-human primates administering 2-8 mg/kg of ibogaine through the subcutaneous route (Aceto et al. 1990). However, when the subcutaneous route was used in morphine-dependent rats to administer 5-40 mg/kg of ibogaine no reductions in withdrawal syndrome were observed, which suggests that ibogaine's pharmacokinetics is relevant for its anti-addictive effects (Sharpe and Jaffe, 1990). The results in mice show differences regarding the dose and timing of ibogaine administration (before or after the induction of withdrawal syndrome using naloxone). Doses ranging from 40 to 80 mg/kg administered before naloxone reduce the withdrawal syndrome (Sharpe and Jaffe, 1990; Popik et al. 1995), while a dose of 30 mg/kg administered after naloxone does not reduce the withdrawal syndrome (Popik et al. 1995; Frances et al. 1992). The evidence in humans is scarce and is mostly based on case series and observational studies. The available studies suggest that ibogaine has significant anti-addictive properties, however, most studies are open-label and therefore causation cannot be established (dos Santos et al. 2016; Luz and Mash, 2021). In a randomized, double-blind, placebo-controlled, single-dose clinical trial (Glue et al. 2016), noribogaine (the main metabolite of ibogaine) was administered to 27 methadone dependent patients, switched off to morphine before treatment. No reductions in the opioid withdrawal syndrome (OWS) were observed. Some authors pointed out that the noribogaine doses used were equivalent to those produced after the administration of a low dose of ibogaine (286 mg), and that more than a single dose would be required to eliminate the OWS (dos Santos et al. 2016; Luz and Mash, 2021). Importantly, serious adverse events, such as dose-dependent QTc prolongation, were reported in this trial. QTc prolongation can be induced by several drugs (including alcohol, opioids, antihistamine or antipsychotic drugs), and it is associated with

bradycardia and arrhythmia, which can be fatal. Indeed, one of the main preoccupations with ibogaine administration, especially in non-controlled clinical settings, is its possible cardiotoxic effects.

In this regard, two systematic reviews evaluated reports of fatalities or serious adverse events related to Iboga/ibogaine use (Alper et al. 2012; Koenig and Hilber, 2015), while a third review also analyzed the anti-addictive potential of ibogaine (dos Santos et al. 2016). Alper et al. (2012) collected the ibogaine-associated fatalities (n=19) reported between 1990 and 2008, while Koenig & Hilber (Koenig and Hilber, 2015) collected the fatalities (n=3) and serious adverse events (n=8) associated with ibogaine reported between the years 2009-2014. All the cases came from case reports in which ibogaine was used in non-controlled settings, including private residences or private ibogaine clinics. Cases reported by Alper et al. (2012) consisted of 15 men and 4 women aged 24 to 54 years (mean 39.1 years). There were 8 men and 3 women aged 25 to 63 years (mean 38 years) in cases collected by Koenig & Hilber (2015). Most of the subjects of both reviews used ibogaine for detoxification purposes, mainly from opioid drugs, but also from cocaine and alcohol, among others. Notably, only 12 out of the 19 cases collected by Alper et al. (2012) described the exact dose of ibogaine taken, which ranged from 4.5 to 29 mg/kg (mean dose of 14.3 ± 6.1 mg/kg). This information was available for 9 out of 11 cases reported by Koenig & Hilber (2015), where doses ranged from 1.5 to 35 mg/kg. The authors of both reviews stated that the main metabolite of ibogaine (noribogaine) could be more directly involved in fatalities and adverse events, since ibogaine has a short half-life (4-7 hours) and deaths occurred at ≥8 hours (Alper et al. 2012) and 24-48 hours post-ingestion (Koenig and Hilber, 2015), respectively. Finally, both reviews coincide in having found pre-existing medical conditions and the presence of one or more drugs of abuse that explained or contributed to most deaths. For instance, Alper et al. (2012) reported that 12 out of 19 cases had cardiovascular diseases, liver diseases, peptic ulcer disease, brain neoplasm, hypertension, and obesity, and the concomitant presence of benzodiazepines, cocaine, opioids, or ephedrine. Koenig & Hilber (2015) found that all the fatalities reported had hypokalemia, and 50% of them had hypomagnesemia. Thus, authors emphasized the need of carefully screening electrolyte levels before administering ibogaine, as well as performing proper drug screenings and even genotyping subjects for CYP2D6 activity, since poor metabolizers would be at a greater risk of cardiotoxic effects of ibogaine/noribogaine (Alper et al. 2012; Koenig and Hilber, 2015).

Dos Santos et al. (2016) performed a systematic review of human studies assessing the anti-addictive potential of ibogaine. Most studies consisted in case series of individuals with opioid and stimulant use disorders seeking treatment, while there was one randomized, placebo-controlled clinical trial using noribogaine in methadone-dependent patients. According to authors, the results found in case series suggest that ibogaine significantly reduces OWS symptoms, since most subjects could remain drug-free for several days after treatment. Most of these case series did not differentiate between heroin/methadone users and given the absence of control groups and informal settings in which the treatments were performed, it is challenging to suggest causation. Importantly, most of these cases did not report significant adverse reactions. However, in most cases there was no detailed information on how adverse events were measured (if they were measured at all). Thus, the absence of serious adverse events in these studies should be interpreted with caution. Regarding the clinical trial in which

noribogaine was administered to methadone-dependent patients [15], non-significant effects on withdrawal syndrome were found. For this study, single doses of 60, 120, or 180 mg of noribogaine were used. Dos Santos et al. (2016) suggest that this absence of effects could be attributed to several factors. First, it should be noted the lack of knowledge regarding the equivalence between therapeutic doses of ibogaine and noribogaine. Additionally, since methadone has a long half-life, a single dose of noribogaine would be hardly able to interrupt the withdrawal syndrome. The subjects included in this clinical trial also showed dose-dependent QT prolongation, raising concerns regarding the safety profile of noribogaine. Dos Santos et al. (2016) concluded that the toxicity of both alkaloids (ibogaine and noribogaine) is an important limitation to their clinical use, and the absence of proper medical screening and monitoring procedures increases the possibility of hazardous situations.

Considering that the previous reviews were published including information until 2015/16, the aim of this manuscript was to perform an updated (2015-2020) systematic review of literature of the serious adverse events (SAEs) and fatalities associated with ibogaine administration. Moreover, special attention was given to those cases in which ibogaine was combined with other drugs, since in the previous reviews concomitant use of other drugs was associated with serious adverse reactions and/or fatalities.

Methods

Data for this systematic review were collected in accordance with the Systematic Reviews and Meta-Analyses guidelines (Moher et al. 2009).

Data Acquisition

We attempted to identify all human studies available to review from July 2nd, 2015 to July 23, 2020 in which the adverse events of ibogaine or noribogaine were analyzed. We used this criterion because other systematic reviews have been recently published (dos Santos et al. 2016; Alper et al. 2012; Koenig and Hilber, 2015).

Search Strategy

Electronic searches were performed using PubMed, Scopus, Web of Science, Scielo, Google Scholar, and Core.ac.uk databases. The following keywords were used: ibogaine OR noribogaine AND humans OR addiction OR dependence. References were retrieved through searching electronic databases and manual searches through reference lists of identified literature. All the studies published from July 2nd, 2015 to July 23, 2020 were included without any language restriction.

Eligibility Criteria

The following inclusion and exclusion criteria were established prior to the literature search.

Article Type

All studies published in peer-reviewed journals involving the use of ibogaine in humans were included. These included case reports, clinical studies, observational studies, and letters.

Preclinical studies (including *in vitro* and *in vivo*), reviews, abstracts, comments, and editorials were excluded.

Study Design

The review included case reports, observational and clinical studies that reported ibogaine- or noribogaine-associated adverse events, serious adverse events, fatalities, as well as potential drug-drug interactions with other drugs or prescribed medications.

Participants/sample

All subjects that used at least one dose of ibogaine or noribogaine were included.

Interventions

All designs evaluating the adverse events, serious adverse events, fatalities, and drug-drug interactions between ibogaine or noribogaine and other drugs or medications were included.

Outcomes

We included all reports that assessed adverse events systematically (with standardized scales and/or physical and biological measures) or non-systematically (any subjective or physical effect described by the authors as adverse or negative) and reports of intoxications and deaths.

Data Extraction

Two independent reviewers screened all studies with discrepancies resolved by a third reviewer. From the articles included, we recorded the names of authors, year of publication, study location (city and country), study design (open label or controlled, observational, letters and cases), characteristics of the context (hospital, clinic, private place, home) and participants (sample size, age, and gender), response criteria (adverse events), type of intervention (dose and other drugs), and type of outcome measure (adverse events, serious adverse events, and interactions with other drugs). Adverse events were further categorized as acute (<24 hours) or prolonged (>24 hours) effects.

NIH Evaluation of Selected Studies

To grade and compare the studies found in our search in a standardized manner, we have utilized the NIH (National Heart, Lung and Blood Institute) checklists and guidelines for clinical trials as a template (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools). We used three checklists: “Quality assessment of case series studies”, “Quality assessment for observational cohort” and “Quality assessment of controlled interventions studies”. All articles were analyzed to see whether they contained or not all the items presented in the checklist. Items from the checklist that were present within each article provided one positive point for the respective article. The final score was calculated by adding the positive points. In the case of “Quality assessment of case series studies” the total items to be added were 9 points; in the “Quality assessment for observational cohort” 14 points; and in the “Quality assessment of controlled interventions studies” 14 points. In cases of disagreement on subjective items, the reviewers discussed their reasons for giving positive, negative or not applicable points, and if a consensus was not reached, a third author/reviewer was consulted. The overall grade of each

article was calculated by dividing the positive points by the difference of the total number of points less the not applicable points. Grades go from 0 to 1 with 0 being the worst and 1 being the best.

Results

Selected studies

A flow diagram illustrating the different phases of the article selection for the systematic review is presented in Figure 1.

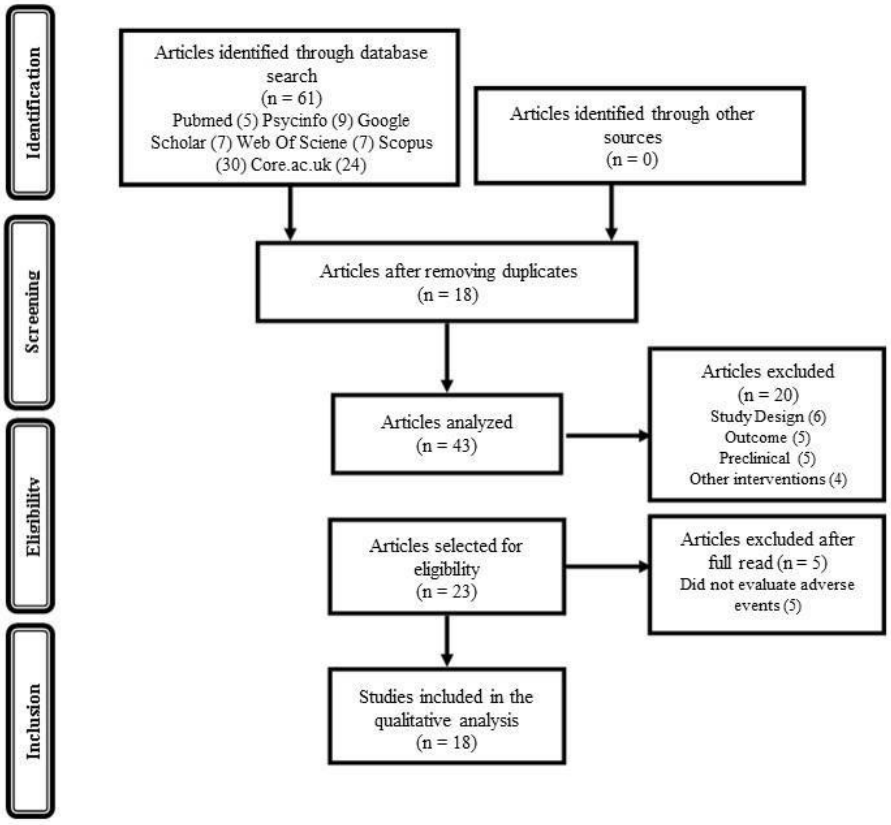


Figure 1. Flowchart showing the different phases of the article selection for the systematic review.

The bibliographic search was performed in the PubMed, Scopus, Web of Science, Scielo, Google Scholar, and Core.ac.uk databases from July 2nd, 2015 to July 23, 2020, with 61 articles found in this first stage. After removing duplicates and reading the titles and abstracts of the articles

found, 23 references were selected for full reading considering the inclusion and exclusion criteria. After full reading, five studies were excluded since they did not report adverse events. In summary, a total of 18 articles were included in the systematic review. Of the 18 selected articles, 15 were case reports or case series (Breuer et al. 2015; Marta et al. 2015; O'Connell et al. 2015; Cloutier-Gill et al. 2016; Hildyard et al. 2016; Meisner et al. 2016; Henstra et al. 2017; Wilkins et al. 2017; Knuijver et al. 2018; Mash et al. 2018; Steinberg and Deyell, 2018; Barsuglia et al. 2018; Grogan et al. 2019; Matamoros-Castillo et al. 2019; Wilson et al. 2021). There were also two randomized, double-blind clinical trials (Glue et al. 2016, 2015), and one observational study (Brown and Alper, 2018). Sample sizes were mostly small. In the case reports and cases series, except for one case series with 191 patients (Mash et al. 2018), most cases described a single subject. There were 22 patients in the clinical trials (n = 21 (Glue et al. 2015); n = 27 (Glue et al. 2016)) and 30 in the observational study (Brown and Alper, 2018). The age range of the case reports ranged between 22-60 years. Regarding gender, in both clinical trials (Glue et al. 2015, 2016), in an open label case series (Mash et al. 2018), and in the observational study (Brown and Alper, 2018), a higher number of males was found. The main reason for the use of ibogaine was the search for a treatment for opioid dependence, and in two cases a search for spiritual cleansing was reported (Breuer et al. 2015; Marta et al. 2015). In the case of the clinical trials, the first one was planned to assess pharmacokinetics and safety of ibogaine in healthy volunteers (Glue et al. 2015). The other clinical trial, carried out by the same group, aimed to evaluate the safety, tolerability, and pharmacokinetics of noribogaine in the treatment of methadone detoxification (Glue et al. 2016). In the observational study, the main objective was to evaluate the efficacy of ibogaine in the treatment of opioid dependence (Brown and Alper, 2018). Finally, in the open label case series, the authors sought to evaluate the safety and efficacy of ibogaine during abstinence due to the use of opioids (Mash et al. 2018).

The dose of ibogaine used in the case reports ranged from 725 mg of ibogaine (Wilson et al. 2021) to 38 g of dried *T. iboga* root bark (Breuer et al. 2015). The main route of administration was orally through capsules. It is important to highlight that in most cases there is a lack of detailed information about the ingested substance, such as appropriate analyses quantifying the alkaloid content. Additionally, the samples were acquired through unreliable sources, making it even more difficult to access reliable information. Regarding adverse events, the results obtained after analyzing the articles were divided between acute (<24 hours) and long-lasting effects (>24 hours). Almost half of the citations (8 in 18) reported absence of effects after the first 24 hours (Glue et al. 2016; O'Connell et al. 2015; Wilkins et al. 2017; Mash et al. 2018; Barsuglia et al. 2018; Wilson et al. 2021; Glue et al. 2015; Brown and Alper, 2018). In the case reports, most cases required hospital intervention, some of them being admitted to intensive care units (Breuer et al. 2015; Steinberg and Deyell, 2018; Grogan et al. 2019). One case, despite the attempt of hospital intervention, died within the first 24 hours (Meisner et al. 2016). Given the high heterogeneity of the studies with different designs, different dosages, and most of them with no description of quality analysis of the ibogaine, this review will contemplate only a qualitative analysis of the results. Findings are discussed in detail below and summarized in Supplementary material 1, 2 and 3.

Characteristics of the subjects

Among all the case reports (n= 15), there were only two subjects with no history of drug abuse/dependence or medical/psychiatric disorders (Breuer et al. 2015; Marta et al. 2015). The remaining subjects reported mainly drug abuse/dependence, consisting in opioids (n= 163), polysubstance use (n= 5), cocaine use disorder (n= 89) and alcohol use disorder (n= 1). In addition, five subjects had medical conditions such as hepatitis C virus (HCV), dyslipidemia, or different types of pain. Regarding psychiatric diagnosis, these were reported by five subjects, mainly consisting in attention deficit disorder (ADHD), depression or anxiety. Moreover, in the Mash et al. (2018) study (n= 191) a high number of patients with depression was observed (52.9% meeting clinical criteria for major depressive disorder or depression) and bipolar disorder. In one of the clinical trials, noribogaine was administered to patients under methadone treatment who were switched off to morphine before the trial (Glue et al. 2016). The observational study published by Brown & Alper (2018) consisted in subjects with a history of opioid dependence without any other medical condition.

Ibogaine/noribogaine information

Detailed information on ibogaine/noribogaine is shown for acute (<24 h) and prolonged (>24 h) adverse reactions in Supplementary material 1 and 2, respectively.

Only in five out of 15 case reports the presence of ibogaine could be determined (O'Connell et al. 2015; Henstra et al. 2017; Wilkins et al. 2017; Mash et al. 2018; Grogan et al. 2019), and in only one both the presence and the quantity of ibogaine were measured (O'Connell et al. 2015). Iboga root bark was supposedly used in three cases (Breuer et al. 2015; Grogan et al. 2019; Wilson et al. 2021), while the other ones supposedly used ibogaine HCl (O'Connell et al. 2015; Cloutier-Gill et al. 2016; Hildyard et al. 2016; Meisner et al. 2016; Henstra et al. 2017; Wilkins et al. 2017; Mash et al. 2018; Steinberg and Deyell, 2018; Barsuglia et al. 2018; Brown and Alper, 2018), or it was unknown (Marta et al. 2015; Matamoros-Castillo et al. 2019). The clinical trials used ibogaine HCl (Knuijver et al. 2018; Glue et al. 2015) or noribogaine HCl (Glue et al. 2016). Regarding toxicological analyses, the presence of ibogaine or noribogaine in hair samples was confirmed in one case (Breuer et al. 2015), in serum in another case (Henstra et al. 2017), and in both urine and serum in another case O'Connell et al. 2015). In the remaining cases where toxicological analyses were performed, the presence of ibogaine/noribogaine was not specifically measured.

Acute adverse events (<24 h)

Detailed information on the acute adverse events of ibogaine/noribogaine is shown in Supplementary material 2. The most common acute adverse event described in the selected articles consisted of QTc prolongation (527 ms (O'Connell et al. 2015); 730 ms (Hildyard et al. 2016; Meisner et al. 2016); 647 ms (Henstra et al. 2017); 516 ms Knuijver et al. 2018); 714 ms (Steinberg and Deyell, 2018); 788 ms (Grogan et al. 2019); 512 ms (Wilson et al. 2021)). Other cardiac alterations were also reported, including tachycardia, hypotension, wide QRS complex (defined as a tachyarrhythmia with alternating morphologies of the QRS complex with irregular R-R intervals) (Jebberi et al. 2019), and Torsades de Pointes (Hildyard et al. 2016; Meisner et al. 2016; Henstra et al. 2017; Steinberg and Deyell, 2018; Grogan et al. 2019). Gastrointestinal symptoms, mainly nausea and vomit, were also observed in the case series (Breuer et al. 2015;

O'Connell et al. 2015; Meisner et al. 2016; Wilkins et al. 2017; Mash et al. 2018; Steinberg and Deyell, 2018; Barsuglia et al. 2018). Clinical symptoms associated with alteration of consciousness, such as visions/hallucinations or space/time disorientation (Breuer et al. 2015; Knuijver et al. 2018; Grogan et al. 2019; Matamoros-Castillo et al. 2019), were also noted. Moreover, a high number of physical symptoms were reported, including ataxia, muscle tension, weakness, diaphoresis, akathisia, or tremors, among others (Breuer et al. 2015; O'Connell et al. 2015; Cloutier-Gill et al. 2016; Wilkins et al. 2017; Kuijver et al. 2018; Mash et al. 2018; Barsuglia et al. 2018). Finally, neurological alterations including seizures (Breuer et al. 2015; Hildyard et al. 2016; Grogan et al. 2019) dysmetria (O'Connell et al. 2015), anoxic brain injury (Meisner et al. 2016), and unconsciousness (Henstra et al. 2017) were also reported.

The first clinical study included in this review aimed to evaluate the pharmacokinetics of ibogaine and noribogaine in 21 healthy individuals divided into two groups previously treated with placebo or paroxetine (CYP2D6 inhibitor) (Glue et al. 2015). Both groups ingested 20 mg of ibogaine and demonstrated a rapid peak of noribogaine (4 hours in the placebo group and 3 hours in the paroxetine group), as well as similar profiles regarding the extent of exposure to noribogaine. However, the group pre-treated with paroxetine showed two times more exposure to ibogaine + noribogaine. This suggests that genetic variations of CYP2D6 could be clinically relevant in ibogaine treatments, and that drugs that inhibit this metabolic pathway could produce interactions with ibogaine/noribogaine (Glue et al. 2015). Subsequently, another clinical study (randomized, double-blind, placebo-controlled) carried out by the same group evaluated the safety, tolerability, and pharmacokinetics of noribogaine in 27 patients undergoing treatment to discontinue treatment of opioid substitution (OST) with methadone (Glue et al. 2016). Three different doses of noribogaine (60, 120 or 180 mg) or placebo were administered. Noribogaine produced only a non-significant effect in opioid withdrawal symptoms and showed a slow elimination time (24-30 hours). In both clinical trials, ibogaine and noribogaine were well tolerated. No subjective effects were noted at the doses used. Only transitory changes in light perception, headache, and nausea were observed. No serious adverse events were reported (Glue et al. 2015, 2016).

Prolonged adverse events (>24 h)

Detailed information on the prolonged adverse events of ibogaine/noribogaine is shown in Supplementary material 3. The most common prolonged adverse events described in the selected articles were mainly associated with psychiatric, neurological, and cardiac alterations. The mean number of days that patients remained hospitalized was 7.8 (range: 3–13 days). Among psychiatric alterations, insomnia (persisting 5-14 days), alterations in speech, delusions, aggressiveness, irritability, dissociation, and hallucinations were the most mentioned (Marta et al. 2015; Grogan et al. 2019; Matamoros-Castillo et al. 2019). Psychomotor slowness, bilateral ptosis, dysarthria, psychomotor agitation, and amnesia were reported as neurological signs (Breuer et al. 2015; Marta et al. 2015; Matamoros-Castillo et al. 2019). Lastly, among cardiac alterations, QTc prolongation remaining for seven days was reported in some cases (Hildyard et al. 2016; Steinberg and Deyell, 2018). With the controlled administration of noribogaine and ibogaine, prolonged adverse events were not observed (Glue et al. 2015, 2016). Alterations in the blood status were described in two studies and included increased C- reactive protein, white

blood cell, and creatinine levels (first days) (Breuer et al. 2015), and hypokalemia and hypomagnesaemia the day after admission (Henstra et al. 2017).

Interventions used to manage adverse events

Three case reports informed about admissions in intensive care units (Breuer et al. 2015; Steinberg and Deyell, 2018; Grogan et al. 2019). Regarding the administered medications, benzodiazepines and antipsychotics were the most used (Breuer et al. 2015; Marta et al. 2015; Wilkins et al. 2017; Matamoros-Castillo et al. 2019; Wilson et al. 2021). Anticonvulsants (Marta et al. 2015), atomoxetine (Marta et al. 2015), atropine (Hildyard et al. 2016), isoprotenerol (Hildyard et al. 2016; Henstra et al. 2017), and magnesium/sodium and saline (Hildyard et al. 2016; Henstra et al. 2017; Barsuglia et al. 2018; Grogan et al. 2019) were also reported. Cannabis oil was used in one case in a non-medical setting (Wilkins et al. 2017). The use of electrical cardioversion, a pacemaker, defibrillation, and intubation were necessary in five cases (Hildyard et al. 2016; Meisner et al. 2016; Henstra et al. 2017; Steinberg and Deyell, 2018; Grogan et al. 2019). One fatality was reported after concomitant administration of naloxone, vasopressors, and morphine (Meisner et al. 2016). In the open-label series cases, administration of intravenous fluids 1 hour prior to ibogaine administration was employed to try preventing orthostatic hypotension and bradycardia, but this was not effective since these adverse events were observed anyway (Mash et al. 2018).

Potential drug-drug interactions

Marta et al. (2015) reported that one subject tested positive for benzodiazepines, another one for benzodiazepines, opioids, and methadone, and another one for cannabinoids. Meisner et al. (2016) found a positive result for opioids. Grogan et al. (2019) reported a positive test for opioids, cannabinoids, and cocaine. Remarkably, in five reports these analyses were not performed (Cloutier-Gill et al. 2016; Hildyard et al. 2016; Wilkins et al. 2017; Wilson et al. 2021; Brown and Alper, 2018). Regarding medications used at hospital or medical settings, some of them might also have caused potentially dangerous interactions. This is the case of Meisner et al. (2016), where vasopressors and morphine were administered upon arrival at hospital, after the administration of naloxone (2 mg) in the field, which resulted in a fatality. However, it is important to observe the substances used after the dose of ibogaine used to handle with psychiatric symptoms (one of the most adverse events observed in the studies). It was reported the use of benzodiazepines (diazepam 2-10 mg) (Wilkins et al. 2017; Wilson et al. 2021), cannabis oil (Wilkins et al. 2017) and antipsychotics (quetiapine, risperidone, olanzapine and others) (Marta et al. 2015; Matamoros-Castillo et al. 2019; Wilson et al. 2021), these medications seem to be well tolerated in the cases described, but it is important to highlight that there was a lack of information about this use, such as the dose and a follow-up treatment. The administration of intravenous fluids, magnesium sulfate and anti-emetics was well tolerated (Henstra et al. 2017; Wilkins et al. 2017; Mash et al. 2018; Barsuglia et al. 2018; Grogan et al. 2019; Matamoros-Castillo et al. 2019).

Quality assessment

Our evaluation of the experiments assessed in the current review yielded an average of 80% in Quality assessment of case series studies, 78% in Quality assessment of controlled interventions

studies, and 64% in Quality assessment for observational cohort completion. Therefore, we considered the evidence of most studies with moderate-to-high quality. However, it is important to consider the limitations present in cases, case series, and observational studies, such as the lack of control of external factors and the difficulty of translating the results to the general population. It is important to note that we were very strict in our considerations, and that the checklist was made to ensure that all information was given. Further studies should be aware of this checklist and try to fulfill its requirements as much as possible to ensure that all important information is present and to ease reproducibility. Detailed evaluation of the quality of the selected references is presented in Supplementary material 4.

Discussion

In this systematic review, we have collected the adverse events and fatalities associated with ibogaine and noribogaine reported in the last five years (2015-2020). Most of the included references were case reports. Two clinical trials (a Phase-I trial with ibogaine and a Phase-II trial with noribogaine) and one observational study were also included.

The case reports in the literature are the first source of evidence for new therapies and rare adverse effects, in addition to help in the formulation of new questions (Celeste, 2008). This fits well with the purpose of the present review. However, as most studies are case reports, it is also important to consider the limitations of this study design. The main disadvantages involving this type of design are mainly related to the difficulty of drawing wide conclusions and translating the results to the general population, making it impossible to establish a cause-relationship due to the small sample and the lack of a control group (Celeste, 2008). Since we are still lacking Phase-I clinical trials for the assessment of the safety of higher (>20 mg) doses of ibogaine, the information provided in these cases is essential in the light of the emerging interest in this substance as a potential way to address the current opioid epidemic (Larsen 2019).

The clinical trials (Glue et al. 2015, 2016) were carried out in a hospital context, under the supervision of professionals, cardiac monitoring, and using low doses of pure ibogaine or noribogaine. Adverse events reported in these trials were mild/moderate (i.e., hallucinations/visual alterations, non-serious cardiovascular, motor, and gastrointestinal alterations). These same adverse events were reported in the case reports, but serious events were also observed (i.e., seizures, prolonged cardiovascular alterations). Case reports were described in non-controlled contexts and with a wide variation on ibogaine/noribogaine dose and purity. Thus, these cases are relevant in the context of the naturalist use of ibogaine and emphasize the importance of using these drugs in controlled contexts.

The doses of ibogaine differed widely between cases, and thus this becomes an important limitation to describe adverse events associated with certain doses. This is an issue previously mentioned in other reviews (dos Santos et al. 2016). In some cases, the root bark of *T. iboga* was used, so the content of ibogaine is not known. The lack of information regarding the safety of root bark or other iboga extracts as compared with pure ibogaine makes this practice especially risky. While there are plenty of examples in which the use of herbal extracts may reduce some of the adverse events found when using purified compounds (e.g., for the specific case of QTc prolongation, quinimax, a standardized mixture of cinchona alkaloids, produce less QTc

prolongation than quinidine alone; (Sowunmi et al. 1990)), the presence of antagonistic or more toxic compounds in the herbal extract is also possible. A recently published article (Bouso et al. 2020) provides an illustrative example regarding the uncertainty when it comes to iboga/ibogaine use in uncontrolled settings. In this study, 16 products used by treatment providers were analyzed and highly heterogeneous results were found. In root bark materials, an ibogaine concentration ranging from 0.6% to 11.2% was found, in contrast to previous studies which claimed a concentration of approximately 7% (Mazoyer et al. 2013). In samples labelled as "total alkaloid", which supposedly consisted of *T. iboga* extracts, the concentration of ibogaine ranged from 8.2% to 32.9%. In one sample labelled as "purified total alkaloid" there was a concentration of ibogaine of 73.7%. Finally, in products labelled as ibogaine HCl, the ibogaine concentration ranged from 0% to 73.4%. The authors noted that one sample did not contain any ibogaine, and that other alkaloids and unknown substances were present in almost all samples (Bouso et al. 2020).

Apart from this high degree of uncertainty, the unknown dosages, and the high doses commonly used in non-medical settings (Brown and Alper, 2018; Alper et al. 2008; Luz and Mash, 2021), the presence of medical conditions and the concomitant use of other drugs should be mentioned among other risk factors that can contribute to adverse events. In the articles reviewed, only two subjects had no history of drug abuse/dependence or medical/psychiatric disorders (Breuer et al. 2015; Henstra et al. 2015). The relevance of previous health conditions, especially cardiac alterations, has been previously highlighted in two reviews (Alper et al. 2012; Koenig and Hilber, 2015). Alper et al. (2012) found that in 12 out of 19 deaths associated with ibogaine, previous cardiovascular, liver, and ulcerative alterations, among other diseases, were reported. Koenig & Hilber (2015) reported that all fatalities were associated with hypokalemia, and 50% of them with hypomagnesemia. This information is crucial when evaluating the most susceptible people that can be at risk of suffering adverse events or even potential fatalities due to ibogaine or noribogaine. Therefore, performing adequate screenings is essential to enhance the safety of these compounds. Furthermore, even after an adequate screening the risk of serious adverse events related with cardiotoxicity will remain, as observed in some case reports where subjects had no personal or familiar history of cardiac issues (Hildyard et al. 2016; Pleskovic et al. 2012; Vlaanderen et al. 2014). This risk can be attributed to both ibogaine and noribogaine potential of inhibiting hERG potassium channels and the subsequent prolongation of the cardiac action potential (Koenig and Hilber, 2015; Ruan et al. 2014; Alpern et al., 2016). A recent study showed the IC50 values for hERG blockade for ibogaine were 4.09 ± 0.69 μM (manufactured by semisynthesis via voacangine) and 3.53 ± 0.16 μM (by extraction from T. iboga), while for noribogaine it was 2.86 ± 0.68 μM (Alpern et al., 2016). This difference could be related to the observations of persistent QT prolongation and cardiac arrhythmia at delayed intervals of days following ibogaine ingestion, considering the extended half-life of noribogaine. Thus, continued cardiovascular monitoring is mandatory in people receiving ibogaine or noribogaine to ensure safety and reduce the occurrence of serious adverse events.

The reported adverse events were in line with the ones observed in previous reviews (Alper et al. 2012; Koenig and Hilber, 2015). They were mainly associated with gastrointestinal, motor, and cardiovascular alterations, but psychedelic-like effects such as hallucinations/visual alterations or disorientation were commonly reported. Moreover, some adverse events possibly

associated with neurological alterations should be further investigated. Generalized seizures were reported in three cases (Breuer et al. 2015; Hildyard et al. 2016; Grogan et al. 2019), which could be associated with the agonistic effect of ibogaine at 5-HT_{2A} receptors, leading to increases in glutamatergic tone (dos Santos and Hallak, 2020). In this sense, it seems that the antagonistic effect of ibogaine on NDMA receptors was not effective in preventing seizures (Kapur 2018). Breuer et al. (2015) suggested that this phenomenon could be due to an enhanced disinhibition process by suppression of inhibitory interneurons. By this manner, high doses of ibogaine, like occurs with dizocilpine, could stimulate the release of glucocorticoids that eventually increase the susceptibility to seizures. Previous preclinical research has shown the degeneration of Purkinje cells in rats after the intraperitoneal administration of ibogaine at high doses (40-100 mg/kg) (Baumann et al. 2001; Glick et al. 1992). Helsley et al. (1997) did not find any neurotoxic effects after daily administration of low doses of ibogaine (10 mg/kg) over a 60-days period. Similarly, Mash et al. (2018) did not find evidence of neurotoxicity in monkeys after the administration of repeated doses of ibogaine, neither through the oral (5-25 mg/kg) nor the subcutaneous (100 mg/kg) route. Moreover, a neuropathological evaluation of a female volunteer who received four doses of ibogaine revealed no cerebellar damage (Mash et al. 2018). Remarkably, the fact that most hospitalizations and admissions to ICUs provided by case reports suggests that ibogaine-associated SAEs occur more frequently when it is used in unsupervised settings without proper medical control. Indeed, SAEs were observed when the drug was administered by unskilled people in unsafe settings. In the light of the adverse events observed in this review, further studies would be needed to confirm these findings.

The interaction between ibogaine and other medications or drugs is also worth mentioning, since an important percentage of the population that generally uses ibogaine are people with substance use disorders. Previous reports confirmed the presence of other drugs/medications in fatalities associated with ibogaine (Alper et al. 2012; Mazoyer et al. 2013). In the case of Mazoyer et al. (2013), the combination of ibogaine with methadone and diazepam was considered the most probable cause of death. The combination of benzodiazepines and ibogaine seems to be mostly safe as observed in other cases (Breuer et al. 2015; Wilkins et al. 2017; Wilson et al. 2021), but the concomitant use of diazepam and methadone has been associated with increased mortality due to a synergistically prolongation of repolarization (Ernst et al. 2002), as reported in an *in vitro* study (Kuryshev et al. 2010). Thus, ibogaine could indeed have contributed to the fatality.

Additionally, substrates or inhibitors of cytochrome P450 (CYP) liver isoforms, mainly CYP2D6, could hamper the effective O-demethylation of ibogaine, resulting in exposure to potentially toxic concentrations (Glue et al. 2016). Substrates of P-glycoprotein (P-gp) should also be avoided or used with caution when combined with ibogaine, since it has been reported that ibogaine inhibits P-gp (Tournier et al. 2010).

Both the complex pharmacokinetics and pharmacodynamics of ibogaine and noribogaine present a challenge to health professionals that may encounter intoxications related to these drugs at emergency departments. This can be clearly seen in the case reported by Meisner et al. (2016), where the patient received naloxone in the field and morphine and unspecified vasopressors at hospital. While naloxone could possibly be administered under the suspicion of an opioid overdose, the use of morphine and vasopressors would be associated with the

intubation procedure but would not be indicated from a pharmacological point of view considering the above information.

Conclusion

Adverse events and fatalities associated with ibogaine/noribogaine are still a major concern that is challenging to address. The high degree of heterogeneity and uncertainty regarding alkaloid content, the lack of purity of the products used, and considering the limitations of the level of evidence produced by case studies results in a complex picture that prevents us from establishing associations (such as expected adverse events at certain doses). Considering that a growing number of people worldwide are using these drugs in search for a treatment for substance use disorders, Phase I-II trials are urgent needed to assess their tolerance and safety, dose-effect relationships, and possible drug-drug interactions.

References

Aceto M, Bowman E, Harris L. (1990) Dependence studies of new compounds in the rhesus monkey, rat, and mouse. National Institute on Drug Addiction Research Monographs 95:578–631.

Alper KR. (2001) Ibogaine: A review. Alkaloids. Chem Biol 56:1–38. doi: 10.1016/s0099-9598(01)56005-8.

Alper KR, Lotsof HS. (2007) Psychedelic medicine: New evidence for hallucinogenic substances as treatments. Westport (CT): Praeger Perspectives. The use of ibogaine in the treatment of addictions; p. 43–66.

Alper KR, Lotsof HS, Kaplan CD (2008) The ibogaine medical subculture. J Ethnopharmacol 115:9–24. doi: 10.1016/j.jep.2007.08.034.

Alper KR, Stajić M, Gill JR. (2012) Fatalities temporally associated with the ingestion of ibogaine. J Forensic Sci 57(2):398–412. Doi: 10.1111/j.1556-4029.2011.02008.x.

Alper K, Bai R, Liu N, et al (2016) hERG Blockade by Iboga Alkaloids. Cardiovasc Toxicol 16(1):14–22. Doi: 10.1007/s12012-015-9311-5.

Barsuglia JP, Polanco M, Palmer R, et al (2018) A case report SPECT study and theoretical rationale for the sequential administration of ibogaine and 5-MeO-DMT in the treatment of alcohol use disorder. Prog Brain Res 242:121–158. doi: 10.1016/bs.pbr.2018.08.002.

Baumann MH, Rothman RB, Pablo J, et al (2001) In vivo neurobiological effects of ibogaine and its Odesmethyl metabolite, 12-hydroxyibogaine (noribogaine) in rats. J Pharmacol Exp Ther 297:531–539. Doi: 10.1177/0960327107087802

Belgers M, Leenaars M, Homberg JR et al (2016) Ibogaine and addiction in the animal model, a systematic review and meta-analysis. Transl Psychiatry 6(5):e826. doi: 10.1038/tp.2016.71.

Bouso JC, Fornís I, Vilamala MV, et al (2020) An analytical study of iboga alkaloids contained in Tabernanthe iboga-derived products offered by ibogaine treatment providers. Arch Clin Psychiatry 47(2):51–54. Doi: 10.1590/0101-60830000000231

Breuer L, Kasper BS, Schwarze B, et al (2015) "Herbal seizures"-atypical symptoms after ibogaine intoxication: a case report. J Med Case Rep 9:243. doi: 10.1186/s13256-015-0731-4

Brown TK. (2013) Ibogaine in the treatment of substance dependence. Curr Drug Abuse Rev 6(1):3–16. doi: 10.2174/15672050113109990001.

Brown TK, Alper K (2018) Treatment of opioid use disorder with ibogaine: detoxification and drug use outcomes. Am J Drug Alcohol Abuse 44(1):24–36. doi: 10.1080/00952990.2017.1320802

Celeste RK (2008) Erros metodológicos mais comuns encontrados em artigos dos tipos relatos e série de casos. Federação Brasileira das Sociedades de Ginecologia e Obstetrícia.

Cloutier-Gill L, Wood E, Millar T, et al (2016) Remission of Severe Opioid Use Disorder with Ibogaine: A Case Report. J Psychoactive Drugs 48(3):214–217. doi: 10.1080/02791072.2016.1180467

Dos Santos RG, Bouso JC, Hallak JEC. (2016) The antiaddictive effects of ibogaine: A systematic literature review of human studies. J Psychedelic Studies 1(1):20–28. Doi: 10.1556/2054.01.2016.001

Dos Santos RG, Hallak JEC (2020) Therapeutic use of serotoninergic hallucinogens: A review of the evidence and of the biological and psychological mechanisms. Neurosci Biobehav Rev 108:423-434. doi: 10.1016/j.neubiorev.2019.12.001

Dworkin SI, Gleeson S, Meloni D et al (1995) Effects of ibogaine on responding maintained by food, cocaine and heroin reinforcement in rats. Psychopharmacol (Berl) 117(3):257–261. doi: 10.1007/BF02246099.

Dzoljic ED, Kaplan CD, Dzoljic MR. (1988) Effect of ibogaine on naloxone-precipitated withdrawal syndrome in chronic morphine dependent rats. Arch Int Pharmacodyn Ther 294:64–70.

Ernst E, Bartu A, Popescu A (2002) Methadone-related deaths in Western Australia 1993-99. Aust N Z J Public Health 26:364–370. Doi: 10.1111/j.1467-842X.2002.tb00188.x

Fernandez JW (1982) Bwiti: an Ethnography of the religious imagination in Africa. Princeton (NJ): Princeton University Press.

Frances B, Gout R, Cros J, et al (1992) Effects of ibogaine on naloxone-precipitated withdrawal in morphine-dependent mice. Fundam Clin Pharmacol 6(8-9):327–332. doi: 10.1111/j.1472-8206.1992.tb00127.x.

Glick SD, Kuehne ME, Raucci J et al (1994) Effects of iboga alkaloids on morphine and cocaine self-administration in rats: relationship to tumorigenic effects and to effects on dopamine release in nucleus accumbens and striatum. Brain Res 657(1-2):14–22. doi: 10.1016/0006-8993(94)90948-2.

Glick SD, Rossman K, Rao NC, et al (1992) Effects of ibogaine on acute signs of morphine withdrawal in rats: independence from tremor. Neuropharmacol 31:497–500. doi: 10.1016/0028-3908(92)90089-8

Glue P, Cape G, Tunnicliff D, et al (2016) Ascending single-dose, double-blind, placebo controlled safety study of noribogaine in opioid-dependent patients. Clin Pharmacol Drug Dev 5(6):460–468. doi: 10.1002/cpdd.254

Glue P, Winter H, Garbe K, et al (2015) Influence of CYP2D6 activity on the pharmacokinetics and pharmacodynamics of a single 20 mg dose of ibogaine in healthy volunteers. J Clin Pharmacol 55(6):680–687. doi: 10.1002/jcph.471

Goutarel R, Gollnhofer O, Sillans R. (1993) Pharmacodynamics and therapeutic applications of iboga and ibogaine. Psychedelic Monographs and Essays. 6:70–111.

Grogan J, Gerona R, Snow JW, et al (2019) Ibogaine Consumption With Seizure-Like Episodes, QTc-Prolongation, and Captured Cardiac Dysrhythmias. J Emerg Med 57(4):e99-e104. doi: 10.1016/j.jemermed.2019.06.052

Helsley S, Fiorella D, Rabin RA, et al (1997) Effects of ibogaine on performance in the 8-arm radial maze. Pharmacol Biochem Behav 58(1):37–41. doi: 10.1016/s0091-3057(96)00476-5

Henstra M, Wong L, Chahbouni A, et al (2017) Toxicokinetics of ibogaine and noribogaine in a patient with prolonged multiple cardiac arrhythmias after ingestion of internet purchased ibogaine. Clin Toxicol (Phila) 55(6):600–602. doi: 10.1080/15563650.2017.1287372

Hildyard C, Macklin P, Prendergast B, et al (2016) A Case of QT Prolongation and Torsades de Pointes Caused by Ibogaine Toxicity. J Emerg Med 50(2):e83–87. doi: 10.1016/j.jemermed.2015.06.051

Jebberi Z, Marazzato J, De Ponti R, et al (2019) Polymorphic Wide QRS Complex Tachycardia: Differential Diagnosis. Card Electrophysiol Clin 11(2):333–344. doi: 10.1016/j.ccep.2019.02.004

Kapur J (2018) Role of NMDA receptors in the pathophysiology and treatment of status epilepticus. Epilepsia Open 3(2):165–168. doi: 10.1002/epi4.12270

Knuijver T, Belgers M, Markus W, et al (2018) Hallucinogen Persisting Perception Disorder After Ibogaine Treatment for Opioid Dependence. J Clin Psychopharmacol 38(6):646–648. doi: 10.1097/JCP.0000000000000966

Koenig X, Hilber K. (2015) The anti-addiction drug ibogaine and the heart: A delicate relation. Molecules 20(2):2208–2228. doi: 10.3390/molecules20022208

Kuryshev YA, Bruening-Wright A, Brown AM, et al (2010) Increased cardiac risk in concomitant methadone and diazepam treatment: Pharmacodynamic interactions in cardiac ion channels. J Cardiovasc Pharmacol 56:420–430. doi: 10.1097/FJC.0b013e3181f1d21b

Larsen P (2019) The Opioid Epidemic: A Public Health Emergency. Rehabilitation Nursing 44(2):67–68.

Luz M, Mash DC (2021) Evaluating the toxicity and therapeutic potential of ibogaine in the treatment of chronic opioid abuse. Expert Opin Drug Metab Toxicol. Doi: 10.1080/17425255.2021.1944099.

Marta CJ, Ryan WC, Kopelowicz A, et al (2015) Mania following use of ibogaine: A case series. Am J Addict 24(3):203–205. doi: 10.1111/ajad.12209

Mash DC, Duque L, Page B, et al (2018) Ibogaine Detoxification Transitions Opioid and Cocaine Abusers Between Dependence and Abstinence: Clinical Observations and Treatment Outcomes. Front Pharmacol 9:529. Doi: 10.3389/fphar.2018.00529

Matamoros-Castillo JM, Jávega-Manjó C, Sierra-San PM, et al (2019) The paradox of iboga: intoxication by a natural detoxification remedy. Actas Esp Psiquiatr 47(2):70–78.

Mazoyer C, Carlier J, Boucher A (2013) Fatal case of a 27-year-old male after taking iboga in withdrawal treatment: GC-MS/MS determination of ibogaine and ibogamine in iboga roots and postmortem biological material. J Forensic Sci 58:1666–1672. doi: 10.1111/1556-4029.12250

Meisner JA., Wilcox SR, Richards JB (2016) Ibogaine-associated cardiac arrest and death: case report and review of the literature. Ther Adv Psychopharmacol 6(2):95–98. doi: 10.1177/2045125315626073

Moher D, Liberati A, Tetzlaff J, et al (2009) Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Medicine 6(7):e1000097. doi: 10.1371/journal.pmed.1000097

O'Connell CW, Gerona RR, Friesen MW, et al (2015) Internet-purchased ibogaine toxicity confirmed with serum, urine, and product content levels. Am J Emerg Med 33(7):985–986. Doi: 10.1016/j.ajem.2014.12.023

Pleskovic A, Gorjup V, Brvar M (2012) Ibogaine-associated ventricular tachyarrhythmias. Clin Toxicol (Phila) 50(2):157. doi: 10.3109/15563650.2011.647031

Popik P, Layer RT, Fossom LH, et al (1995) NMDA antagonist properties of the putative antiaddictive drug, ibogaine. J Pharmacol Exp Ther 275(2):753–760.

Ruan Y, Bai R, Liu N, et al (2014) Herg blockade by iboga alkaloids. In Proceedings of the European Society of Cardiology Congress, Barcelona, Spain.

Sharpe LG, Jaffe JH. (1990) Ibogaine fails to reduce naloxone-precipitated withdrawal in the morphine-dependent rat. Neuroreport 1(1):17–19.

Sowunmi A, Salako LA, Laoye OJ, et al (1990) Combination of quinine, quinidine and cinchonine for the treatment of acute falciparum malaria: correlation with the susceptibility of Plasmodium falciparum to the cinchona alkaloids in vitro. Trans R Soc Trop Med Hyg 84:626–629. doi: 10.1016/0035-9203(90)90127-z.

Steinberg C, Deyell MW (2018) Cardiac arrest after ibogaine intoxication. J Arrhythm 34(4):455–457. doi: 10.1002/joa3.12061

Tournier N, Chevillard L, Megarbane B, et al (2010) Interaction of drugs of abuse and maintenance treatments with human P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2). Int J Neuropsychopharmacol 13(7):905–915. doi: 10.1017/S1461145709990848

Vlaanderen L, Martial LC, Franssen EJ (2014) Cardiac arrest after ibogaine ingestion. Clin Toxicol (Phila) 52:642–643. doi: 10.3109/15563650.2014.927477

Wilkins C, dos Santos RG, Solá J, et al (2017) Detoxification from methadone using low, repeated, and increasing doses of ibogaine: A case report. J Psychedelic Studies 1(1):29–34. 10.1556/2054.01.2017.005

Wilson C, Millar T, Matieschyn Z (2021) Novel treatment of opioid use disorder using ibogaine and iboga in two adults. J Psychedelic Studies 4(3):149–155. Doi: 10.1556/2054.2020.00133

Supplementary material 1.

References	Country	Design	Context and motivation of use	Subjects and clinical history	Ibogaine information	Toxicological analyses	Outcome	Conclusion
Breuer et al. (2015)	Germany	Case report	Spiritual cleansing at home with family members.	22-year-old man without history of acute or chronic illnesses. No concomitant medication or drug abuse.	38 g (Iboga root bark; internet supplier); Analysis not performed.	Presence of ibogaine and noribogaine in hair samples; presence of noribogaine in urine sample.	Intervention at ICU for 3 days. Treatment included i.v. midazolam (unknown dose) and levetiracetam (1 g). After 5 days in hospital all symptoms had disappeared.	Potential dose-dependent proconvulsive effect of ibogaine at high doses.
Marta et al. (2015)	Germany	Case series	Case 1: Not reported. Main reason: treatment of an opioid use relapse. Case 2: Unstructured setting. Main reason: treatment of an opioid use relapse. Case 3: Mexico (unspecified). Main reason: spiritual reasons.	Case 1: 36-year-old man with a history of depression, ADHD, maniac symptoms and polysubstance dependence. Medications used: divalproex ER. 1,000 mg, risperidone 2 mg, and quetiapine 200 mg, daily. Case 2: 35-year-old woman with a history of opioid dependence in sustained remission. Case 3: 40-year-old man without history of acute or chronic	Case 1: Unknown; Analysis not performed. Case 2: Unknown; Analysis not performed. Case 3: Unknown; analysis not performed.	Case 1: Urine drug screening positive for benzodiazepines . Case 2: toxicology positive for methadone at first intervention; toxicology positive for methadone, opioids, and benzodiazepines at second intervention. Case 3: Urine	Case 1: Intervention at residential chemical dependency treatment (divalproex 1 g, risperidone 2 mg, quetiapine 200 mg daily); Intervention at ED (divalproex 1.5 g daily, risperidone 2 mg, atomoxetine 80 mg). Discharged after 13 days of hospitalization. Case 2: Intervention at hospital for 2	Temporal association between ibogaine ingestion and subsequent development of mania has been described.

				illnesses.		drug screening positive for cannabinoids (admitted to having used an unknown quantity of psilocybin mushrooms days after ibogaine).	weeks (quetiapine, risperidone, and olanzapine at unknown doses); intervention at ED for 3 days (olanzapine at unknown dose). Case 3: Involuntary hospitalization for 6 days but refused all treatments offered.	
O'Connell et al. (2015)	United States	Case report	Home. Main reason: treatment of heroin dependence.	33-year-old man with history of heroin dependence and good health status.	3.8 g (ibogaine capsules; internet supplier); LC-TOF-MS confirmed the presence and quantity of ibogaine in one sample capsule.	Ibogaine was detected in both urine and serum samples.	Discharged from ED after 24 hours with no treatment.	Confirmation of classic physical and psychotropic manifestations seen in previous cases and literature.
Clouter-Gill et al. (2016)	Canada	Case report	Residential ibogaine program in Vancouver with nursing monitoring. No formal psychotherapy was performed. Main reason: treatment of heroin dependence.	37-year-old woman with history of heroin dependence. History of angina, dyslipidemia, obesity, chronic HCV, peripheral vascular disease, and ADHD. Patient's baseline ECG was within normal parameters, including a normal QTc.	Different dosing schedules for 4 days (total of 2.3 g of ibogaine HCL; iboga clinic); Analysis not informed.	Not performed	Unknown	A 4-day treatment with ibogaine successful in achieving long-term remission of a patient with severe OUD.

Hildyard et al. (2016)	United Kingdom	Case report	Home. Main reason: treatment of heroin dependence.	39-year-old man with history of heroin dependence. No regular medication or use of illicit drugs. Use of ibogaine 4 months earlier.	7 g (unknown presentation; internet supplier).	Not performed	Electrical cardioversion, i.v. magnesium 2 g, atropine 1 mg, and isoproterenol 2.5 µg per minute. Transvenous pacing. Patient was discharged after 7 days.	Ibogaine-associated ventricular dysrhythmias should respond to the standard treatments used for drug-induced polymorphic VT with QTc prolongation: supportive care with correction of electrolytes and parenteral magnesium plus anti bradycardia.
Meisner et al. (2016)	United States	Case report	Home. Main reason: treatment of heroin dependence.	40-year-old man with history of heroin dependence	4 g (unknown presentation; internet supplier); Analysis not performed	Serum drug screening positive for opioids and negative for other drugs. Relatives informed of heroin use 4 days before.	Intervention in field by ES (intubation, naloxone 2 mg, and CPR); Intervention in community hospital (morphine, therapeutic hypothermia protocol). Hemodynamic support with vasopressors. Cardiopulmonary death the same day.	Ibogaine induced cardiotoxicity and cardiac arrest showing the significant potential clinical risks of ibogaine
Henstra et al. (2017)	The Netherlands	Case report	Home. Main reason: treatment of drug dependence.	46-year-old woman with history of heroin, cannabis, alcohol, and cocaine dependence. No medical history of cardiovascular disease.	1.4 g (capsules; internet supplier; Analysis not performed.	Urine drug screening negative. LC-MS/MS confirmed the presence of ibogaine and noribogaine.	Treatment in coronary care unit included isoproterenol (unknown dose), pacemaker, i.v. sodium and magnesium. The patient was discharged after 12 days	A direct relationship between the concentration of the metabolite of ibogaine and the duration of cardiac effects.

Wilkins et al. (2017)	Spain	Case report	At home, with support service and remote monitoring. Main reason: detoxification of methadone.	47-year-old woman with history of heroin dependence and HCV. History of cannabis, heroin, amphetamine, and ethanol use. History of unsuccessful nonpharmacological detoxification treatment. No psychiatric diagnosis.	Repeated low doses ranging from 150 to 600 mg of ibogaine HCL; Phytostan Enterprises Inc.); Purity confirmed through unknown method	Not performed	Use of a single dose of diazepam (2 mg) after the first session. After the other sessions use of oral cannabis oil	The use of low doses of ibogaine may be effective for detoxifying patients from methadone. Use of benzodiazepines may be indicated to counteract insomnia and psychostimulant side effects. Clinical trials comparing single doses with multiple doses of ibogaine are necessary.
Knuijver et al. (2018)	The Netherlands	Case Report from Phase II clinical trial on ibogaine treatment for opioid dependence.	Hospital setting. Main reason: evaluate cardiac toxicity of ibogaine in patients with opioid use disorder in substitution therapy.	31-year-old man with opioid use disorder, history of heroin and nicotine dependence and a possible ADHD. No signs of other psychiatric conditions detected at the screening phase of the trial.	700 mg (HCL); Analyses not reported.	Urine drug screening negative. LC-MS/MS confirmed the presence of ibogaine and noribogaine.	Eye Movement Desensitization and Reprocessing (EMDR). Support in a clinical setting and administration of buprenorphine/naloxone 16/4 mg daily. The patient reported substantial relief of symptoms, but severe craving for opioids remained.	This case highlights the importance of psychiatric monitoring during and after treatment with ibogaine

Steinberg & Deyell (2018)	Canada	Case report	Holistic, naturopathic clinic. Main reason: overcome a long-standing opioid dependency related to chronic pain.	61-year-old man. Multiple spine operations resulting in a chronic cervico-lumbar pain syndrome. History of depression, mild hypertension, and dyslipidemia	5.6 g (capsules; iboga clinic); Analysis not performed.	Lab screening negative.	ED and ICU intervention (defibrillation, i.v. supplementation). Patient was discharged after 12 days.	The authors recommended prolonged cardiac monitoring because of active metabolite effect.
Barsuglia (2018)	United States	Case report	A 4-day program at an ibogaine clinic (Mexico) with medical screening and monitoring, SPECT, psychotherapeutic preparation, and post-experience integration. 4 doses of ibogaine were given and one dose of 5-MeO-DMT. Main reason: treatment of alcohol use disorder.	31-year-old male with ADHD, PTSD, depressed mood and alcohol use disorder. Reported personal history of sporadic use of ayahuasca and psilocybin mushrooms and weekly psychotherapy.	1550 mg of ibogaine HCl with the first three doses given in 30 min and the fourth 3h after the prior. Ibogaine was imported from Phytostan Enterprises, Inc.	Urine alcohol toxicology results negative upon arrival.	Administration of 500 mL of intravenous saline for hydration, 1 ampule of magnesium sulfate, and ranitidine 50 mg for nausea prior to the first dose of ibogaine.	Short-term therapeutic outcome was reported (improvement in mood, cessation of alcohol use, and reduced cravings at 5 days post-treatment, effects which were sustained at 1 month, but with a partial return to mild alcohol use at 2 months).
Mash et al. (2018)	United States	Open label case series	Iboga clinic (St. Kitts, West Indies). Main reason: to assess safety and open-label efficacy of ibogaine as a pharmacological treatment for managing withdrawal symptoms. Safety evaluations (for 7 days): physical examinations, laboratory tests, vital signs, 12-lead ECG and ECG telemetry (for	191 participants (men= 144; women= 47) with history of cocaine or opioid-dependence (DSM-IV). All individuals were subjected to a physician' examination, clinical laboratory tests, ECG, HCQ-29, and CCQ-45	8-12 mg/kg of ibogaine HCL; Unknown.	Pharmacokinetic analyses performed.	Administration of i.v. fluids 1 h prior to ibogaine administration to prevent orthostatic hypotension and bradycardia.	Significant improvements in craving, withdrawal, and mood were observed according to self-rated scales (p<.05). The results obtained support the use of ibogaine for the treatment of opioid withdrawal symptoms.

			24h).					
Grogan et al. (2019)	United States	Case report	Home with family members. Main reason: self-withdraw from opioids.	34-year-old woman with heroin and cocaine use disorder.	2 g (ibogaine powder; internet supplier); LC-QTOF/MS confirmed the presence of ibogaine, but quantitative analysis could not be performed.	Urine drug screening positive for cannabinoids, cocaine, and opioids.	Intervention at ED and ICU for 4 days, and 5 additional days in an inpatient psychiatric unit. Treatment included intubation and i.v. magnesium sulfate.	Authors highlight the awareness and education as an important way to offer benefit to patients who seek to use ibogaine and to the providers who care for patients suffering from dangerous sequelae.
Matamoros -Castillo et al. (2019)	Spain	Case report	Home with a family member supervision. Main reason: withdraw from codeine and alprazolam.	31-year-old man with history of anxiety and muscular pain (treated with alprazolam and fluoxetine). He had used codeine as auto-medication. The patient denied illicit drug use.	Unknown; Analysis not performed.	Urine drug screening negative.	Intervention in ED (olanzapine 20 mg); Intervention in psychiatric ward (rehydration and forced diuresis through fluid therapy). Number of days not specified. All clinical signs and symptoms ceased except a partial amnesia of the experience.	This case study shows that the use of ibogaine in informal settings poses serious risks for users and more studies are needed to clarify those risks.

Wilson et al. (2020)	Canada	Case series	Two different clinics at Vancouver area. Case 1: Iboga clinic with support service. Main reason: treatment of opioid dependence. Case 2: Iboga clinic following African traditions. Main reason: treatment of polydrug use.	Case 1: 35-year-old male with a 5-year history of opioid (illicit oxycodone) use disorder secondary to chronic pain. No prior mental health history. Case 2: 34-year-old woman with history of cocaine, heroin, fentanyl, and crystal methamphetamine use, and prescribed opioids.	Case 1: ibogaine/iboga doses not informed. Case 2: *T. iboga* (containing 725 – 1850 mg of ibogaine per ceremony) Unclear analysis	Not performed	Case 1: 10 mg diazepam between treatments to help the patient sleep and an antiemetic (not specified). Case 2: Self-administration of 25 mg of quetiapine orally before first ibogaine session. Intervention in ED (i.v. fluids and zopiclone). Discharged 9 hours later.	The author conclusion is that a better alignment and collaboration between health services are needed. More research is needed to determine optimal and safe ibogaine administration.
Glue et al. (2015)	New Zealand	Clinical trial, randomized, double blind (30 mg paroxetine or placebo) and 20 mg of ibogaine.	Hospital setting. Main reason: assess the influence of CYP2D6 activity on the pharmacokinetics of a single low dose of ibogaine in healthy volunteers.	21 healthy male participants, 20-40-years old.	20 mg of ibogaine (HCL) with 240 ml of water after an overnight fast; Analysis not informed.	Pharmacokinetic analyses performed.	Adverse effects were solved without intervention prior to study completion.	This study has confirmed the role of CYP2D6 in the metabolism of ibogaine and showed that single 20 mg doses of ibogaine were safe and well tolerated in healthy male volunteers. Paroxetine pre-treatment induced a 26-fold increase in the peak concentration of ibogaine. Mean half-life was longer as well (10.2 h vs 2.5 h; $p<0.05$).

Glue et al. (2016)	New Zealand	Clinical trial, randomized, double-blind, placebo-controlled of a single ascending-dose of noribogaine.	Hospital setting. Main reason: evaluate safety, tolerability, and pharmacokinetics of noribogaine in the treatment of methadone detoxification.	27 participants patients seeking to discontinue methadone OST who had been switched to morphine during the previous week.	60, 120, and 180 mg of noribogaine (HCL); Analysis not informed.	-	Adverse effects were solved without intervention prior to study completion.	Non-significant trend toward decreased total score in opioid withdrawal ratings (most notable in 120 mg dose of noribogaine). Dose-dependent QTc prolongation was found.
Brown & Alper (2017)	United States	Observational study. Detoxification and follow-up outcomes at 1, 3, 6, 9, and 12 months were evaluated using the SOWS and ASIC scores.	Two private iboga clinics (Mexico) with cardiac monitoring and pre-treatment tests. Main reason: treatment of opioid dependence	30 subjects with history of drug dependence, without any other use of ibogaine and good health and social status. Subjects were switched to a short acting opioid before ibogaine.	1540 ± 920 mg of ibogaine HCL; five subjects received additional 1610 ± 1650 mg of *T. iboga* root bark; iboga clinic; Purity confirmed through unknown method.	Not performed	No clinically significant cardiovascular or other medical events occurred in this study.	Ibogaine was effective for the treatment of opioid withdrawal symptoms and drug use in subjects for whom other treatments had been unsuccessful.

ICU= Intense Care Unit; ADHD= Attention Deficit Hyperactivity Disorder; ED= Emergency Department; LC-QTOF/MS= Liquid Chromatography-Quadrupole Time-Of-Flight Mass Spectrometry; HCV= Hepatitis C Virus; ECG= Electrocardiogram; Qtc= QT Intervals; HCL= Hydrochloride; OUD= Opioid Use Disorder; VT= Ventricular Tachycardia; ES= Emergency Services; CPR= Cardiopulmonary Resuscitation; LC-MS/MS= Liquid Chromatography With Mass Spectrometry; I.V.= Intravenous; SPECT= Single Photon Emission Computed; 5-Meo-DMT= 5-Methoxy-N,N-Dimethyltryptamine; PTSD = Post-Traumatic Stress Disorder; DMS-IV= Diagnostic And Statistical Manual Of Mental Disorders IV; HCQ-29/ CCQ-45= Craving For Cocaine Or Opioids Using Questions From The Heroin And Cocaine Questionnaires; LC-TOF-MS= Liquid Chromatography–Time-Of-Flight Mass Spectrometry; T. Iboga= *Tabernanthe Iboga*; CYP2D6= Cytochrome P450 2D6; SOWS= Subjective Opioid Withdrawal Scale; ASIC= And Addiction Severity Index Composite.

Supplementary material 2.

References	Acute adverse effects (24h)
Breuer et al. (2015)	Visions, nausea, vomit, muscle tension, cramps, generalized (tonic–clonic) seizure.
Marta et al. (2015)	Case 1: Unknown. Case 2: Unknown. Case 3: Unknown.
O'Connell et al. (2015)	Nausea, vomiting, altered mental status. Gait instability, diffuse myalgia, tremors, significant dysmetria with finger to nose examination, ataxia, QTc prolongation (527 ms).
Clouter-Gill et al. (2016)	Bradycardia, weakness, dizziness, diaphoresis.
Hildyard et al. (2016)	Glasgow Coma Scale score of 14, heart rate of 55 beats/min. Laboratory studies without relevant findings. Seizures, bradycardia, QTc prolongation (730 ms), ventricular tachycardia.
Meisner et al. (2016)	Found unresponsive for an unknown period and covered with emesis. Hypotension, hypothermia, anoxic brain injury (tomography) with nonreactive pupils, QTc prolongation (peak of 588 ms). Diagnosis of brain death and then cardiopulmonary death.
Henstra et al. (2017)	Unconscious for few hours. QTc prolongation (647 ms), atrial tachycardia, ventricular tachycardia, Torsades des Pointes.
Wilkins et al. (2017)	Use of scales during the first 24-hours: OWS, BPRS, UKU. Cardiac monitoring was performed. No psychiatric effects (BPRS) found. Gravity, fatigability, memory impairment, akathisia, and orthostatic dizziness, constipation, tension headache, reduction in the duration of sleep (UKU).
Knuijver et al. (2018)	Visual hallucinations (0-5 h), blunted affect, QTc prolongation (516 ms), ataxia (acute).
Steinberg & Deyell (2018)	Severe vomit and diarrhea. Alteration of level of consciousness. Radial pulse not palpable, and no blood pressure could be measured on arrival at ED. Wide QRS complex, tachycardia (270 bpm), QTc prolongation (714 ms), hypokalemia.

Mash et al. (2018)	Ibogaine was well tolerated, no changes in physical examination and safety laboratory tests. Statistically significant decreases in craving (p<.001) and different depression scales (p<.001). The following adverse events were reported: nausea, vomiting, ataxia of gait, perceptual changes, headache, orthostatic hypotension, bradycardic heart rate.
Barsuglia (2018)	Ataxia after 4.5 hours post-ingestion, vomiting episodes at 5- and 6-hours post-ingestion, and several acute panic attacks at 5 hours post-ingestion.
Grogan et al. (2019)	Hallucinations, seizures, altered mental status, apneic state. QTc prolongation (788 ms), Torsades des Pointes, persistent dysrhythmias.
Matamoros-Castillo et al. (2019)	Panic attack, fluctuating level of consciousness, space-time disorientation, psychomotor unrest.
Wilson et al. (2020)	Case 1: No clinically significant cardiovascular or other medical acute events occurred. Case 2: Increased visual features with negative feelings and sleep deprivation. QTc prolongation (512 ms), bradycardia (53 beats per minute).
Glue et al. (2015)	Decreases in VAS scale of 'sleepy' and increases in 'energetic' during 24 hours after ibogaine. Adverse events that occurred on or after ibogaine consisted in dizziness and nausea. Adverse effects in paroxetine-pretreated subjects were nausea, gastrointestinal symptoms, and dizziness.
Glue et al. (2016)	Headache, visual impairment, nausea. There were no hallucinations, serious adverse effects, nor changes in vital signs or safety laboratory tests.
Brown & Alper (2017)	Statistically significant decreases of ASIc scores (p< .001) between baseline and all the timepoints after ibogaine. No clinically significant cardiovascular or other medical events occurred in this study.

QTc= QT intervals; OWS= Opioid Withdrawal Scale; BPRS= Brief Psychiatric Rating Scale; UKU-SERS= Udvalg for Kliniske Undersogelser, Side Effects Rating Scale; ED= Emergency department; VAS= Visual Analogue Scale.

Supplementary material 3.

References	Prolonged adverse effects (>24 h)
Breuer et al. (2015)	Blood status: Increased C- reactive protein, white blood cell and creatine (first days). Neurological status: dysarthria, mild bilateral ptosis, psychomotor slowness and diffuse encephalopathic changes (day two).
Marta et al. (2015)	Case 1: Mania, irritability, grandiose delusions, rapid tangential speech, aggressive behavior. Awaken for 14 days after ibogaine use. Case of little to no sleep, aggression, impulsivity, psychomotor agitation, emotional lability, hallucinations, pressured and tangential speech, mania. Diagnosed with bipolar I disorder. Case 3: Two weeks of distractibility, irritability, grandiosity, emotional lability, decreased need mania, racing thoughts, suicidal ideation. Diagnosed with bipolar I disorder.
O'Connell et al. (2015)	All the symptoms were normalized during hospitalization.
Clouter-Gill et al. (2016)	Minor concentration deficits were reported during the first few weeks following therapy.
Hildyard et al. (2016)	QTc prolongation for 7 days.
Meisner et al. (2016)	Death in the first 24 hours
Henstra et al. (2017)	Hypokalaemia and hypomagnesaemia the day after admission. Pacemaker removed after 5 days.
Wilkins et al. (2017)	Not reported.
Knuijver et al. (2018)	Persisting hallucinogen perception disorder and negative feelings (48 h after ibogaine administration). Strong craving for opioids.
Steinberg & Deyell. (2018)	QTc prolongation for 7 days
Barsuglia (2018)	No clinically significant cardiovascular or other medical prolonged events occurred in this study.
Mash et al. (2018)	No clinically significant cardiovascular or other medical events occurred.

Grogan et al. (2019)	Extubated on her third day of admission. She showed persistent confusion for 5 days.
Matamoros-Castillo et al. (2019)	Drowsiness and psychomotor inhibition, soliloquies, hallucinations, dissociation, depersonalization, amnesia (two days after last ibogaine).
Wilson et al. (2020)	Case 1: No clinically significant cardiovascular or other medical prolonged events occurred. Case 2: No clinically significant cardiovascular medical prolonged events occurred.
Glue et al. (2015)	One report of cold symptoms (day 12) and conjunctivitis (day 14) in paroxetine-pretreated subjects.
Glue et al. (2016)	Not reported.
Brown & Alper (2017)	No clinically significant cardiovascular or other medical events occurred in this study.

Grogan et al. (2019)	Extubated on her third day of admission. She showed persistent confusion for 5 days.
Matamoros-Castillo et al. (2019)	Drowsiness and psychomotor inhibition, soliloquies, hallucinations, dissociation, depersonalization, amnesia (two days after last iboga
Wilson et al. (2020)	Case 1: No clinically significant cardiovascular or other medical prolonged events occurred. Case 2: No clinically significant cardiov medical prolonged events occurred.
Glue et al. (2015)	One report of cold symptoms (day 12) and conjunctivitis (day 14) in paroxetine-pretreated subjects.
Glue et al. (2016)	Not reported.
Brown & Alper (2017)	No clinically significant cardiovascular or other medical events occurred in this study.

Supplementary material 4.

References	Positive Points	Negative Points	Not Applicable Points	Total	Grade
Case Report Studies					
Breuer et al. (2015)	3	2	4	9	0,6
Marta et al. (2015)	6	1	2	9	0,85
O'Connell et al. (2015)	3	2	4	9	0,6
Clouter-Gill et al. (2016)	4	1	4	9	0,8
Hildyard et al. (2016)	3	2	4	9	0,6
Meisner et al. (2016)	4	1	4	9	0,8
Henstra et al. (2017)	6	0	3	9	1
Wilkins et al. (2017)	6	0	3	9	1
Knuijver et al. (2018)	3	2	4	9	0,6
Steinberg & Deyell (2018)	5	1	3	9	0,8
Mash et al. (2018)	8	1	0	9	0,88
Barsuglia (2018)	6	0	3	9	1
Grogan et al. (2019)	6	0	3	9	1
Matamoros-Castillo et al. (2019)	4	2	3	9	0,6
Wilson et al. (2020)	7	1	1	9	0,87
Total	**74**	**16**	**45**	**135**	**0,8**
Controlled Intervention Studies					
Glue et al. (2015)	10	4	0	14	0,71
Glue et al. (2016)	12	2	0	14	0,85
Total	**22**	**6**	**0**	**28**	**0,78**
Observational Study					
Brown & Alper (2017)	9	5	0	14	0,64
Total	**9**	**5**	**0**	**14**	**0,64**

Study 2

Reversing tolerance in methadone detoxification with a low dose of ibogaine

Genís Ona, Eulàlia Sabater, Carmen Ligero, Neus Vilalta, Edu Beas, Andrés Ferreira, Judit Biosca-Brull, Toni Llort, Josep M. Alegret, Juliana Mendes Rocha, Rafael G. dos Santos, Jaime E.C. Hallak, Miguel Ángel Alcázar-Córcoles, Clare Wilkins, Maria Teresa Colomina, Tre Borràs, José Carlos Bouso

Study II overview:

What do we already know?

Ibogaine has shown therapeutic potential as an "anti-addictive" drug in observational research and open-label trials. In addition, preclinical research and one case report have informed about the ability of ibogaine to reduce drug tolerance non-specifically. However, there are no randomized and double-blind trials confirming these preliminary observations.

What does this study add?

This study evidenced, in a controlled clinical setting, that ibogaine administered at a single low dose (100 mg) drastically reduces methadone tolerance and shows good tolerability and safety profiles.

Highlights

Methadone detoxification can be significantly accelerated using ibogaine.

Ibogaine administration in patients included in methadone maintenance programs is safe.

100 mg of ibogaine produces relaxing and slightly psychoactive effects.

Almost all patients receiving an acute dose of 100 mg of ibogaine were able to reduce the consumption of methadone at a half for one week.

Metabolic correlates of clinical findings were identified using a metabolomic approach.

More research is needed to assess higher/multiple doses and establish long-term efficacy.

Reversing tolerance in methadone detoxification with a low dose of ibogaine

Genís Ona,[1,2,3,4] Eulàlia Sabater,[5] Carmen Ligero,[6] Neus Vilalta,[7] Edu Beas,[5] Andrés Ferreira,[8] Judit Biosca-Brull,[2,9] Antoni Llort,[5] Josep M. Alegret,[6] Juliana Mendes Rocha,[10] Maja Kohek,[1,3] Rafael G. dos Santos,[1,10,11] Jaime E.C. Hallak,[1,10,11] Miguel Ángel Alcázar-Córcoles,[12] Clare Wilkins,[13] Maria Teresa Colomina,[2,9] Tre Borràs,[5] José Carlos Bouso[1,3,10]*

1. International Center for Ethnobotanical Education, Research, and Service (ICEERS), Barcelona, Spain.

2. Universitat Rovira i Virgili, Department of Psychology and Research Center for Behavior Assessment (CRAMC), Tarragona, Spain.

3. Universitat Rovira i Virgili, Medical Anthropology Research Center (MARC), Tarragona, Spain.

4. Parc Sanitari Sant Joan de Déu, Fundació Sant Joan de Déu, Institut de Recerca Sant Joan de Déu, Barcelona, Spain.

5. University Hospital Sant Joan de Reus. Servei de Drogodependències i Salut Mental. Pla D'Accions Sobre Drogues de Reus, Reus, Spain.

6. Department of Cardiology, University Hospital Sant Joan de Reus, University of Rovira i Virgili, Reus, Spain.

7. University Hospital Sant Joan de Reus. Emergency Department. Reus, Spain.

8. Independent researcher.

9. Universitat Rovira i Virgili, Research in Neurobehavior and Health (NEUROLAB), Tarragona, Spain.

10. Department of Neuroscience and Behavior, Ribeirão Preto Medical School, University of São Paulo, Hospital das Clínicas, Terceiro Andar, Av. Bandeirantes, Ribeirão Preto, SP 3900, Brazil.

11. National Institute for Translational Medicine (INCT-TM), CNPq, Ribeirão Preto, Brazil.

12. Department of Biological and Health Psychology, School of Psychology, Autonomous University of Madrid, Madrid, Spain.

13. Pangea Biomedics, Nayarit, Mexico.

*Corresponding author: José Carlos Bouso. International Center for Ethnobotanical Education, Research, and Service (ICEERS). Sepúlveda, 65, Bajos 2. Barcelona, Spain. 08015. Mail: jcbouso@iceers.org

Abstract

This study explores the impact of a single administration of ibogaine (100 mg) on methadone tolerance in patients enrolled in methadone maintenance programs. 20 subjects (3 women) were included in this study.

Methods: Subjects received a single oral dose of ibogaine and were closely monitored during a 24h period. Withdrawal symptoms, safety measures (electrocardiogram (ECG), blood pressure), and subjective effects were assessed. Additionally, metabolomic analysis of plasma samples pre- and post-ibogaine offered insights into potential biomarkers of its impact.

Results: Seventeen out of 20 participants were able to reduce their methadone dose by 50% compared to their pre-ibogaine daily dose and maintained it during the week after. There were three drop-outs after one week. No serious adverse events were reported. The dose of 100 mg of ibogaine was slightly psychoactive. The metabolomic analysis suggested that ibogaine could partially restore the energetic metabolism affected by the chronic use of methadone.

Conclusion: These findings indicate that a low-dose of ibogaine is safe and can enhance the detoxification process and offer cost-saving advantages. Larger, controlled studies are warranted to evaluate safety for higher or repeated doses, and determine long-term efficacy, especially in the current context of the dramatic fentanyl crisis facing a large part of the world.

Keywords: Ibogaine, methadone, opioid withdrawal syndrome, tolerance, psychedelics.

Introduction

The plant *Tabernanthe iboga* has been used for centuries, playing a central role in various spiritual and healing practices such as Bwiti and other African equatorial traditions [1,2]. The main alkaloid of the plant, ibogaine (IBO), is a psychoactive compound with putative anti-addictive properties primarily found in the root bark [3]. IBO is subjected to extensive first-pass metabolism in the liver, primarily by the cytochrome P4502D6 (CYP2D6) enzyme. IBO is O-demethylated to its active metabolite, noribogaine (NOR), which also contributes to its anti-addictive properties [4,5]. While IBO is currently administered in dozens of private clinical settings around the world, its safety is largely unknown. Its anti-addictive properties make it a candidate for the treatment of opioid dependence.

Since its registration in a patent by Ciba Pharmaceutical Products [6], the potential of IBO to counteract opiate tolerance has been recognized. Five years later, in 1962, Howard Lotsof serendipitously discovered the effect of IBO on opioid withdrawal syndrome (OWS). Lotsof explored the effects in an informal setting with a group of 20 subjects, seven of them being heroin users. The IBO dose was 19 mg/kg and was found to alleviate the symptoms of physical dependence and cravings. The most interesting effect was the suppression of OWS in five out of the seven heroin-dependent subjects, who remained abstinent for six months [7]. The first report documenting the effect of IBO in reversing opioid tolerance in humans was published in a case study. In this report, multiple and ascending doses of IBO were administered while reducing the methadone (MTD) dose by 50% after each dose [8].

Several studies investigating the potential anti-addictive effect of IBO have been carried out in animal models. Belgers et al. [9] performed a meta-analysis on preclinical research with IBO for the treatment of substance use disorders (SUDs). The results revealed a reduction in the self-administration of some drugs —such as cocaine, ethanol, or opioids— during the 72 hours after IBO administration. In humans, open-label case series and clinical trials in opioid-dependent subjects highlight the potential of IBO to reduce OWS. For example, the results of a study conducted by Mash et al. [10] with 32 opioid-dependent patients showed that after administering 800 mg of IBO, craving symptoms decreased over 3-9 days, and withdraw symptoms decreased after 12-24 hours. Another open-label study) published by the same author reported reductions in OWS and drug cravings after a single oral dose (8-12 mg/kg) of IBO [11]. Most of the studies evaluating the efficacy of IBO on OWS were performed on heroin, MTD, or cocaine-dependent subjects. The treatment of MTD dependence has significant importance due to the particular challenges posed by its long half-life, which makes the dishabituation process more difficult.

Regarding the safety of IBO and NOR, while some studies have shown no adverse events [11,12], others reported nausea, gastrointestinal symptoms, dizziness, or headaches after administering 20 mg of IBO [13,14] or 3-60 mg of NOR in healthy volunteers [15]. A randomized, double-blind study by Glue et al. [16] involving patients upon MTD maintenance programs demonstrated that NOR administration at doses of 60 mg, 120 mg, and 180 mg resulted in visual impairment, headache, and nausea. Additionally, a dose-dependent QTc interval prolongation (28 ms, 120 mg; 42 ms, 180 mg) was observed. Lastly, an open-label trial where 10 mg/kg of IBO were used reported relevant QTc prolongations (50% of patients reached QTc above 500 ms) and severe ataxia [17]. Interestingly, Koenig et al. [18] describe how IBO alters the repolarization of the action potential by inhibiting the hERG (human Ether-a-go-go-Related Gene) channels. These data are gathered in three systematic reviews [19-21]. Ona et al. [21] divided the adverse events into acute (occurring < 24h after dosing) and prolonged (> 24h after dosing). The most common

acute effects were QTc prolongation, tachycardia, hypotension, nausea, and vomiting. The prolonged effects included QTc prolongation, insomnia, alterations in speech, delusions, psychomotor slowness, bilateral ptosis, or dysarthria [21]. The conclusion of these reviews is that more research is needed to identify factors predisposing to IBO/NOR-associated adverse events.

In the 1980s, Spain faced a significant heroin crisis. To address this, many heroin users were enrolled in methadone maintenance programs (MMP). Methadone, a long-acting opioid, is prescribed to reduce cravings and withdrawal symptoms, helping users stabilize and decrease their use of needles for illegal opioids. However, missing a daily MTD dose can result in OWS [22-25], making it difficult to discontinue its use in most cases. In addition, MTD has long-term adverse events such as cognitive decline [26,27], oral health issues [28], a decrease in quality of life [29], or potential risk of respiratory depression and subsequent death [30]. Spain presently has a total of 49,014 individuals enrolled in these programs [31], with 8,062 of them in Catalonia specifically [32]. In Spain in 2020, opioids were detected in 59.5% of deaths caused by acute drug reactions, with MTD being present in 28.8% of cases. Methadone was the only opioid present in 13.3% of cases [31].

The safety, tolerability, and mechanisms of IBO in humans need thorough investigation, especially given its potential anti-addictive properties and the growing informal use. Despite safety concerns and numerous adverse events, high doses of IBO (15-20 mg/kg) are commonly administered to induce strong psychoactive effects. However, low and safer doses are rarely used. Some researchers suggest testing lower doses of IBO in humans [33]. These lower doses could aid in the lengthy detox process, as preliminary evidence indicates IBO can reduce drug tolerance [34-37]. This study aims to investigate the safety and efficacy of a low, single oral dose of IBO (100 mg) in reducing tolerance to MTD.

Materials and methods

Study Design

The present results belong to the first administrations of a randomized and double-blind study involving a maximum of six weekly IBO administrations. The randomized, double-blind study involves two parallel groups assessing the safety and efficacy of IBO for the treatment of OWS in the context of MTD detoxification. Each group received six weekly doses of IBO. Group 1 received six fixed IBO doses (100 mg), while Group 2 received six ascending IBO doses (100-200-300-400-500-600 mg), a protocol previously published in a case report [8]. Each IBO administration took place 24 hours after the last MTD dose. The current manuscript describes the initial administration (100 mg) of the whole sample (20 subjects). It is worth noting that, to date, the only randomized clinical trial reporting on the safety of IBO used a dose of 20 mg [13]. We used the CONSORT reporting guidelines for randomized trials [38].

Participants

In this study, data from 20 patients (17 males, 3 females) were included. They were recruited between the years 2019 and 2024 via treatment providers, referrals, and word of mouth through the Addictions and Mental Health Service (Servei d'Addiccions i Salut Mental) at the University Hospital Sant Joan, Reus, Spain. The profile included subjects from MMPs wishing to stop their MTD use. All of them were taking MTD daily at around 8 am. Once informed consent

was obtained, the participants were assessed for their eligibility. Inclusion criteria included body mass index within a range of 19-27, and no alterations in laboratory tests or in the electrocardiogram (ECG). Exclusion criteria included viral activity for Hepatitis B or C, HIV, a history of severe psychiatric diseases like psychosis, bipolar disorder, or dissociative disorders. The complete criteria can be found at Clinicaltrials.gov [NCT04003948, 01/07/2019]. The study protocol was amended on two occasions. It was first done to change the experimental drug provider before recruitment started. The second was to change the age range of patients from "18-60" to "18-67."

Randomization

The method of randomization used was block randomization with a block size of 20. This approach ensured that each of the two groups received exactly 10 subjects, maintaining balance between the groups. The random allocation sequence was implemented using a computer-based random number generator to ensure unbiased assignment. The sequence was concealed by generating and storing the allocation assignments in a secure, password-protected database. It was not revealed until the study was completed, ensuring that neither the participants nor the researchers could influence the allocation process. This approach preserved blinding and maintained the integrity of the randomization throughout the study. The allocation sequence was generated by the pharmacist, while the recruitment of participants was carried out by the co-PI, Dr. Tre Borràs, and the medical anthropologist, Dr. Antoni Llort. The assignment of patients to interventions was determined by the randomization process. All personnel involved in the treatment process were blinded to the study. Only the pharmacist, who provided the medication to the clinical staff, had access to the patient codes. However, for this manuscript, all subjects were aware that they were receiving the 100 mg dose.

Psychometric Measures

Screening

The Wave test [39] was used with exploratory purposes since it is a tool under development. This test contains two sub-scales measuring the risk of having a psychotic break or a bipolar break, respectively. It includes 30 Likert-type items that are scored from 1 to 4. The outcome is reported on a Z-score scale. A cut-off point of around 1.8 can be considered indicative.

Adverse events

Each adverse event was collected and appropriately registered. In addition, the UKU scale [40] (Spanish version [41]) was administered to systematically register them. The UKU consists of 54 items grouped into four distinct subscales. It measures the presence and causal relationship of various medication symptoms: psychological effects (9 items), neurological effects (8 items), autonomic effects (11 items), and other effects (25 items). The last two items (55 and 56) assess the degree that the side effects impact the patient's daily functioning and the consequences of medication intake (none, dose reduction, withdrawal, or change). Each item presents four possible responses ranging from 0 to 3 (0: not present or unlikely present, 1: mild, 2: moderate, 3: severe).

Opioid withdrawal syndrome

Three different scales were administered to assess the presence of OWS symptoms. Due to measures not collected, data of these questionnaires were reported for 18 subjects. The OWS scale [42] (Spanish version [43]) consists of 32 items and was administered right before IBO administration and again after 12 hours. The Short Opioid Withdrawal Scale (SOWS [44]; Spanish version [41]) consists of 10 items and was administered right before IBO, every hour for the first 8 hours, and 10 and 12 hours after IBO administration. Finally, the Subjective Opioid Withdrawal Scale (SbOWS [45]; Spanish version [41]) consists of 16 items and was administered right before IBO was given and again after 12 hours.

Visual Analogue Scale

For this study, 15 Visual Analogue Scales (VAS) were used to capture the subjective experience elicited by IBO. Due to subject dropout and measures not collected, VAS data were reported for 17 subjects. VAS scales consisted of 15 items that were administered 12 hours post-IBO. Each one was placed above a 100 mm horizontal line (from 0, “no effect” or “I did not feel anything at all,” to 100, “extremely intense, I felt that”). See Supplementary material for a list of VAS items and for a diagram of all the measurements.

Psychiatric safety

The Hospital Anxiety and Depression scale (HAD [46]; Spanish version [47]) was used to monitor mood along the study. Due to subject dropout, HAD data were reported for 15 subjects. The questionnaire was given at the beginning of each IBO session. The HAD is a self-report questionnaire consisting of 14 items. Seven items assess depression and the other seven anxiety. Scores on both scales range from 0 to 21 and the total score is formed by combining the two scales.

The Brief Psychiatry Rating Scale (BPRS [48]; Spanish version [41]) was used to measure psychopathological signs along the study. Due to subject dropout and measures not collected, BPRS data were reported for 14 subjects. It consists of 18 items that are scored according to a 5-point Likert scale of intensity or severity. Each item has a definition and operational criteria for evaluation and scoring. It provides an overall score and scores in two sections: negative symptoms and positive symptoms.

See Supplementary Table 1 for a schematic diagram of all the procedures within each IBO session.

Experimental drug

Two batches of IBO HCl were used for this study. They were purchased from CAPE Labs, South Africa. This laboratory extracts IBO from *Voacanga africana* instead of *Tabernanthe iboga*, since the former is a more ecologically sustainable option. The provider reported the purity of batch 1 was > 96% using thin layer chromatography (TLC). A reanalysis performed by Eurecat Reus (Spain) through nuclear magnetic resonance (NMR) found a purity of 97.9% (±0.1%). Reported purity of batch 2 by the provider through TLC was > 96%. A reanalysis performed by Eurecat Reus through NMR resulted in a purity of 98.4% (±0.3%).

Procedures

After patients gave their informed consent, they were screened via a psychiatric interview and given psychometric questionnaires and a medical assessment (which included a physical examination, blood and urine analysis, and neurological assessment). 47 people were contacted,

30 were screened, and 20 were randomized. They were included and randomized in the study if all inclusion criteria were met and there were no exclusion criteria. They took the last dose of MTD 24 hours before the first session of IBO. On the day of the session, patients arrived at the hospital at around 8:30 am. A urine test and breathalyzer were performed. If both tested negative, blood pressure (BP) and ECG were obtained. Once the cardiologist (CL) checked the results, the patient was accompanied to their hospital room. They stayed there for 24 hours where psychometric tests were done before IBO was administered. The room contained no special decorations. Patients could not leave the room during the 24-hour hospital stay. The hospital provided a low-fat diet without spices to not interfere with IBO pharmacokinetics. A psychologist (GO) or a psychiatric nurse (JMR) were present in the room for safety reasons and to provide support (if needed) during the entire 24-hour period. No formal psychotherapy techniques were employed.

After IBO administration, an ECG was conducted by the nurse members of our team (EB, NV, AF and JMR) every hour for the first eight hours and at 10 and 12 hours. Half of the last MTD dose was available for patients if OWS symptoms were present. Patients could ask for MTD when they needed it. Paracetamol or diazepam was available if needed. Emergency medications for potential serious cardiac events (bisoprolol 5 mg p.o., magnesium sulfate 150 mg/ml i.v.) were available inside the room. Our team was coordinated with the nurse's ward to activate established hospital protocols in case of serious adverse events.

The last ECG was performed and the patient left the hospital 24 hours after IBO administration. Participants were provided with the daily doses of MTD needed until the next IBO session. The participants were sequentially included in the study one at a time except for participants 16-20, who shared the same room in pairs.

The daily dose of MTD was reduced by 50% compared to the dosage administered prior to the IBO session. This reduction was done due to IBO's putative capacity for reducing drug tolerance [34-37]. The protocol allowed an increase in the MTD dose between IBO sessions upon subject's demand.

Metabolomic analysis

A total of 26 plasma samples from thirteen patients were analyzed by the Center for Omic Science (COS, Universitat Rovira i Virgili) (Reus, Spain). Samples were collected in each subject at baseline (around 1 week before IBO administration) and 24 hours after administration. The metabolic profile was determined by a polar (aqueous metabolites) and apolar (lipid metabolites) extraction using proton nuclear magnetic resonance (1H NMR). See Supplementary material for a detailed description of methods.

Statistical analysis

The data were registered and stored in the electronic Case Report Form created and hosted by the W3-Nexus platform at the Fundació Institut Català de Farmacologia (currently the Catalan Pharmacovigilance Center) and followed anonymity, traceability, and all Good Clinical Practices guidelines. As this is an exploratory study, the analyses were performed following a completer analysis strategy. The analyses including measures before the session and seven days after were performed with those participants who attended session 2 (n = 17) and participants with missing values in any variable were excluded from the analysis of that specific variable. Descriptive statistics were used to present demographic data, the MTD dose used during enrollment and after IBO administration, the use of other medications, adverse events, and the results of psychometric questionnaires. A

student *t*-test or *the Wilcoxon* signed-rank test were used to compare OWS and SbOWS (before IBO administration and +12h post-IBO administration) and HAD (before IBO administration and +7 days) scores when the studied variable was normally or non-normally distributed respectively. Repeated measures ANOVA (RM-ANOVA) or the *Kruskal-Wallis* test were used for analyzing the data on SOWS scores, BP, and QTc intervals. Post-hoc pairwise Student *t* test with Bonferroni correction was done for RM-ANOVA results since significant results were observed. A Wilcoxon matched pairs signed rank test was conducted to explore differences in the sub-sample of women (n= 3) in some cases. Significance was two-tailed and set at $p < 0.05$. Adjustment for multiple comparisons was applied in a post-hoc analysis of repeated measures ANOVA with QTc measurements (0.05/11= 0.004). We used IBM SPSS Statistics 21 to run the analyses.

Metabolomics statistical analysis was carried out using the Metaboanalyst 6.0 web tool (www.metaboanalyst.ca). Aqueous and lipid metabolome databases were uploaded and normalized by sum and pareto scaling [49]. Afterwards, outliers screening was performed using heat maps. Samples that had very different color or shades from the rest of the matrix were removed, as well as the complementary baseline or 24h post-IBO group. For all the analyses, the sample was first analyzed as a whole, and then divided by the MTD dose used (low or high; doses below 50 mg are considered low, and above 50 mg high). Supplementary Table 2 shows the total number of subjects in the analysis. Principal component analysis (PCA) was used to perform a general metabolic screening and to determine the differences between pre- and post-intervention. In addition, random forest analysis was used to quantify the most significant metabolites to explain differences between baseline and post-IBO administration, while the discriminative ability of each metabolite to distinguish between the pre- and post-intervention was assessed by receiver operating characteristic (ROC) curve, measuring the area under the ROC curve (AUC). Better discriminatory abilities were associated with higher AUC values, thus discriminatory ability was classified as excellent when AUC values were 0.90 or above, good with values between 0.80 and 0.90 and fair when AUC was between 0.70 and 0.80, while values below 0.70 were considered poor. Biological interpretation of the altered metabolites was performed by functional pathway analysis. In parallel, a paired sample t-test using the SPSS 26.0 software (IBM Corp., Chicago, IL, USA) was conducted to confirm statistically significant differences between groups in that metabolites presented in the random forest. Graph were made with GraphPad Prism 8.0 (GraphPad software, San Diego, CA, USA) and represented as the mean ± S.D. Statistical significance was set at $p < 0.05$.

Results

The recruited sample age ranged between 27 and 59 years (with a mean of 42.7 years). The dose of MTD used at inclusion ranged between 5 and 100 mg, with a mean of 46.5 mg (see Table 1). Doses of MTD in women ranged between 5 and 75 mg, with a mean of 40 mg. Among the whole sample, 18 were smokers, and 10 people were using additional medications that were not interrupted for the study. Nine of them were prescribed some type of medication during the 24h hospital stay (see Table 3 in the Supplementary materials for further details).

Three subjects abandoned the study after the first session. Subject R-003 consumed heroin during the week and was excluded for safety reasons. Subject R-007 reported intense withdrawal effects after few hours of IBO administration and decided to interrupt their study participation upon completion of first session. Subject R-011 was excluded for safety reasons.

Their QTc raised from 446 ms at baseline to 484 ms after 6 hours of IBO administration, and the cardiologist of the team considered it was not safe for them to continue in the study.

Results of the Wave test were obtained from only 15 participants. The risk score of developing psychotic disorders measured with the WAVE ranged between -2.01 and 1.56, and the range for bipolar disorders ranged between -2.16 and 0.20. None of the participants were excluded due to their scores on this test.

Safety

No serious adverse events were reported as assessed through the UKU or through the observation of other adverse events not included in this scale. 19 subjects reported some kind of adverse event. The most common adverse events were fatigability (n = 7), constipation (n = 3), restlessness (n = 3), dizziness (n= 3), photosensitivity (n= 3), memory disturbances (n = 2), anxiety (n = 2), and insomnia (n = 2). The adverse events reported by women were dizziness (n = 1), constipations (n = 1), anxiety (n = 1), photosensitivity (n = 1), sleepiness (n = 1), increased sleep duration (n = 1), and concern (n = 1). Moreover, one of the women showed bronchial infection symptoms after session 1.

The general mean of HAD anxiety was 5.67 (min. 0; max. 13) (women: mean = 6.67; min = 4; max = 11), and the general mean of HAD depression was 4.39 (min. 0; max. 14) (mean of 3.67; min. 0; max. 7 in the case of women) at baseline. One week later, mean of HAD anxiety was 5.60 (min = 1 max = 13) (women: mean = 5.33; min = 2; max = 11), and mean of HAD depression was 4.07 (min = 0 max = 14) (women: mean = 3.67, min = 2; max = 6). Neither HAD depression (Z= 152.0, p = 0.56) nor HAD anxiety scores (t(14)= 0.36, p = 0.73) differed significantly from baseline scores at +1 week. Mean total score of HAD was 10.1 (min: 0; max = 27) (women: mean = 10.3; min = 4; max = 18). At +1 week, total score of HAD was 9.67 (min = 2; max = 24) (women: mean = 9.0; min = 5; max = 17), being the difference not statistically significant (t(14)= 0.33, p = 0.75). Results Mean BPRS score before IBO administration was 24.6 (min = 20; max = 32) (women: mean = 27.0; min = 24; max = 32), being 23.2 (min = 18; max = 31) (women: mean = 25.0; min = 25; max = 25) at +1 week. This difference was not statistically significant (t(12)= 1.58, p = 0.14).

QTc values changed significantly over the 12 times they were measured in the 24-hour period, as informed by repeated measures ANOVA ($F_{(11,\ 207)}$= 3.56, p = 0.001). Post-hoc pairwise t test showed significant Bonferroni corrected differences between +4h and +10h timepoints (432.5 (20.9) *vs.* 420.9 (24.9); p= 0.01), between +2h and +10h (436.5 (23.4) *vs.* 420.9 (24.9); p = 0.04) and between +2h and +12h (436.5 (23.4) *vs.* 422.2 (18.4); p = 0.04). The general tendency of QTc was to slightly increase after IBO administration and return to baseline after 10 hours, as observed in Figure 1. Women showed a mean QTc of 413.7 at baseline (compared to 422.5 in men) and 408.5 after 12 hours (compared to 419.7 in men). Notably, one subject had to be excluded because of his QT raised to 484 ms after 6 hours from a QT baseline of 446 ms.

A significant time effect was observed for diastolic ($F_{(11,\ 179)}$= 2.37, p = 0.01) but not for systolic ($F_{(11,\ 179)}$= 1.81, p = 0.06) BP. The post-hoc pairwise t test with Bonferroni correction performed to analyse the significant effect of systolic BP showed only significant differences between pre-session and +3h (125.7 (17.0) vs. 116.0 (15.6); p = 0.02). See Supplementary Table 4 for BP details.

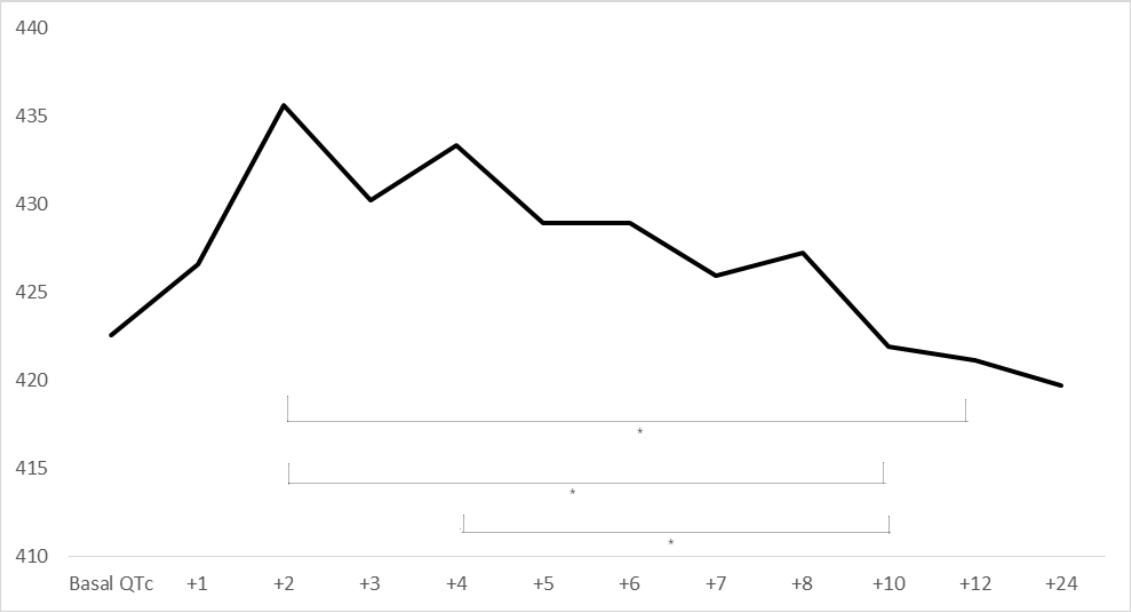


Figure 1. Hourly QTc values obtained across the 24h period of ibogaine sessions. *Indicates significant differences (p<0.05).

Efficacy

Most subjects could interrupt their MTD use for several hours (M= 1081.9 min. or 18.03 h, SD= 305.8 min.). The mean time for men was 1052.8 min (SD= 289), and 1246.6 min (SD= 414) for women. While most of them claimed that the withdrawal syndrome was much softer, the OWS score was slightly higher at the 12h administration post-IBO (M= 8.33; SD= 6.6; women= 7.33; SD= 10.1) as compared with baseline (M= 7.44; SD= 7.1; women: M= 3.67; SD= 4.0). However, this difference was not statistically significant for the general sample ($t(17)= 0.56$, $p = 0.58$) or for women only ($Z= 3.0$, $p > 0.99$). The SbOWS score was also higher at 12h post-IBO (M= 5.50; SD= 6.5; women: M= 4.33; SD= 7.5) than at baseline (M= 4.72; SD= 3.9; women: M= 2.67; SD= 3.8). This difference was, again, not statistically significant in the general sample ($Z= 74.0$, $p = 0.92$) or in the case of women ($Z= 3.0$, $p > 0.999$). As SOWS scores registered throughout the IBO session did not show a normal distribution, Kruskal-Wallis test was performed to assess the change in scores during the session. There was no statistically significant change across measurements ($\chi^2_{(10)} = 9.55$, $p = 0.48$). Nevertheless, a first decrease was observed after one hour of IBO administration, as seen in Figure 2. This was clinically noticeable, as subjects showed clear signs of relaxation and relief. SOWS scores then increased again and there was an overall tendency to decrease across subsequent measurements up to 12 hours after IBO administration. As observed in Table 1, one participant (R-010) did not tolerate the 50% reduction of the MTD basal dose. Moreover, as mentioned at the beginning of the results section, three participants abandoned the study within the week following IBO administration (See Table 1).

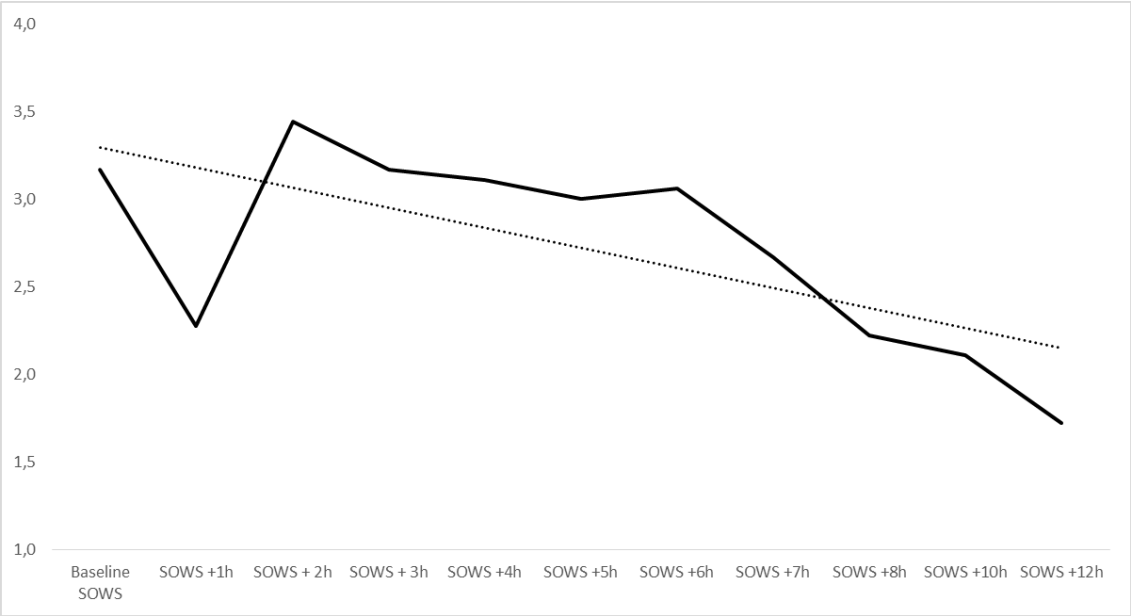


Figure 2. The SOWS scores obtained over the 12 hours after ibogaine administration. Mean values are expressed as tendency in a dotted line. No statistically significant differences were observed.

Table 1. Decreases in methadone dose use after ibogaine administration

Participant code	Initial dose of MTD (mg)	Dose of MTD (mg) during 6 days after ibogaine administration
R-001	20	10
R-002	100	50
R-003	30	15
R-004	50	23
R-005	45	23
R-006	60	30
R-007	60	30
R-008	55	28
R-009	60	30
R-010	32	26
R-011	40	20
R-012	28	14
R-013	40	20
R-014	100	50
R-015	75	35
R-016	14	7
R-017	16	8
R-018	60	30
R-019	5	3
R-020	40	20

Visual Analogue Scale

Regarding the VAS scores, the highest scores were obtained in "I would like to take this drug again", and "I felt relaxed" (See Figure 3). "I have experienced psychoactive effects / 'high'", and "Overall intensity of the experience" obtained a mean of 14 and 25 scores, respectively, suggesting that 100 mg of IBO was slightly psychoactive.

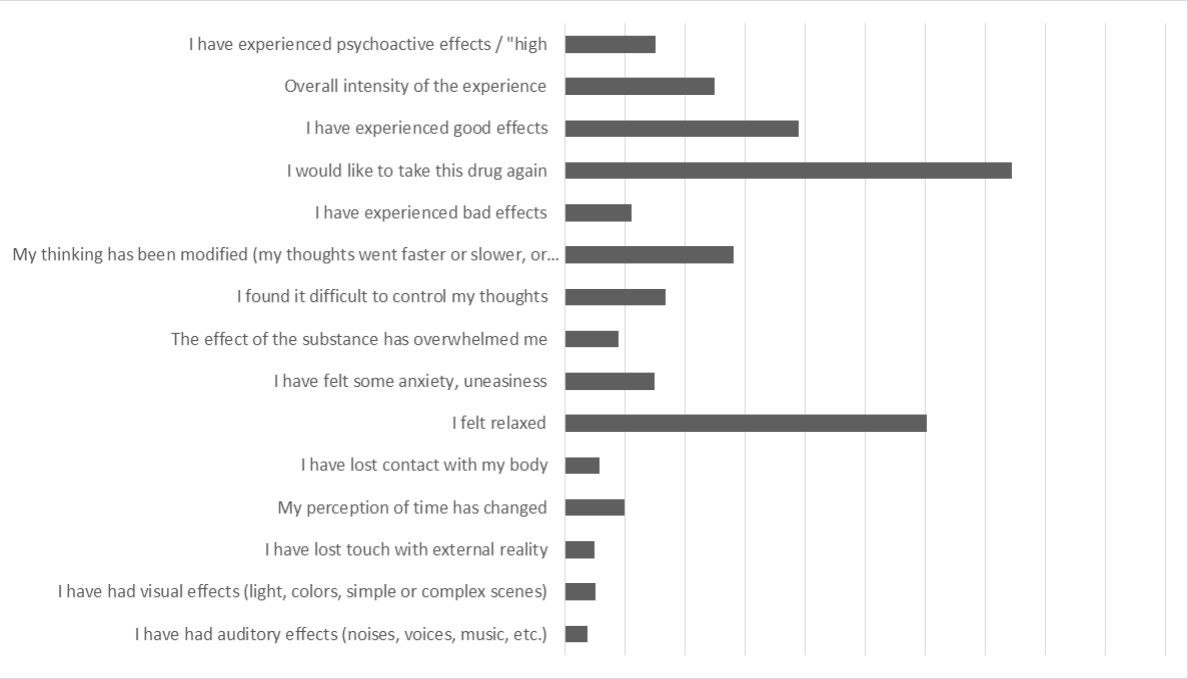


Figure 3. Mean VAS scores obtained 12 hours after ibogaine (100 mg) administration.

Metabolomic analysis

Aqueous metabolites

Results from the polar extraction showed two well-defined groups ("baseline" and "24h post-IBO administration") in the graphical representation of the PCA analysis (Figure 4A). However, these groups did not differ from each other, as they overlap in the graphical representation. This may be attributed to the heterogeneity manifested in post-treatment outcomes, as evidenced by the increased dispersion of scores.

As can be seen in Figure 4B, random forest analysis indicated that lactate and 2-oxo isocaproate are the best metabolites to discriminate between pre- and post-IBO administration, with lactate levels decreased in the 24h post-IBO administration, while 2-oxo isocaproate levels increased.

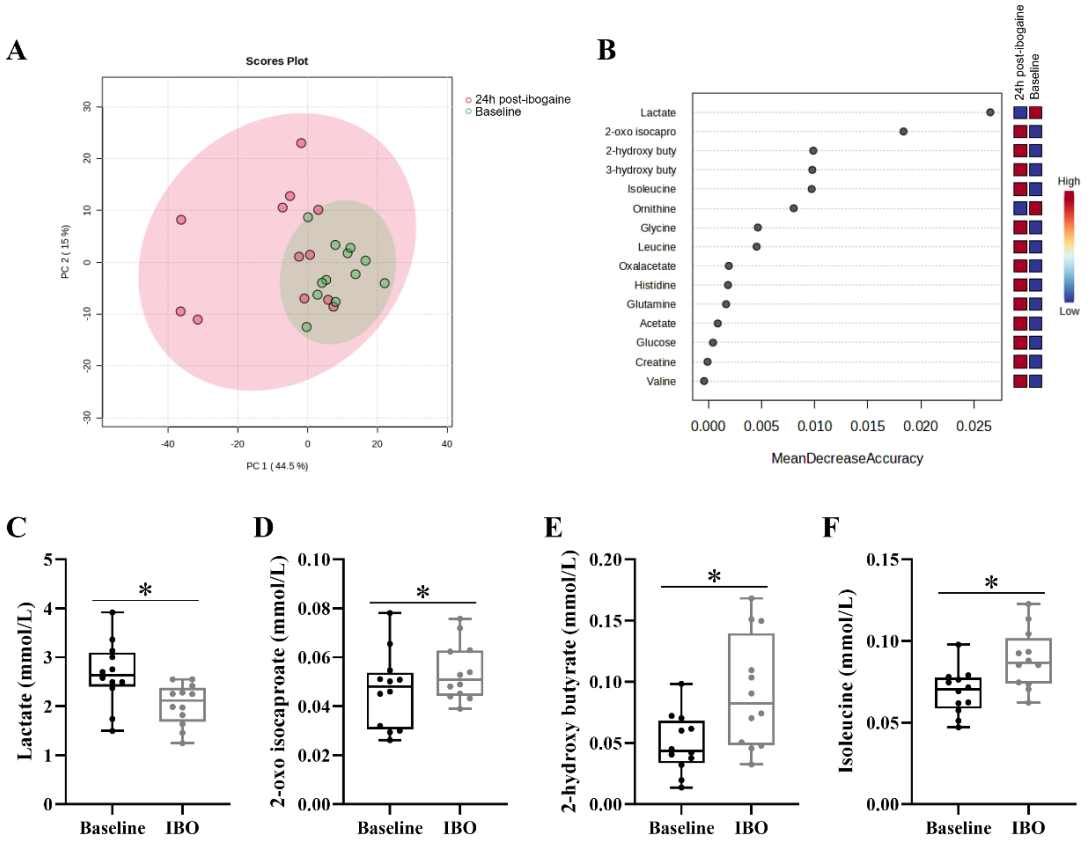


Figure 4. Principal component analysis (A), random forest representation (B) and Student's *t* test results (C–F) of changes in aqueous metabolites 24h after 100 mg of ibogaine administration. *Indicates differences between pre- and post-administration at $p<0.05$.

In order to evaluate the discriminative ability of lactate and 2-oxo isocaproate, as well as other metabolites, ROC curves were performed, and AUC scores were taken into account (Supplemtary Table 5). Paired sample t-test was carried out to confirm differences between groups in those metabolites with AUC higher than 0.80. This analysis confirmed that IBO administration decreased lactate [t_{11} = 3.183, p = 0.009] (Figure 4C), but increased 2-oxo isocaproate ([t_{11} = -2.501, p = 0.029]), 2-hydroxy butyrate ([t_{11} = -4.367, p = 0.001]), and isoleucine ([t_{11} = -4.586, p = 0.001]) (Figure 4D to 4F).

The analysis considering patients with low doses of MTD showed two well-defined groups in the PCA, but again they overlap in the graphical representation (Figure 5A). Metabolites such as 2-oxo isocaproate and 2-hydroxy butyrate were the most affected showing an increase after IBO administration (Figure 5B)., The paired sample t-test for metabolites with AUC values higher than 0.80, showed significant differences in 2-oxo isocaproate [t_6 = -2.477, p = 0.048] and isoleucine levels [t_6 = -2.625, p = 0.039] (Figure 5C to 5H and Supplementary Table 5),

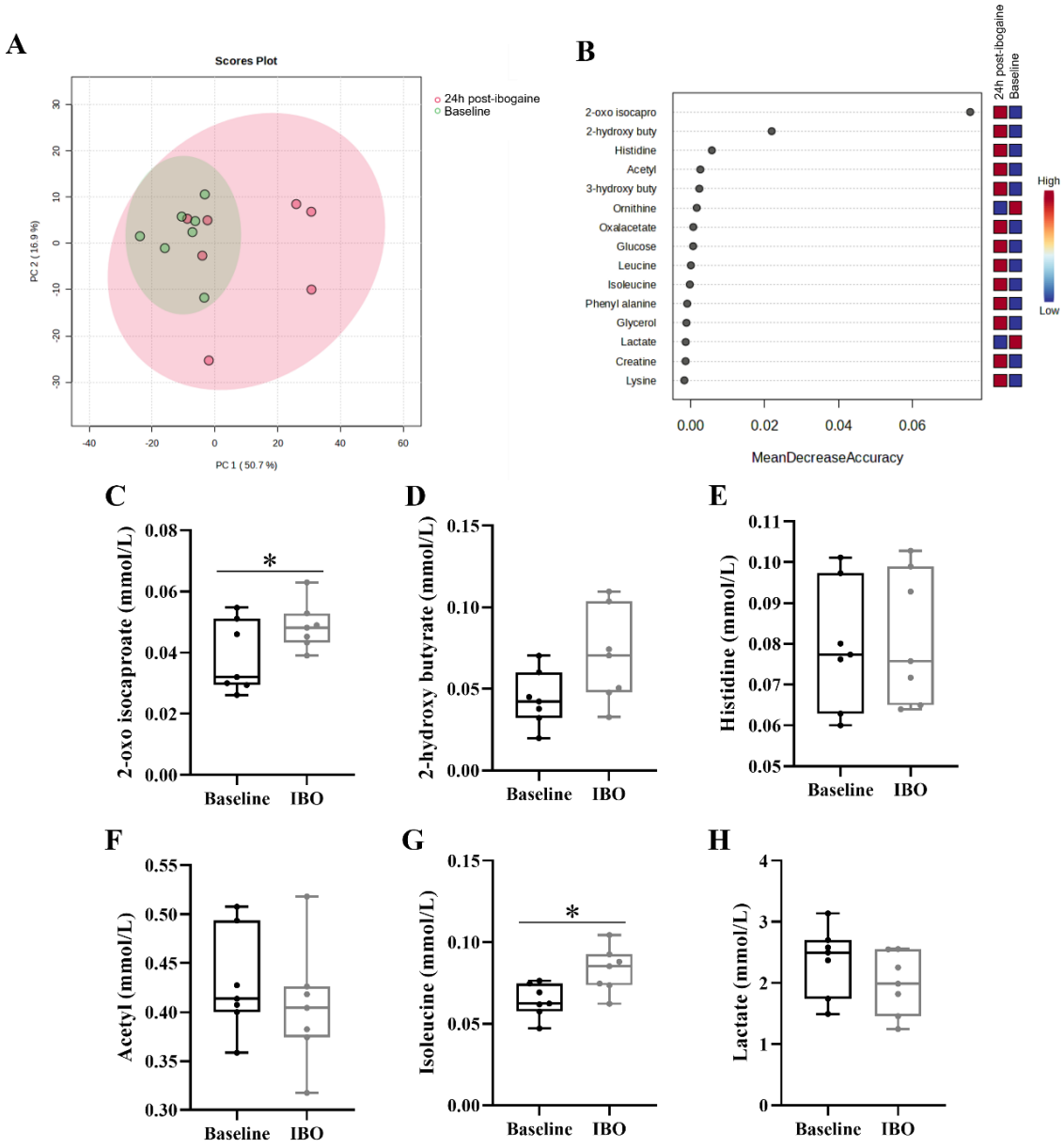


Figure 5. Principal component analysis (A), random forest representation (B) and Student's *t* test results (C–H) of changes in aqueous metabolites after 24h of 100 mg ibogaine administration in patients using low, daily MTD doses (<50 mg). *Indicates differences between pre- and post-administration at $p<0.05$, while trend is indicated with a t.

Administration of IBO after a high dose of MTD showed a clearer difference between baseline and 24h post-IBO groups in PCA analysis (Figure 6A). Random forest analysis pointed that valine, phenylalanine and lactate were the metabolites which presented higher differences between groups. Specifically, valine and phenylalanine increased after IBO administration, whereas lactate decreased (Figure 6B). ROC curve showed AUC values higher than 0.90 in those metabolites (Supplementary Table 5) and paired-sample t-test showed significant differences in valine [$t_4 = -3.543$, $p = 0.024$] lactate [$t_4 = 3.976$, $p = 0.016$], and isoleucine [$t_4 = -5.373$, $p = 0.006$] which presented an AUC higher than 0.80, but not in phenylamine, acetate and tyrosine (Figures 6C, 6D, 6E, 6F, 6G, and 6H).

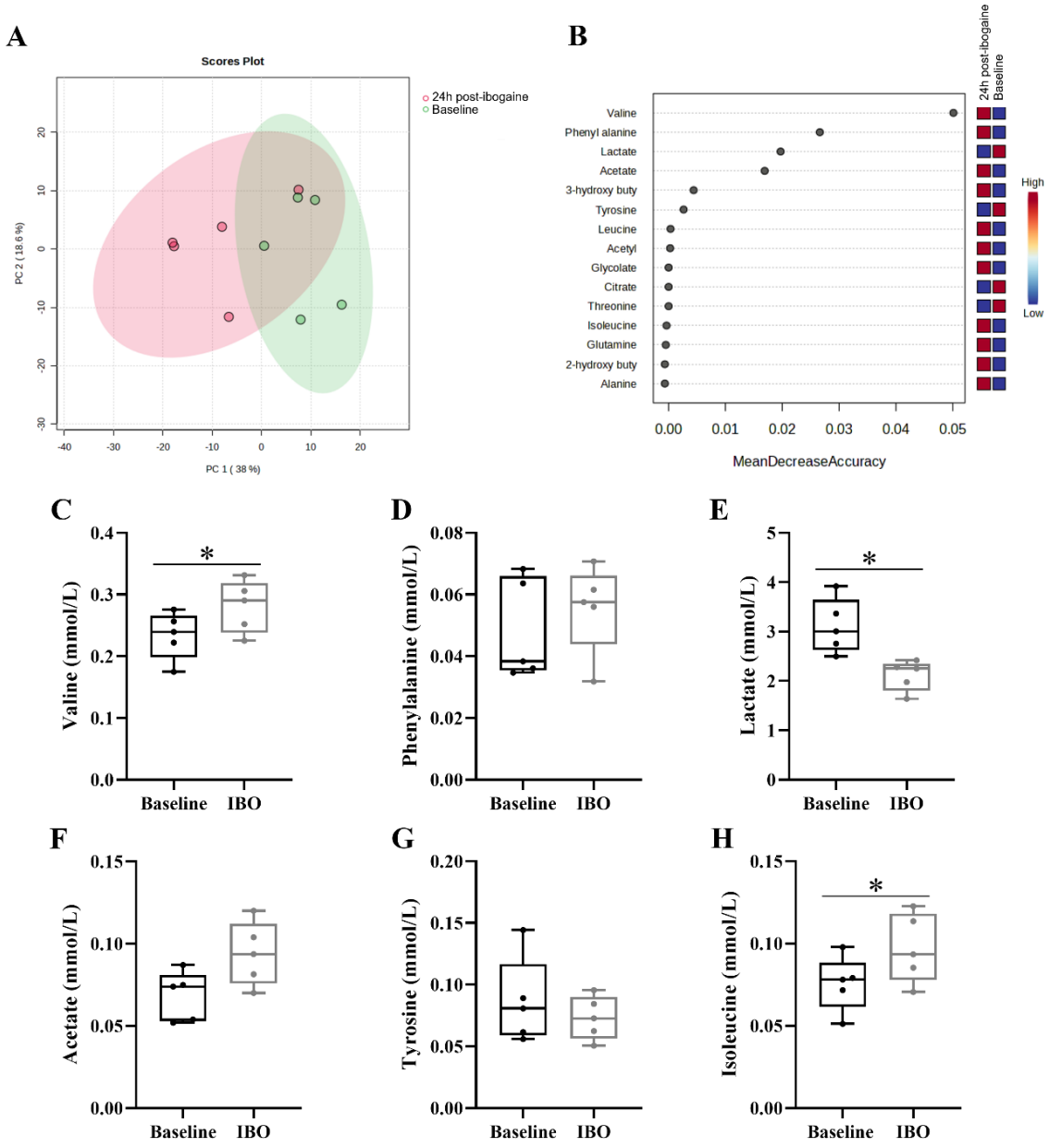


Figure 6. Principal component analysis (A), random forest representation (B) and Student's *t* test results (C–H) of changes in aqueous metabolites after 24h of 100 mg ibogaine administration in patients using high, daily MTD doses (>50 mg). *Indicates differences between pre- and post-administration at $p<0.05$, while trend is indicated with a t.

Metaboanalyst web tool revealed the most relevant metabolic pathways affected by IBO. Valine, leucine and isoleucine degradation (impact value: 0.011, p=0.025) and biosynthesis (impact value: <0.001, p = 0.035), as well as pyruvate metabolism (impact value: 0.081, p = 0.002) and glycolysis and gluconeogenesis (impact value: 0.028, p = 0.002) were especially affected in aqueous metabolites (Supplementary Figure 1). Additionally, it was observed that high and low doses of MTD affected different metabolic pathways, with an emphasis on amino acids and energy-related metabolites at high doses, and glycolysis and specific amino acids metabolites at

low doses (Supplementary Figure 1). See Supplementary material for the results on lipid metabolites.

Discussion

The putative efficacy of IBO in the treatment of SUDs has been presumed for decades without robust clinical trial evidence. This is the first time to our knowledge that the anti-addictive effects of IBO are assessed in a randomized clinical trial (RCT) setting, although the nature of the data presented here is open-label, as previously stated. Previous RCTs have administered IBO to healthy volunteers [13], its metabolite NOR to opioid users [16], or a *T. iboga* extract [50]. Overall, IBO showed good safety and tolerability profiles in this study. As expected [8], a single dose of 100 mg of IBO was able to reduce MTD tolerance, reduction that was sustained in the following week in most subjects.

The dose of IBO used in the present study (100 mg) allowed to interrupt MTD use for a mean time of 18.03h, therefore allowing patients to cease MTD for at least 42h. This suggests a promising potential for IBO to interrupt OWS. Most importantly, the MTD dose was successfully reduced by 50% during the following week in 16 out of 20 subjects, showing a potential effect of IBO on drug tolerance. This outcome aligns with the patent of Ciba Pharmaceutical Products [6] and the case report by Wilkins et al. [8], which initially showcased the consistent inhibitory effect of IBO on opioid tolerance. The reduction of MTD dose could potentially be associated with an increased quality of life [29]. This finding challenges the presupposed need to administer “flood doses” (high doses) of IBO when looking for therapeutic outcomes [51]. In small doses, IBO may not exert powerful subjective experiences that could play a role in its overall anti-addictive effects [52]. However, it is worth noting that certain patients may not require or desire the psychological effects associated with IBO, particularly those with a long history of MTD use and who no longer have SUD. Instead, they may be primarily interested in its pharmacological effects.

Regarding the effects of 100 mg of IBO in reduction of SOWS, in the study conducted by Wilkins et al. [8], a comparable dosage of IBO (150 mg) also led to a significant reduction in the SOWS score, although the observed pattern differed. In the case report, the baseline score was considerably higher (9), but significantly decreased during the initial five hours following IBO administration and rose again between 6 and 12h. It is crucial to acknowledge that this information is based on a single subject, thus necessitating caution when extrapolating these findings to other observations. In our subjects, basal SOWS were relatively low, which may explain the absence of significant differences along time points. In another study by Glue et al. [16], single administrations of various NOR doses (60, 120, 180 mg) did not result in significant changes in OWS measurements. However, subjects receiving 120 mg remained free from OWS for a greater duration. The authors suggested that certain design issues might have influenced these results since patients from different NOR dosage groups were housed in the same ward, potentially making the presence of OWS in some subjects evident for others.

As methadone has a long half-life (8-60h), OWS will appear again when plasmatic concentrations of IBO are low, likely around 17h post-administration. Unfortunately, the pharmacokinetic (PK) data on IBO is currently limited. The impact of CYP2D6 activity on the PK of IBO was evaluated by Glue et al. [13] administering a single 20 mg dose of IBO to healthy volunteers. Individuals whose intrinsic CYP2D6 activity was diminished through prior administration of the CYP2D6

inhibitor paroxetine exhibited a substantial increase in the mean peak concentration (Cmax), the mean area under the concentration-time curve (AUC0-t) and the mean half-life (t½) of IBO [13]. Rapid absorption of IBO from the gastrointestinal tract was observed in a clinical, non-randomized setting where IBO was administered in doses ranging from 500 to 1,200 mg. This was indicated by mean tmax values ranging from 1.75 to 4h for IBO and from 4 to 10h for NOR [11]. Interestingly, SOWS scores clearly decreased between +5 and +12h. Given the PK data available from other studies, it is possible that this decrease corresponds more to the action of NOR (tmax = 4-10h) rather than the parent drug. In any case, this first study with MTD suggests that IBO might be especially helpful in the detoxification of shorter-acting opioids, like buprenorphine, morphine, or heroin. This is highly relevant considering the “opioid epidemic” that is unfolding in the United States of America and other countries.

In the present study, IBO also reduced withdrawal symptoms for at least 12h, as indicated by SOWS measurements. However, it is important to acknowledge that the baseline scores of SOWS were initially low and did not increase significantly at each time point. This may be due to MTD long half-life. In future studies, IBO’s effect on abstinence symptoms should be assessed in people with higher measurement scores. In addition, the scores of both SbOWS and OWS were higher after 12h post-IBO than at baseline. Thus, a single dose of IBO might not be sufficient to completely disrupt MTD OWS. A similar explanation was provided by the authors of a single-dose NOR design [16].

In terms of safety and tolerability, an IBO dose of 100 mg was not associated with serious adverse events. The primary risk of IBO is the potential prolongation of QT intervals [18,20,21,53]. This risk is also shared with various other medications, including MTD and certain antipsychotic, antihistaminic, or antidepressant drugs [54]. In an ECG, the QT interval assesses ventricular repolarization, encompassing both ventricular depolarization (QRS interval) and repolarization (JT = QT – QRS ventricular) [55]. QT prolongation is a predictor of malignant ventricular arrhythmias, such as polymorphic ventricular tachycardia, which can be potentially lethal [56]. Methadone also prolongs the QT interval through the inhibition of the voltage-gated potassium channels, the same mechanism as IBO. The inhibition of voltage-gated potassium channels by MTD and IBO elicits an extension in the repolarization time, thereby causing a lengthening of both the action potential and the corresponding QT interval [57]. The potential additive effect of both substances on QT intervals did not produce clinically significant effects. However, one of the subjects experienced a non-clinically significant QT prolongation that limited their participation in the study. This suggests that certain subjects may be especially vulnerable to the QT-prolonging effects of IBO and should be further studied. It also highlights the need of a cardiovascular monitoring during IBO administration and start using low doses. We suggest that future research should identify risk biomarkers in clinical samples.

The data provided by VAS is highly valuable given the current lack of VAS measurements in IBO studies. It appears that 100 mg of IBO has a mild psychoactive effect, although this can be the result of suggestibility, since patients knew that IBO is a psychoactive drug. It has been claimed that low doses of IBO exert stimulant effects [58]. However, a previously published report did not find any stimulant effect when a low dose of IBO (20 mg) was administered to healthy subjects [14]. Our results corroborate this observation, as the administration of 100 mg of IBO elicited significant relaxation responses, accompanied by minimal instances of "uneasiness or anxiety," which might be associated with potential stimulant effects.

Data separated by gender showed that the three women included in the study had much lower baseline OWS and SbOWS scores than the overall mean. Preclinical research has suggested that

IBO has a higher bioavailability in females [59,60]. Thus, further studies should clarify IBO pharmacokinetics in females.

Results from the metabolomic analysis showed mainly alterations in the aqueous extract as compared with the lipid one. Summarizing the results, levels of lactate decreased, and 2-oxoisocaproate increased 24h after IBO. Results were further analyzed distinguishing those who were taking a high or low MTD dose, observing that variations in daily MTD use could cause differing changes in metabolome. Nevertheless, all compounds indicated significant changes in energy metabolism.

Interestingly, the energy metabolism has been increasingly associated with substance use disorders, including OUD [61]. In an early metabolomics study involving morphine-treated monkeys and controls, it was observed that levels of various compounds, including lactate, changed between groups [62]. Furthermore, it was found that administering MTD or clonidine after morphine treatment in monkeys reverses these metabolic changes, highlighting the usefulness of metabolomics in understanding the molecular basis of opioid withdrawal and treatment [62]. In this regard, a preclinical study reported a dysregulation in brain energy homeostasis in long-term morphine administration [63]. Another study reported an increase in lactate after chronic administration of morphine in rats [64].

Lactate is actually one of the metabolites more frequently modified in these studies. In the current trial, we observed a statistically significant decrease in lactate in the subgroup receiving high doses of MTD and in the whole sample.

Our results support the statement that IBO exerts its effects partly by modifying brain energy metabolism [65,66]. Thus, we propose that IBO treatment is reversing opioid effects on brain energy metabolism and that can be related to its anti-addictive effects. The effects observed in lactate levels, which can be interpreted as a situation of enhanced metabolic efficiency, together with the increased acetate levels in subjects receiving high daily doses of MTD, suggests a situation similar to hyperactive glucose metabolism, where higher levels of glucose are required by cells [67]. This would suggest an acute reversal of dampened energy metabolism induced by the chronic use of opioids.

This study has certain limitations that must be mentioned. First, the sample only included three female participants. According to the data on Catalan individuals in treatment for heroin use [32], this proportion is the same as found in this specific population, so it mirrors the real world. However, it is essential to emphasize the need of collecting data not only from men to conduct gender-sensitive research. Furthermore, the protocol required participants to stay in the hospital for 24 hours per week over a period of six weeks. This schedule posed significant challenges for some participants, including women who needed childcare for those 24 hours. For this reason, the sponsor provided the possibility of covering the costs of these services, although they were not requested. Future studies should design more flexible protocols in order to allow participants to complete the treatment. Another limitation is the absence of a placebo group in the study. While it is a methodological limitation, it would not be ethical for us to let a group of patients without any medication, especially for those who have been on MMT for years and expressed their desire to get off MTD. Additionally, due to the lack of pharmacokinetic data, it is difficult to accurately determine the extent to which the parent drug (IBO) and its metabolite (NOR) contribute to the anti-addictive effects.

Conclusion

This study provided evidence that a low dose of 100 mg of IBO exhibited both safety and efficacy in substantially reversing MTD tolerance. Additionally, it showcased promising effectiveness of IBO in the detoxification process of MTD by interrupting its usage for over 18 hours and significantly diminishing MTD tolerance in the subsequent week. These findings challenge the notion of a solitary "flood dose" while distinctly pointing towards the potential requirement for repeated administrations of IBO for MTD detoxification. Moreover, our data support that IBO restores energetic metabolism in heavy opioid users. Future studies should focus on providing more comprehensive descriptions of appropriate multiple-dose protocols to identify the minimum effective/maximum tolerated IBO doses. These data can be used for designing subsequent studies to encourage further exploration of the potential of this overlooked molecule for the treatment of substance use disorders.

Statement of Ethics

This study protocol was reviewed and approved by the Comitè Ètic d'Investigació amb Medicaments Pere Virgili, approval number 038/2019. All patients gave their informed consent after being provided with the complete information on the study. This clinical study was conducted in accordance with the principles of the Declaration of Helsinki and with the Biomedical Research Law 2007. This clinical trial was registered before patient enrolment [Preliminary Efficacy and Safety of Ibogaine in the Treatment of Methadone Detoxification, ClinicalTrials.gov ID NCT04003948, 01/07/2019].

Acknowledgments

We are grateful for the data management support by Xavier Barroso, from the Fundació Institut Català de Farmacologia. We extend our thanks to Farmàcia Bello for the encapsulation of ibogaine.

We would like to acknowledge Pep Cuñat, Josep Maria Fabregas and Nico Piñeiro for their recruitment assistance. Their dedication and efforts in identifying suitable participants have significantly contributed to the success of our study.

We express our appreciation to the Doctorats Industrials (AGAUR) scholarship program, from which GO benefitted.

We thank Dr. Maria Guirro, Dr. Hector Palacios, Dr. Miguel Ángel Rodriguez and Iris Samarra from the Metabolomics facility of the Centre for Omic Sciences (COS) Joint Unit of the Universitat Rovira i Virgili-Eurecat, for their contribution to metabolomics analysis and purity and stability analyses of the molecule.

We sincerely thank all the involved individuals and organizations for their valuable contributions to this study. This research would not have been possible without them. Finally, we thank the Support for the Requalification of the Spanish University System, the Ministry of Universities, the Recovery, Transformation, and Resilience Plan, and the Autonomous University of Madrid (reference: CA5/RSUE/2022-00157), from which MAA-C has benefitted.

Declarations of competing interest

The authors have no conflicts of interest to declare.

Funding sources.

This study was funded by Dr. Bronner's, Riverstyx Foundation, Etheridge Foundation, Multidisciplinary Association for Psychedelic Studies (MAPS), Nikean Foundation, and Doctorats Industrials program, AGAUR (Catalan Government). No donors have participated in any phase of the study including study design, and data analysis and reporting.

Author contributions

GO performed clinical practices, study coordination, data collection, statistical analyses, and writing – original draft. ES assisted in supervision, clinical practice and data collection. CL assisted with clinical practice – electrocardiography, and methodology. Authors NV, EB, and AF assisted in Clinical practice and data collection. JBB conducted the metabolomic analyses. TL assisted in recruitment and methodology. JMA assisted in clinical practice, and methodology. Author JMR assisted in clinical practice and data collection. MK, RGdS, JECH, and MAAC assisted in conceptualization, methodology, and supervision. CW assisted in conceptualization and supported the delivery of didactic training to the team. MTC assisted in conceptualization and supervision. TB served as co-Principal Investigator, and assisted in conceptualization, recruitment, clinical practice and supervision. JCB served as co-Principal Investigator, conceptualized and designed the clinical trial, wrote the protocol, assisted in obtaining grant support, and provided methodology and supervision. All authors contributed to writing and approved the paper.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

References

1. De Veciana A. La secta del Bwiti en la Guinea española. CSIC, Madrid; 1958.

2. Wasko MJ, Witt-Enderby PA, Surratt CK. DARK Classics in Chemical Neuroscience: Ibogaine. ACS Chem. Neurosci. 2018;9(10):2475–83.

3. Dos Santos RG, Bouso JC, Hallak JEC. The antiaddictive effects of ibogaine: a systematic literature review of human studies. J. Psychedelic Studies. 2016;1(1):20–8.

4. Litjens RP, Brunt TM. How toxic is ibogaine?. Clin Toxicol (Phila). 2016;54(4):297–302.

5. Ona G, Reverté I, Rossi G, dos Santos RG, Hallak JEC, Colomina MT, Bouso JC. Main targets of ibogaine and noribogaine associated with its putative anti-addictive effects: a mechanistic overview. J Psychopharmacol. 2023. Under review.

6. United Stated Patent Office (USPO). Tabernanthine, ibogaine containing analgesic compositions. 1957. Available online in: https://patentimages.storage.googleapis.com/ba/89/87/82bbfbe3ffd993/US2817623.pdf

7. Alper KR. Ibogaine: a review. Alkaloids Chem Biol. 2001;56, 1–38.

8. Wilkins C, dos Santos RG, Solá J, Aixalá M, Cura P, Moreno E, Alcázar-Córcoles MA, Hallak JEC, Bouso JC. Detoxification from methadone using low, repeated, and increasing doses of ibogaine: A case report. J Psychedelic Studies. 2017;1(1):29–34.

9. Belgers M, Leenaars M, Homberg JR, Ritskes-Hoitinga M, Schellekens AF, Hooijmans CR. Ibogaine and addiction in the animal model, a systematic review and meta-analysis. Transl Psychiatry. 2016;6(5):e826

10. Mash DC, Kovera CA, Pablo J, et al. Ibogaine in the treatment of heroin withdrawal. Alkaloids Chem Biol. 2001;56:155–71.

11. Mash DC, Duque L, Page B, Allen-Ferdinand K. Ibogaine Detoxification Transitions Opioid and Cocaine Abusers Between Dependence and Abstinence: Clinical Observations and Treatment Outcomes. Front Pharmacol. 2018;9:529.

12. Mash DC, Kovera CA, Buck BE, et al. Medication development of ibogaine as a pharmacotherapy for drug dependence. Ann N Y Acad Sci. 1998;844:274–92.

13. Glue P, Winter H, Garbe K, et al. Influence of CYP2D6 activity on the pharmacokinetics and pharmacodynamics of a single 20 mg dose of ibogaine in healthy volunteers. J Clin Pharmacol. 2015a;55(6):680–7.

14. Forsyth B, Machado L, Jowett T, et al. Effects of low dose ibogaine on subjective mood state and psychological performance. J Ethnopharmacol. 2016;189:10–3.

15. Glue P, Lockhart M, Lam F, Hung N, Hung CT, Friedhoff L. Ascending-dose study of noribogaine in healthy volunteers: pharmacokinetics, pharmacodynamics, safety, and tolerability. J Clin Pharmacol. 2015b;55(2):189–94.

16. Glue P, Cape G, Tunnicliff D, et al. Ascending Single-Dose, Double-Blind, Placebo-Controlled Safety Study of Noribogaine in Opioid-Dependent Patients. Clin Pharmacol Drug Dev. 2016;5(6):460–8.

17. Knuijver T, Schellekens A, Belgers M, et al. Safety of ibogaine administration in detoxification of opioid-dependent individuals: a descriptive open-label observational study. Addiction. 2022;117(1):118–28.

18. Koenig X, Kovar M, Boehm S, Sandtner W, Hilber K. Anti-addiction drug ibogaine inhibits hERG channels: a cardiac arrhythmia risk. Addict Biol. 2014;19(2):237–9.

19. Alper KR, Stajić M, Gill JR. Fatalities temporally associated with the ingestion of ibogaine. J Forensic Sci. 2012;57(2):398–412.

20. Koenig X, Hilber K. The anti-addiction drug ibogaine and the heart: a delicate relation. Molecules. 2015;20(2):2208–28.

21. Ona G, Rocha JM, Bouso JC, et al. The adverse events of ibogaine in humans: an updated systematic review of the literature (2015-2020). Psychopharmacology (Berl). 2022;239(6):1977–87.

22. Mattick RP, Breen C, Kimber J, Davoli M. Buprenorphine maintenance versus placebo or methadone maintenance for opioid dependence. Cochrane Database Syst Rev. 2014;(2):CD002207.

23. Schuckit MA. Treatment of Opioid-Use Disorders. New Eng J Med. 2016;375(4):357–68.

24. Ward J, Mattick RP, Hall W. Key Issues in Methadone Maintenance Treatment. New South Wales University Press, Sydney; 1992.

25. Amato L, Davoli M, Minozzi S, Ferroni E, Ali R, Ferri M. Methadone at tapered doses for the management of opioid withdrawal. Cochrane Database Syst Rev. 2013;2013(2):CD003409.

26. Baldacchino A, Armanyous M, Balfour DJ, Humphris G, Matthews K. Neuropsychological functioning and chronic methadone use: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2017;73:23–38.

27. Wang GY, Wouldes TA, Russell BR. Methadone maintenance treatment and cognitive function: a systematic review. Curr Drug Abuse Rev. 2013;6(3):220–30.

28. Brondani M, Park PE. Methadone and oral health--a brief review. J Dent Hyg. 2011;85(2):92–8.

29. Pedrero-Pérez EJ, MethaQoL G. Methadone dosage and its relationship to quality of life, satisfaction, psychopathology, cognitive performance and additional consumption of non-prescribed drugs. Dosis de metadona y su relación con calidad de vida, satisfacción, psicopatología, rendimiento cognitivo y consumo adicional de sustancias no prescritas. Adicciones. 2016;29(1):37–54.

30. Drummer OH, Opeskin K, Syrjanen M, Cordner SM. Methadone toxicity causing death in ten subjects starting on a methadone maintenance program. Am J Forensic Med Pathol. 1992;13(4):346–50.

31. Observatorio Español de las Drogas y las Adicciones (OEDA). Informe 2022. Alcohol, tabaco y drogas ilegales en España. Madrid: Ministerio de Sanidad. Delegación del Gobierno para el Plan Nacional sobre Drogas.

32. Sistema d'informació sobre drogodependències a Catalunya (SIDC). Informe 2014. Available online in: https://drogues.gencat.cat/web/.content/minisite/drogues/professionals/epidemiologia/docs/SIDC-Informe-2014-v_09-03-2016.pdf

33. Schep LJ, Slaughter RJ, Galea S, Newcombe D. Ibogaine for treating drug dependence. What is a safe dose?. Drug Alcohol Depend. 2016;166:1–5.

34. Barber M, Gardner J, Savic M, Carter A. Ibogaine therapy for addiction: Consumer views from online fora. Int J Drug Policy. 2020;83:102857.

35. Cao YJ, Bhargava HN. Effects of ibogaine on the development of tolerance to antinociceptive action of mu-, delta- and kappa-opioid receptor agonists in mice. Brain Res. 1997;752(1-2):250–4.

36. Schneider JA, McArthur M. Potentiation action of ibogaine (BogadinTM) on morphine analgesia. Experientia. 1956;12:323–324.

37. Sunder Sharma S, Bhargava HN. Enhancement of morphine antinociception by ibogaine and noribogaine in morphine-tolerant mice. Pharmacology. 1998;57(5):229–32.

38. Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials.

39. Ona G. Elaboración y validación de un cuestionario para la detección del riesgo de reacciones adversas graves por empleo de drogas psicodélicas en terapia. Int J Psychol Psychological Therapy. 2020;20(2):211–22.

40. Lingjaerd O, Ahlfors UG, Bech P, Dencker SJ, Elgen K. The UKU side effect rating scale. Acta Psychiatr Scand. 1987;76:1–100.

41. Bascarán MT, Bobes J, Bousoño M, García-Portilla MP, Parellada M, Sáiz PA. Banco de instrumentos básicos para la práctica de la psiquiatría clínica, 6ª edición. Majadahonda: CYESAN; 2011.

42. Bradley BP, Gossop M, Phillips GT, Legarda JJ. The development of an opiate withdrawal scale (OWS). Br J Addict. 1987;82(10):1139–42.

43. Jiménez-Lerma JM, Ariño J, Iraurgi I, Landabaso M, Ramírez MJ, Herrera A. Instrumentos e indicadores para la evaluación de los procesos de desintoxicación/abstinencia supervisada a sustancias, in: Iraurgi Castillo, I., González Sáiz, F. (eds.) Instrumentos de evaluación en drogodependencias. Aula Médica, Barcelona; 2002.

44. Gossop M. The development of a Short Opiate Withdrawal Scale (SOWS). Addict Behav. 1990;15(5):487–90.

45. Hiltunen AJ, Lafolie P, Martel J, et al. Subjective and objective symptoms in relation to plasma methadone concentration in methadone patients. Psychopharmacol. (Berl). 1995;118(2):122–6.

46. Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand. 1983;67:361–370..

47. Quintana JM, Padierna A, Esteban C, Arostegui I, Bilbao A, Ruiz I. Evaluation of the psychometric characteristics of the Spanish version of the Hospital Anxiety and Depression Scale. Acta Psychiatrica Scandinavica. 2003;107(3):216-21.

48. Overall JE, Gorham DR. The brief psychiatric rating scale. Psychol. Rep. 1962;10:799-812.

49. Biswas B, Kumar N, Hoque A, Alam A. Weighted Scaling Approach for Metabolomics Data Analysis. ArXiv. 2022. Doi: 10.48550/arXiv.2208.00603

50. Prior PL, Prior SL. Ibogaine effect on cocaine craving and use in dependent patients - A double-blind, placebo-controlled pilot study. J Addict Ther. 2014;1(1):003.

51. Davis AK, Barsuglia JP, Windham-Herman AM, Lynch M, Polanco M. Subjective effectiveness of ibogaine treatment for problematic opioid consumption: Short- and long-term outcomes and current psychological functioning. J Psychedelic Stud. 2017;1(2):65–73.

52. Rodríguez-Cano BJ, Kohek M, Ona G, et al. Underground ibogaine use for the treatment of substance use disorders: A qualitative analysis of subjective experiences. Drug Alcohol Rev. 2023;42(2):401–14.

53. Corkery JM. Ibogaine as a treatment for substance misuse: Potential benefits and practical dangers. Prog Brain Res. 2018;242:217–57.

54. Li M, Ramos LG. Drug-Induced QT Prolongation And Torsades de Pointes. P T. 2017;42(7):473–7.

55. Ponte ML, Keller GA, Di Girolamo G. Mechanisms of drug induced QT interval prolongation. Curr Drug Saf. 2010;5(1):44–53.

56. Viskin S, Chorin E, Viskin D, Hochstadt A, Schwartz AL, Rosso R. Polymorphic Ventricular Tachycardia: Terminology, Mechanism, Diagnosis, and Emergency Therapy. Circulation. 2021;144(10):823–39.

57. Treece JM, Madani MA, Khoury GE, Khraisha O, Martin JE, Baumrucker SJ, Neglia CA, Paul TK. Comprehensive Review on Methadone-Induced QT Prolongation and Torsades. J Pharmacol Pharmacotherapeutics. 2018;9(2):66–75.

58. Kargbo RB. Ibogaine and Their Analogs as Therapeutics for Neurological and Psychiatric Disorders. ACS Med Chem Lett. 2022;13(6):888–90.

59. Pearl SM, Hough LB, Boyd DL, Glick SD. Sex differences in ibogaine antagonism of morphine-induced locomotor activity and in ibogaine brain levels and metabolism. Pharmacol Biochem Behav. 1997;57(4):809–15.

60. Tatalović N, Vidonja Uzelac T, Mijović M, et al. Ibogaine Has Sex-Specific Plasma Bioavailability, Histopathological and Redox/Antioxidant Effects in Rat Liver and Kidneys: A Study on Females. Life (Basel). 2021;12(1):16.

61. Chen XL, Lu G, Gong YX, et al. Expression changes of hippocampal energy metabolism enzymes contribute to behavioural abnormalities during chronic morphine treatment. Cell Res. 2007;17(8):689-700.

62. Deng Y, Bu Q, Hu Z, et al. (1) H-nuclear magnetic resonance-based metabonomic analysis of brain in rhesus monkeys with morphine treatment and withdrawal intervention. J Neurosci Res. 2012;90(11):2154-2162.

63. Ujcikova H, Vosahlikova M, Roubalova L, Svoboda P. Proteomic analysis of protein composition of rat forebrain cortex exposed to morphine for 10 days; comparison with animals exposed to morphine and subsequently nurtured for 20days in the absence of this drug. J Proteomics. 2016;145:11-23.

64. Sharma SK, Yashpal K, Fundytus ME, Sauriol F, Henry JL, Coderre TJ. Alterations in brain metabolism induced by chronic morphine treatment: NMR studies in rat CNS. Neurochem Res. 2003;28(9):1369-1373.

65. Paskulin R, Jamnik P, Zivin M, Raspor P, Strukelj B. Ibogaine affects brain energy metabolism. Eur J Pharmacol. 2006;552(1-3):11-14.

66. Levant B, Pazdernik TL. Differential effects of ibogaine on local cerebral glucose utilization in drug-naive and morphine-dependent rats. Brain Res. 2004;1003(1-2):159-167.

67. Liu X, Cooper DE, Cluntun AA, Warmoes MO, Zhao S, Reid MA, Liu J, Lund PJ, Lopes M, Garcia BA, Wellen KE, Kirsch DG, Locasale JW. Acetate Production from Glucose and Coupling to Mitochondrial Metabolism in Mammals. Cell 2018;175(2):502-513.

Supplementary material.

List of VAS items.

“I have experienced psychoactive effects / "high"” indicated the presence of psychoactive effects. “Overall intensity of the experience” indicated the intensity of the experience elicited by IBO. “I have experienced good effects” indicated any effect the person perceived as positive. “I would like to take this drug again” refers to the willingness to take IBO again. “I have experienced bad effects” indicated any effect the person perceived as negative. “My thinking has been modified (my thoughts went faster or slower, or were richer)” indicated alterations in thought processes. “I found it difficult to control my thoughts” indicated disrupting alterations in thought processes. “The effect of the substance has overwhelmed me” indicated disturbing effects in terms of potency. “I have felt some anxiety, uneasiness”' indicated the presence of anxious states. “I felt relaxed” indicated the overall presence of relaxation. “I have lost contact with my body” indicated dissociation between mind and body. “My perception of time has changed” indicated alterations in time perception. “I have lost touch with external reality” indicated separation from their surroundings. “I have had visual effects (light, colors, simple or complex scenes)” indicated the presence of visual modifications. And “I have had auditory effects (noises, voices, music, etc.)” indicated the presence of auditory modifications.

Supplementary Table 1. Psychometric measures administered over a 12h timeframe in ibogaine sessions.

	-1 week	-1h	Rx (IBO admin.)	+1h	+2h	+3h	+4h	+5h	+6h	+7h	+8h	+10h	+12h
Wave	X												
HAM-D		X											
OWS		X											X
SbOWS		X											X
SOWS		X		X	X	X	X	X	X	X	X	X	X
VAS													X
UKU													X

Metabolomic analysis.

Methodology.

For aqueous metabolites determination 200 µL of plasma samples were placed in 2 mL 96 deepwell plates by the Bravo liquid handler (Agilent, Santa Clara, USA), while in lipid extraction 100 µL were used. Subsequently, 1400 µL of a solution of methanol and water (8:1) and 1580 µL of a mixture of methanol, methyl tert-butyl ether and water (323:1077:180) were added to polar and apolar extractions, respectively. In both cases, samples were briefly homogenate and centrifugate. In aqueous extraction, 1000 µL of the supernatant were collected in a separate plate and speed dried. The pellet was redissolved in 650 µL of 0.05 M PBS buffer D2O using the Gilson liquid handler (Gilson, Middleton, WI, USA) and 600 µL were transferred to an NMR tube with 5 mm of diameter for measurement. On the other hand, in lipid extraction, 900 µL of the supernatant were transferred to another plate and speed dried. To redissolve the pellet, we used 700 µL of CDCl3 (Panreac, Barcelona, Spain) and MeOD (Merck, Darmstadt, Germany) (2:1) with tretamethyl silane (Sigma-Aldrich, Barcelona, Spain), 650 µL of the mixture were introduced in an NMR tube with 5 mm of diameter using the Bravo liquid handler (Agilent, Santa Clara, USA) for measurement.

Samples quantification was made calibrating the electronic reference to access in vivo concentration signal by a reference sample of 2 mM sucrose. 1H NMR spectra were recorded using the Avance III 600 spectrometer (Bruker, Billerica, MA, USA) at 300 K with a proton frequency of 600.20 MHz and 5 mm PABBO gradient probe.

Measures and registration of samples were made with procno 11 and 22, in aqueous and lipid samples, respectively. For aqueous extracts we carried out a one-dimensional 1H pulse experiment by the nuclear Overhauser effect spectroscopy and the presaturation sequence RD-90º-t1-90º ACQ to suppress residual water peak. The mixing time was set at 100 ms and solvent presaturation with irradiation power of 160 mW was applied during recycling delay of 5 s and mixing time. The 90º pulse length was calibrated for each sample and varied from 9.72 to 10.06 µs. A total of 256 transients were collected into 64 K data points with a spectral width of 20 ppm for each 1H spectrum. In the case of lipid extracts, we used a 90º pulse with the presaturation sequence of zgpr to suppress water residual and methanol signal. The acquisition time was set at 2.94 s. The 90º pulse length was calibrated for each sample between 9.92 and 10.04 µs. A total of 128 scans were collected into 64 K data points with a spectral width of 18.6 ppm.

Before Fourier transformation the exponential line broadening applied was of 0.3 Hz. All the frequency domain spectra were phased, baseline-corrected and references to 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt or tetramethyl silane signal (d=0 ppm) by a TopSpin 3.6 (Burker, Billerica, MA, USA).

All the 1H NMR obtained were compared with the database AMIX (Buker, Billerica, MA, USA), the human metabolome data and Chenomx for metabolite identification. Moreover, metabolites were assigned using 1H-1H homonuclear correlations (COSY and TOCSY) and 1H-13C heteronuclear 2D NMR experiments, as well as correlations with pure compounds run in-house when were needed. After pre-processing, the AMIX 3.9 software package (Buker, Billerica, MA, USA) helped us to integrate specific 1H NMR regions in the spectra, as well as curated regions. Finally, all the data were exported to excel spreadsheet in order to evaluate robustness of the different 1H NMR signals and give concentrations.

Supplementary table 2. Number of samples per group in the metabolomic analysis. MTD= methadone.

	Aqueous metabolites	Lipid metabolites
Whole samples	12	12
Low MTD dose	7	6
High MTD dose	5	6

The total number of subjects are 7 in the Low MTD dose and 6 in the high MTD dose. One outlier was removed in the high MTD dose for Aqueous and one in the low MTD for Lipid metabolites.

Supplementary Table 3. Information on smokers and medications used. (w) indicates a female gender.

	Smoker (Yes;No)	Additional medications prescribed during the hospital stay
R-001	Y	Three nicotine gums of 2 mg
R-002	Y	Four nicotine gums of 2 mg
R-003	Y	One nicotine gum of 2 mg
R-004	Y	Three nicotine gums of 2 mg
R-005	Y	-
R-006	Y	Paracetamol 1 g twice One nicotine gum of 2 mg
R-007	N	Alprazolam 2 mg (4 mg total dose)
R-008	Y	Lormetazepam 2 mg
R-009	Y	Lorazepam 1 mg
R-010	Y	Diazepam 2 mg, twice Two nicotine gums of 2 mg
R-011	Y	Diazepam 5mg
R-012	Y	Diazepam 5 mg
R-013(w)	Y	Diazepam 5 mg Paracetamol 1 g
R-014	N	-
R-015(w)	Y	-
R-016	Y	-
R-017	Y	
R-018	Y	Paracetamol 1 g twice, almagate twice
R-019(w)	Y	-
R-020	Y	-

Supplementary Table 4. Blood pressure means at each time point.

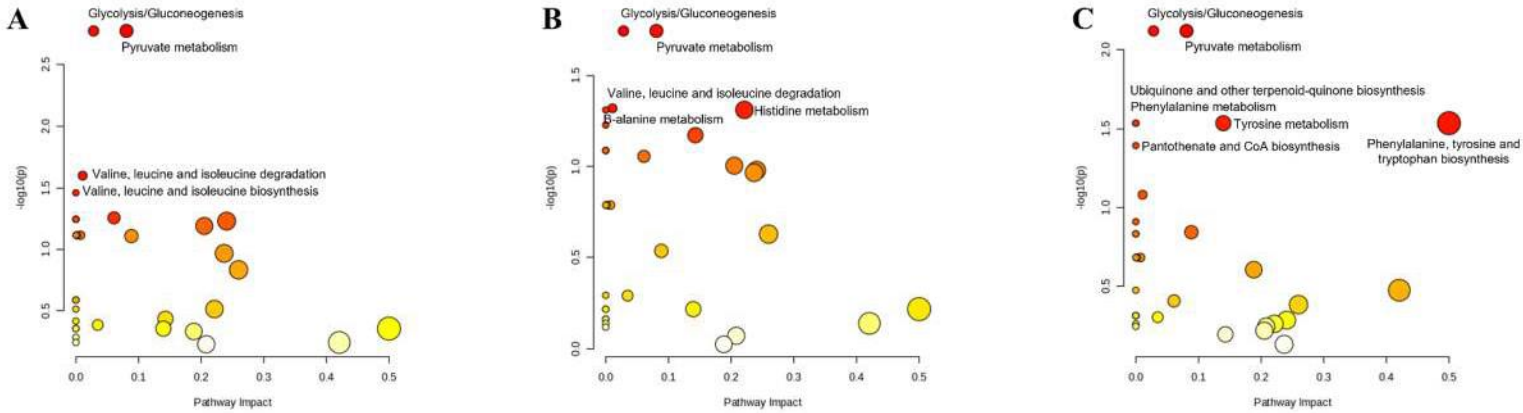
	Systolic BP	Diastolic BP
Baseline	125.7	74.3
+1	120.9	69.4
+2	119.3	70.8
+3	116.0	67.2
+4	121.6	69.4
+5	118.3	67.1
+6	120.4	67.3
+7	119.6	69.7
+8	116.8	68.1
+10	120.2	66.2
+12	121.9	68.1
+24	121.1	71.0

Supplementary Table 5. AUC scores in each metabolite for aqueous and lipid extractions and high or low MTD dose. MTD= methadone.

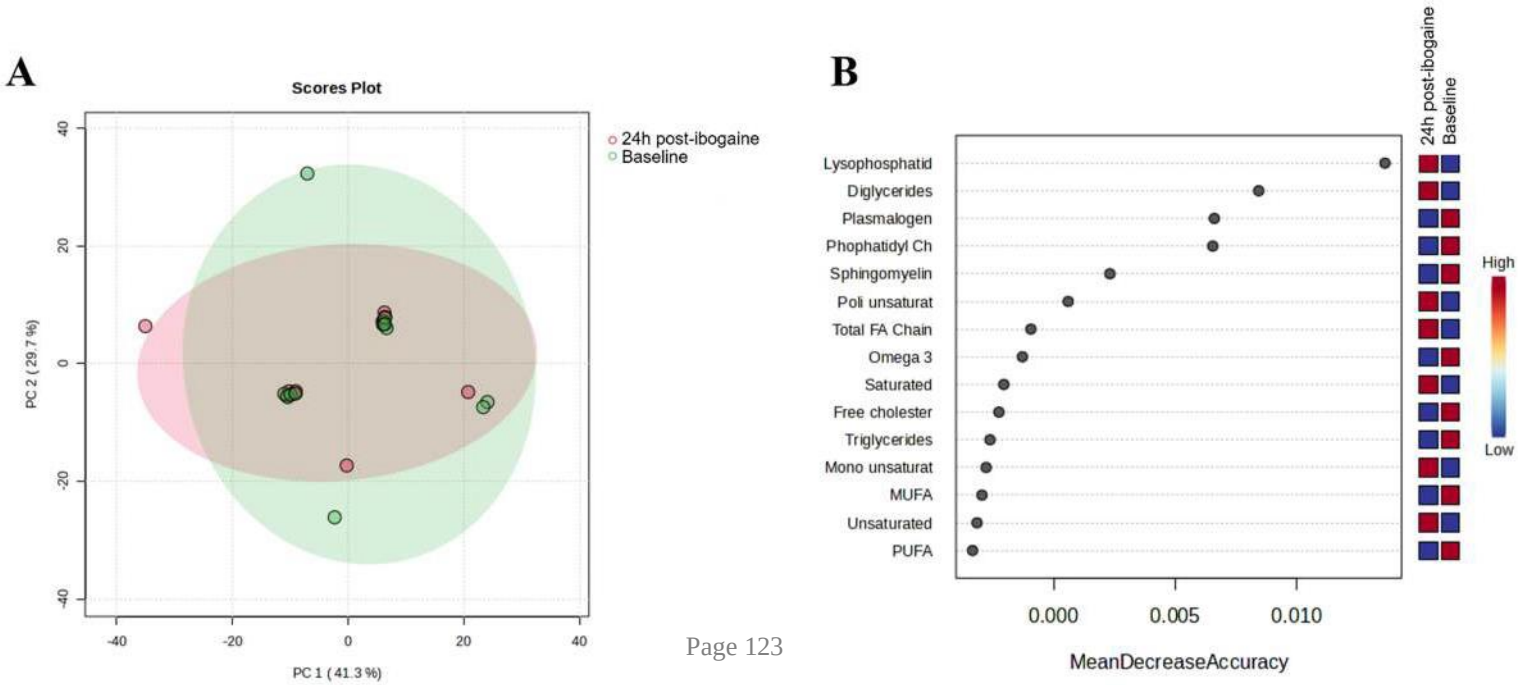
Aqueous metabolites					
Whole sample		*High MTD dose*		*Low MTD dose*	
Name	AUC	Name	AUC	Name	AUC
Lactate	0,88889	Valine*	1	2-oxo isocaproate*	0,97959
2-oxo-isocaproate	0,86111	Phenyl alanine*	0,96	2-hydroxy butyrate*	0,91831
2-hydroxy butyrate	0,80556	Lactate*	0,96	Histidine	0,83673
3-hydroxy butyrate	0,70139	Acetate	0,8	Acetyl	0,81633
Isoleucine	0,80556	3-hydroxy butyrate	0,8	3-hydroxy butyrate	0,65306
Ornithine	0,625	Tyrosine	0,84	Ornithine	0,53061
Glycine	0,68056	Leucine	0,76	Oxalacetate	0,5102
Leucine	0,76389	Acetyl	0,6	Glucose	0,61224
Oxalacetate	0,51389	Glycolate	0,56	Leucine	0,77551
Histidine	0,61806	Citrate	0,64	Isoleucine	0,81633
Glutamine	0,69444	Threonine	0,6	Phenyl alanine	0,53061
Acetate	0,65278	Isoleucine	0,8	Glycerol	0,71429
Glucose	0,625	Glutamine	0,56	Lactate	0,83673
Creatine	0,58333	2-hydroxy butyrate	0,76	Creatine	0,59184
Valine	0,65972	Alanine	0,6	Lysine	0,65306
Lipid metabolites					
Whole sample		*High MTD dose*		*Low MTD dose*	
Name	AUC	Name	AUC	Name	AUC
Lysophosphatidiyl choline	0,66667	Unsaturated	0,77778	Plasmalogen	0,77778
Diglycerides	0,6875	Phosphatidyl choline	0,55556	Saturated	0,66667
Plasmalogen	0,67361	DHA	0,80556	Triglycerides	0,66667
Phosphatidyl choline	0,59028	Diglycerides	0,80556	Cholesterol oxidized	0,61111
Sphingomyelin	0,63889	Esterified cholesterol	0,63889	Omega 3	0,61111
Poli unsaturated	0,65278	Saturated	0,66667	Phosphatidyl choline	0,63889
Total FA chain	0,52778	Plasmalogen	0,52778	Diglycerides	0,52778
Omega 3	0,61111	Total FA chain	0,63889	ARA + EPA	0,72222
Saturated	0,52778	Total FA methyl	0,66667	MUFA	0,69444
Free cholesterol	0,56944	Oleic acid	0,63889	Linoleic acid	0,55556
Triglycerides	0,51389	Lysophosphatidyl choline	0,75	Total cholesterol	0,55556
Mono unsaturated	0,61111	Triglycerides	0,69444	Total FA methyl	0,61111
MUFA	0,61111	PUFA	0,5	Total FA chain	0,63889
Unsaturated	0,51389	Sphingomyelin	0,61111	Unsaturated	0,69444

PUFA	0,50694	Linoleic acid	0,55556	PUFA	0,5

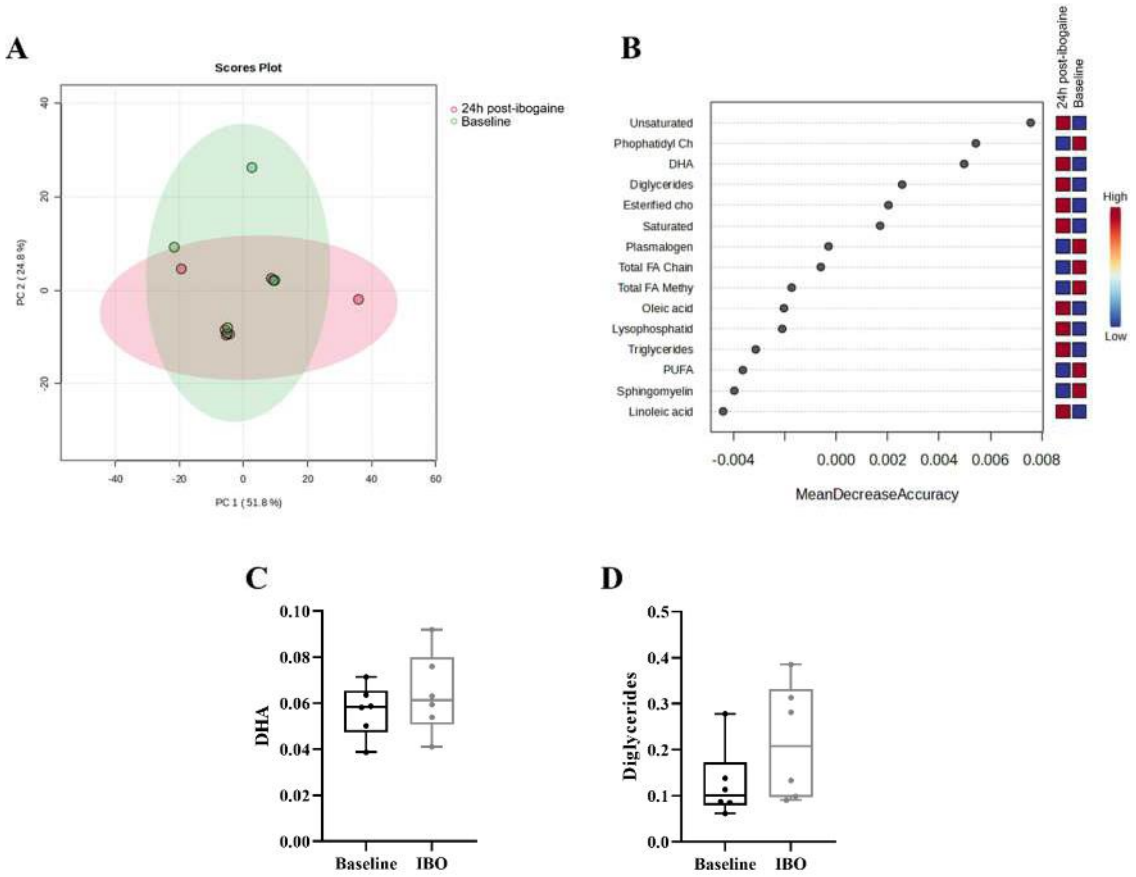
Supplementary Figure 1. Main metabolic pathways modulated by IBO. A= Whole sample; B= Group using a low, daily MTD doses; C= Group using high, daily MTD doses. High dose MTD affected pathways: pyruvate metabolism (impact value: 0.081, p=0.007), glycolysis/gluconeogenesis (impact value: 0.028, p=0.007), phenylalanine, tyrosine and tryptophan biosynthesis (impact value: 0.500, p=0.029), tyrosine metabolism (impact value: 0.140, p=0.029), ubiquinone and other terpenoid-quinone biosynthesis (impact value: <0.001, p=0.029), phenylalanine metabolism (impact value: <0.001, p=0.029) and pantothenate and CoA biosynthesis (impact value: <0.001, p=0.040). Low dose MTD affected pathways: glycolysis/gluconeogenesis (impact value: 0.028, p=0.018), pyruvate metabolism (impact value: 0.081, p=0.018), valine, leucine and isoleucine degradation (impact value: 0.011, p=0.048), histidine metabolism (impact value: 0.221, p=0.048) and β-alanine metabolism (impact value: 0.000, p=0.048).



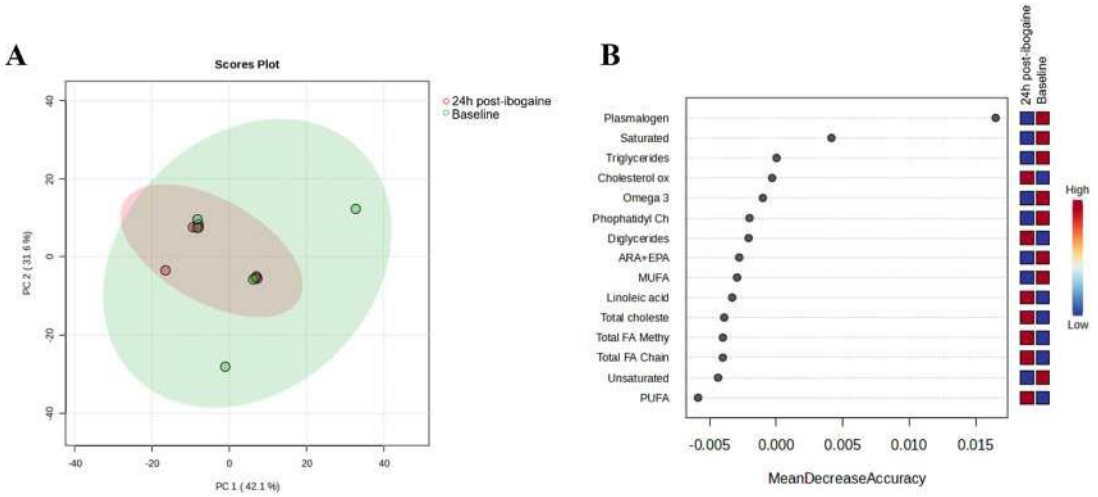
Supplementary Figure 2. Principal component analysis (A) and random forest representation (B) of lipid metabolites changes after 24h of 100 mg ibogaine administration.



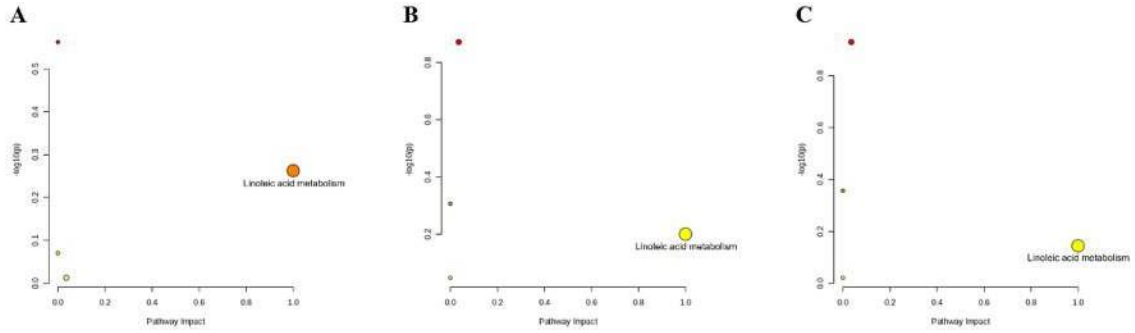
Supplementary Figure 3. Principal component analysis (A), random forest representation (B), and Student's *t* test results (C,D) of changes in lipid metabolites after 24h of 100 mg ibogaine administration in the group using low, daily doses of MTD.



Supplementary Figure 4. Principal component analysis (A) and random forest representation (B) of changes in lipid metabolites after 24h of 100 mg ibogaine administration in the group using high, daily doses of MTD.



Supplementary Figure 5. Main metabolic pathways modulated by IBO. A= Whole sample; B= Group using a low, daily MTD doses; C= Group using high, daily MTD doses. Impact values of linoleic acid metabolism: Whole sample (impact value: 1.000, *p*=0.546), low MTD dose (impact value: 1.000, *p*=0.631) and high MTD dose (impact value: 1.000, *p*=0.717).



Results of the metabolomic analysis on lipid metabolites

In the case of apolar extraction, PCA analysis showed two well-defined groups ("baseline" and "24h post-IBO administration") (Supplementary Figure 2). Nevertheless, the variations in metabolites observed in these groups overlap, probably due to the heterogeneity observed after IBO administration. Only minor changes on the lipid metabolites were observed, for the whole group or for either low or high doses of MTD groups. See for more detail text in supplementary material and supplementary Table 4 and supplementary; Figure 2, 3 and 4. In the same line, the analysis of affected metabolic pathways did not reveal any significant alteration (Supplementary Figure 5).Random forest analysis reported an increase in lysophosphatidyl choline and diglycerides in the 24h post-IBO group and a decrease in plasmalogen and phosphatidyl choline metabolites. However, ROC analysis showed AUC values below 0.70 which indicate that those metabolites presented poor ability to discriminate between groups (Supplementary Table 4 and Supplementary Figure 2).

Regarding the analysis differentiating patients with low and high dose of MTD, PCA analysis showed pre- and post-intervention groups but those did not differ from each other (Supplementary Figure 3 and Supplementary Figure 4). Random forest analysis predicted different metabolites as the best to differentiate between group, but only DHA and diglycerides, in the case of high MTD dose, presented AUC values higher than 0.80 (Supplementary Figure 3, Supplementary Figure 4 and Supplementary Table 4). However, paired sample t-test did not show significant differences in those metabolites (DHA [t_5=-1.219, p=0.277] and diglycerides [t_5=-1.358, p=0.233]) (Supplementary Figure 4). Therefore, these results indicated that a single dose of IBO produce minor changes on the lipid metabolites. In the same line, the analysis of affected metabolic pathways did not reveal any significant alteration (Supplementary Figure 5).

Publication 3

Main targets of ibogaine and noribogaine associated with its putative anti-addictive effects: a mechanistic overview

Genís Ona, Ingrid Reverte, Giordano N. Rossi, Rafael G. dos Santos, Jaime E.C. Hallak, Maria Teresa Colomina, José Carlos Bouso

J Psychopharmacology, 2023; 37(12):1190-1200

https://doi.org/10.1177/02698811231200882

Study III overview:

What do we already know?

Several targets are potentially related with the putative anti-addictive effects of ibogaine. Throughout *in vitro/in vivo* research, the action of ibogaine in G protein-coupled receptors and other targets have been proposed.

What does this study add?

The most exhaustive review showing all the targets with which ibogaine can interact was published in 1995. Since then, the body of knowledge not only related to ibogaine but also to the targets associated with substance use disorders has substantially expanded. In this narrative review, we gathered the available information in the literature for those targets associated with substance use disorders showing binding affinity for ibogaine to provide further mechanistic explanations.

Highlights

Multiple targets and synergistic effects resulting from their complex modulation by ibogaine should be considered involved in the anti-addictive effects of this molecule.

Ibogaine research can benefit from complex approaches like the polypharmacology paradigm when describing its mechanisms of action.

Abstract

Background:

There is a growing interest in studying ibogaine as a potential treatment for substance use disorders. However, its clinical use has been hindered for mainly two reasons: First, the lack of randomized, controlled studies informing about its safety and efficacy. And secondly, ibogaine's mechanisms of action remain obscure. It has been challenging to elucidate a predominant mechanism of action responsible for its anti-addictive effects.

Objective:

To describe the main targets of ibogaine and its main metabolite, noribogaine, in relation to their putative anti-addictive effects, reviewing the updated literature available.

Methods:

A comprehensive search involving MEDLINE and Google Scholar was undertaken, selecting papers published until July 2022. The inclusion criteria were both theoretical and experimental studies about the pharmacology of ibogaine. Additional publications were identified in the references of the initial papers.

Results:

Ibogaine and its main metabolite, noribogaine, can modulate several targets associated with substance use disorders. Instead of identifying key targets, the action of ibogaine should be understood as a complex modulation of multiple receptor systems, leading to potential synergies. The elucidation of ibogaine's pharmacology could be enhanced through the application of methodologies rooted in the polypharmacology paradigm. Such approaches possess the capability to describe multifaceted patterns within multi-target drugs.

Conclusion:

Ibogaine displays complex effects through multiple targets. The information detailed here should guide future research on both mechanistic and therapeutic studies.

Keywords: Iboga, ibogaine, noribogaine, substance use disorders, anti-addictive, hallucinogen, psychedelic

Introduction

Ibogaine (IBO) is the main alkaloid of the shrub *Tabernanthe iboga* (Lavaud and Massiot, 2017). While traditionally part of West Central African medicine (Faura and Langlois, 2022), IBO is now gaining recognition as a potential treatment for Substance Use Disorders (SUDs) (Belgers et al., 2016; dos Santos et al., 2016). A substantial body of preclinical research supports IBO's effectiveness in reducing drug self-administration and decreasing the likelihood of drug relapse involving cocaine, ethanol, nicotine, and opioids (Belgers et al., 2016). The reported effects in preclinical models are in line with those reported in humans.

Two studies have assessed the pharmacokinetics of IBO in humans. In healthy volunteers, IBO administered at a single dose of 20 mg (0.285 mg/kg) has a half-life of 2-5 hours (Glue et al 2005a). In comparison, IBO administered to heroin users at a higher dose (10 mg/kg) has a half-life of 7.45 hours (Mash, 2001). Remarkably, the active metabolite noribogaine (NOR) has a longer half-life than IBO and may be implicated in its anti-addictive effects. Indeed, it has been described that the half-life after a single administration of NOR in healthy volunteers (0.14 mg/kg) is about 49 hours, while higher doses (0.85 or 2.33 mg/kg) had a shorter half-life between 27 and 29 hours (Glue et al., 2005b).

Both preclinical and clinical studies have reported noteworthy effects that surpass the half-life of either IBO or NOR. These findings suggest long-lasting changes in the reward or stress systems linked to withdrawal states.

Notwithstanding, no randomized and controlled clinical trials have explicitly addressed the “anti-addictive” properties of IBO in humans. However, there is promising evidence from case reports and observational research (Brown and Alper, 2018; Köch et al., 2022; Malcolm et al., 2018; Noller et al., 2018). There is only one randomized and placebo-controlled study that assessed the efficacy of a T. iboga extract (1800 mg, single dose) in the treatment of cocaine dependence (Prior and Prior, 2014). These authors report that the extract elicited powerful psychoactive effects for patients in the IBO group that lasted 72 hours after administration. No cardiac monitoring was done, or at least was not reported. It seems that cravings and dependence associated with cocaine use disorder were drastically reduced over a period of 24 weeks.

In spite of the dearth of randomized and controlled studies, IBO is offered as a treatment for SUDs in several countries around the world, often without medical supervision. This phenomenon has been described as a “vast, uncontrolled experiment” (Vastag, 2005). A systematic review of these studies supports IBO’s therapeutic potential to significantly reduce drug withdrawals, cravings, and self-administration in patients with SUDs (dos Santos et al., 2016). The duration of the therapeutic effect varies highly between studies, ranging from 24h to weeks or months (dos Santos et al., 2016).

Despite this encouraging scenario, a number of safety concerns have hindered the clinical development of IBO. Three systematic reviews reported that ibogaine poses certain risks (i.e., QT prolongation), and fatalities have occurred in rare cases (Alper et al., 2012; Koenig and Hilber, 2015; Ona et al., 2021). The most common situations include QT (electrocardiogram parameter) prolongation, gastrointestinal symptoms (vomiting, nausea), or physical symptoms (ataxia, muscle tension, weakness) (Ona et al., 2021; Corkery, 2018). The QT prolongation is regarded as one of IBO’s most dangerous effects. Koenig et al. (2015) describe how IBO inhibits the hERG (human Ether-a-go-go-Related Gene) channels, which play an important role in the

repolarization of the action potential. This inhibition delays repolarization, producing a prolongation of the QT interval and, consequently, inducing arrhythmias and sudden death. Koenig et al. (2015) also demonstrated that IBO inhibits sodium and calcium flow in the ventricular cardiomyocyte (Koenig et al., 2013). Notably, it appears that NOR blocks the hERG channel with a potency comparable to its parent compound IBO (Alper et al., 2016). Given the longer half-life of NOR, this might explain the persistent QT prolongations observed in several case reports (Alper et al., 2012; Koenig and Hilber, 2015; Ona et al., 2021). Innovative methodologies have been implemented In that regard to predict NOR-induced cardiotoxicity (Shi et al., 2021). In addition, a recently published open label trial stated that this substance can induce a clinically relevant QT prolongation (50% of patients reached QT above 500 ms) and severe ataxia (Knuijver et al., 2022). However, a valuable contribution by Luz and Mash (2022) noted that this report had several limitations and could be potentially flawed technically. Specifically, it underscores inappropriate methods of QT correction or the co-administration of drugs (metoclopramide) inhibiting CYP2D6, the main enzyme responsible for IBO metabolism.

This complicated scenario has led researchers to develop non-hallucinogenic, non-toxic analogues of IBO. This challenging research is currently emerging and starting to bear fruit (Cameron et al., 2021; Gassaway et al., 2016). For instance, tabernanthalog (TBG) showed promise in preliminary research due to its pharmacokinetics and lack of hallucinogenic effects. It has thus been proposed as an IBO alternative with therapeutic potential (Lu et al., 2021).

Given IBO's supposed "anti-addictive" potential and its unclear safety profile, it is crucial to understand the central mechanisms of this substance. The atypical multi-target profile of IBO (See Figure 1) poses a challenge to elucidate the primary mechanism of action responsible for its anti-addictive potential. The most comprehensive review of IBO's mechanisms was published more than 25 years ago (Popik et al., 1995). It is therefore necessary to update the description of the main targets of IBO and its metabolite. The aim of this manuscript is to provide an overview of the pharmacological targets of IBO/NOR and discuss the most recent advances. Because the relevance of the target may depend on the drug (e.g., opioids, amphetamines, etc.), each target's relevance for different drugs will be discussed when necessary.

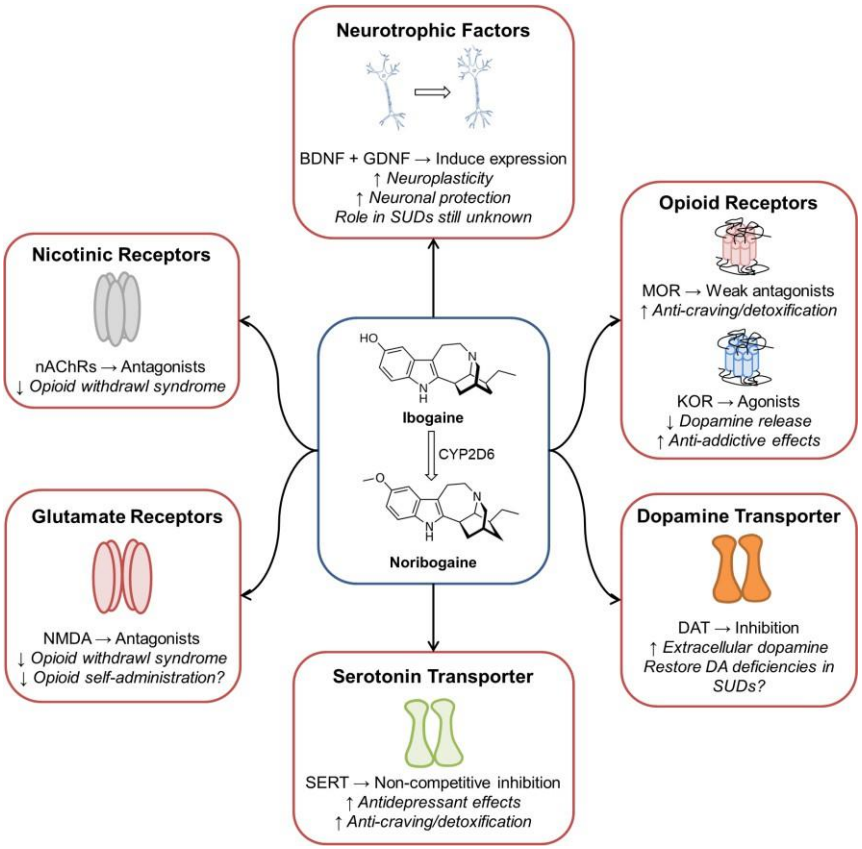


Figure 1. Main targets of ibogaine and noribogaine related to the treatment of substance use disorders and the respective effects they convey (in italics).

BDNF: Brain-Derived Neurotrophic Factor; GDNF: Glial Cell-Derived Neurotrophic Factor; DA: Dopamine; DAT: Dopamine Transporter; KOR: Kappa Opioid Receptor; MOR: Mu Opioid Receptor; nAChRs: Nicotinic Acetylcholine Receptors; NMDA: N-methyl-D-aspartate Receptor; SERT: Serotonin Transporter; SUDs: Substance Use Disorders.

Main receptors / targets

Opioid receptors

Early radioligand binding analyses (Sweetnam et al., 1995) and preclinical studies (Codd, 1995) suggested that both IBO and its metabolite NOR bind to the mu opioid receptor (MOR) with affinities in the low micromolar (μM) range. It has therefore been suggested that the anti-craving and detoxification mechanisms of IBO share similarities with other effective treatments for opioid dependence, such as buprenorphine (a partial agonist of MOR; Lutfy et al., 2003) and methadone (a MOR agonist; Kreek et al., 2011).

The hypothesis that IBO and NOR could act as MOR agonists was suggested on the basis of naloxone-sensitive stimulation of guanosine-5´-O-(γ-thio)-triphosphate ([35S]GTPγS) binding (Pablo and Mash, 1998). However, a seminal study by Alper in 2013 demonstrated unequivocally that neither IBO nor NOR act as partial or full agonists at MOR (Antonio et al., 2013). In this study, the authors examined the effects of IBO and NOR by measuring the activation of MOR using [35S]GTPγS in cell cultures that overexpressed the recombinant MOR. Additionally, they

evaluated MOR activity in rat thalamic membranes and performed autoradiography on the brain slices. Both IBO and NOR displayed MOR antagonists features with Ki (equilibrium dissociation constant) values of 3 μM (IBO) and 13 μM (NOR) (Antonio et al., 2013). Based on the above, both IBO and NOR should be considered weak MOR antagonists. MOR antagonists have been used to mitigate the abuse potential of opioid drugs and treat non-opioid drug dependence and addictive behaviors (Goodman et al., 2007).

Both IBO and NOR interact with the kappa opioid receptor (KOR) (Staley et al., 1996; Maillet et al., 2015). Both act on this receptor as agonists but NOR exerts a stronger effect on KOR (Staley et al., 1996). In the study conducted by Maillet et al. (2015), it was shown that NOR is a partial G-protein biased kappa agonist. KOR agonists display analgesic, antidepressant, and neuroprotective effects. But clinical use has been limited due to their side effects, consisting mainly of dysphoria and psychotomimetic effects (Stein, 2016). Indeed, the psychoactive effects of IBO may be related to KOR agonism to some extent, taking into account that salvinorin-A, another "atypical psychedelic," acts as a highly selective KOR agonist that produces powerful hallucinogenic effects (González et al., 2006; Ona et al., 2022; Roth et al., 2002).

KOR agonists have also attracted attention for the treatment of SUDs (Butelman et al., 2012; dos Santos et al., 2014) since they prevent both behavioral and neurochemical responses evoked by drug dependency. Furthermore, it has been proposed that the dynorphin/KOR system may function as an anti-reward system after acute drug use (Tejeda and Bonci, 2019). However, chronic drug users might present with a dysregulated dynorphin/KOR system, which can promote negative affective states and a higher sensitivity to stress (Tejeda and Bonci, 2019). In this regard, it is possible to speculate that IBO/NOR regulates KOR function by inducing distinct dynamics in KOR signaling pathways. In fact, it has been observed that in the presence of dynorphin, NOR is able to modulate the functional selectivity of dynorphin (Maillet et al., 2015). According to the authors of the study, this mechanism could potentially contribute to the antagonism of negative affective states triggered by an excessively active dynorphin-KOR system (Maillet et al., 2015).

DAT

Early preclinical studies demonstrated the effects of IBO on DA depends on the brain region involved (Maisonneuve et al., 1991) and the dose of IBO administered. These studies examined the biphasic effects on DA levels depending on the IBO concentration used (Reid et al., 1996). It has also been reported that IBO blocks DA uptake through the inhibition of the DA transporter (DAT), potentially increasing extracellular DA levels (Wells et al., 1999). However, later studies found that IBO has a low affinity for DAT, resulting in a weak inhibition of DA uptake (Baumann et al., 2001a). In addition, IBO induces marked and sustained decreases in DA concentrations, in conjunction with elevations in the metabolites 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Ali et al., 1996; Baumann et al., 1998). These effects of ibogaine are specific to the dopamine system, and are consistent with disruption of synaptic vesicle storage via inhibition of VMAT2 (Staley et al., 1996).

This finding holds particular significance in the context of SUDs involving psychostimulants like cocaine and methylphenidate, as these drugs inhibit DAT, leading to increased synaptic DA levels, particularly in the ventral tegmental area (VTA) and nucleus accumbens (NAc) (Zahniser and Sorkin, 2004). More recently, several studies utilizing in vitro and Drosophila models have

provided additional evidence that both IBO and NOR, along with IBO analogs, interact with DAT and restore functional activity in DAT mutations. These effects are due to the influence on transporter folding (Kasture et al., 2016; Beerepoot et al., 2016; Bhat et al., 2021). The implications of these findings regarding SUDs are not yet clear. But the authors suggested that both IBO and NOR can exert additional non-described effects on DAT, which might be relevant in the context of SUDs.

However, it is challenging to predict the eventual effects of IBO/NOR on DA neurotransmission, due to both of these molecules inhibiting DAT and being KOR agonists, as noted in the previous section. Acute activation of KOR inhibits DA release specifically at the NAc and dorsal striatum (Escobar et al., 2020). In this sense, two preclinical studies reported that IBO decreases drug-induced DA efflux in the NAc and striatum after chronic cocaine or morphine use (Szumlinski et al., 2000; Pearl et al., 1996).

The influence of IBO/NOR on dopamine signaling is unquestionable given the evidence provided in the literature. It is plausible that both mechanisms, DAT inhibition and KOR activation, counteract each other. Depending on the dose of IBO/NOR administered, DA levels could either be increased or decreased. Therefore, further research should be performed with the equivalent of standardized clinical doses to assess the implications of IBO-associated DAT inhibition in the context of SUDs.

SERT

Unlike other hallucinogens, IBO/NOR have weak affinities for serotonin (5-HT) receptors, including the 5-HT2A receptor which is the main target of classic hallucinogens such as LSD, psilocybin, and DMT (Kyzar et al., 2017). Acute IBO administration to rats does not elicit the characteristic head twitch response typically associated with the administration of psychedelics (González et al., 2018). IBO therefore produces psychedelic effects through a different biological mechanism which has not yet been elucidated. Recently, researchers suggested that IBO induces psychedelic effects by altering gamma oscillations in rats (González et al., 2021).

IBO is an inhibitor of the 5-HT transporter (SERT) (Toll et al., 1998; Repke et al., 1994). NOR is a SERT inhibitor approximately 10 times stronger than IBO (Baumann et al., 2001a) and increases in 5-HT levels have been observed in different in vivo studies (Baumann et al., 2001a, 2001b). Recently, IBO has been categorized as an active-site-binding inhibitor that demonstrates non-competitive inhibition. (Coleman et al., 2019). This is an exception in enzymology, since inhibitors that bind to the active site tend to do so by competition (Blat, 2010).

The inhibition of SERT can be associated with antidepressant effects, which can be useful in the context of drug detoxification. For instance, a preclinical study found antidepressant effects after a single administration of IBO to rats (Rodriguez et al., 2020). This finding is consistent with IBO's antidepressant effects reported in an uncontrolled human study (Mash et al., 2018). The regulation of 5-HT levels through SERT inhibition is a mechanism that may be directly involved in the anti-addictive properties of IBO/NOR, especially in the case of opioids. Indeed, withdrawal from chronic MOR agonist exposure is associated with reduced 5-HT levels, which are partially responsible for withdrawal syndrome (Kirby et al., 2011). Thus, the inhibition of SERT by IBO/NOR would sustain 5-HT levels and soften the detox process.

NMDA

IBO is a competitive N-methyl-D-aspartate (NMDA) receptor antagonist, as demonstrated by both in vitro and in vivo studies (Chen et al., 1996; Popik et al., 1994). However, NOR shows a lower affinity for that target (Maillet et al., 2015). In rats, the systemic administration of NMDA receptor antagonists tends to attenuate the rewarding effects of drugs (Shelton and Balster, 1997; Allen et al., 2005). However, divergent results have been observed with opioids. In certain studies, NMDA receptor antagonists like dizocilpine and ketamine increased heroin self-administration in rats when administered systemically (Xi and Stein, 2002). In others, NMDA receptor antagonists (memantine) diminished opioid withdrawal syndrome (OWS) in humans (Bisaga et al., 2001). Notably, when ketamine and AP5 (d-(–)-2-amino-5-phosphonopentanoic acid, a competitive NMDA receptor antagonist) were administered directly to the VTA of rats, heroin self-administration was effectively blocked (Bisaga et al., 2001).

More direct evidence on the relationship between NMDA and OWS was first provided by Zhu and Ho (1998). Following chronic exposure to morphine, OWS was induced in rats through naloxone administration. Subsequently, a ventricular administration of an antisense oligonucleotide was used to knockdown the NMDA-R1 (NR1) subunit. The observed results indicated a significant attenuation of OWS, suggesting that functional NMDA receptors are necessary for full OWS expression (Zhu and Ho, 1998).

The mechanisms through which functional NMDA receptors allow the expression of OWS are highly complex. It appears that NMDA signaling plays a crucial role in plasticity processes associated with neural adaptation or the regulation of both DA release and ΔFosB expression, which are linked to OWS via NMDA antagonists (Glass, 2011).

Additionally, there seem to be critical brain regions where NMDA antagonism might be more related to anti-addictive effects. For instance, the blockade of NMDA receptors at the NAc and VTA was associated with the inhibition of OWS (Glass, 2011; Wang et al., 2005). Therefore, future human neuroimaging studies with IBO to describe its specific neuropharmacological effects would help better understand its anti-addictive attributes.

It is important to highlight that the NMDA antagonistic properties exhibited primarily by IBO, and to a lesser extent by NOR, may have implications for potential antidepressant effects similar to ketamine. This is indicated by the overall psychoactive effects of IBO/NOR, due to similarities recently being found in the cortical activity between ketamine and IBO-treated animals. (González et al., 2021).

Nicotinic receptors

A prominent theory that has emerged to explain the IBO's anti-addictive effects is its ability to modulate nicotinic acetylcholine receptors (nAChRs), particularly the α3β4 subtype, by functioning as a noncompetitive antagonist (Popik et al., 1995).

In recent decades, it has been increasingly accepted that nicotinic receptors are crucial for OWS. Preclinical evidence has shown that nicotinic antagonists attenuate naloxone-precipitated morphine withdrawal (Taraschenko et al., 2005; Hall et al., 2011). In humans, two datasets have shown that variants of the CHRNA3 gene (which is coding for the α5, α3, and β4 nAChRs subunits) are associated with opioid dependence and withdrawal (Muldoon et al., 2014). In

addition, a recent study suggested that IBO inhibits (±)-epibatidine-induced Ca2+ influx in the α3β4 receptor with an estimated potency 9 times stronger than phencyclidine (PCP). Both IBO and PCP bind to overlapping sites located between the serine and valine/phenylalanine rings, blocking the nAChR ion channel (Arias et al., 2010). In the case of IBO, it maintained nAChR in a desensitized state for a longer period of time (Arias et al., 2010). A putative explanation for the relevance of nAChRs to IBO's anti-addictive effects is their connection to the VTA. This brain region is critical for behavioral activation and sensitization by morphine, and its activity is mostly regulated by nAChRs (Vezina and Stewart, 1984). Notably, nAChRs also mediate the rewarding effects of morphine (Rezayof et al., 2007). Additionally, cholinergic activation at VTA modulates dopamine release in the NAc and extends midbrain dopaminergic systems (Mansvelder and McGehee, 2002; Bajic et al., 2015), making it a highly relevant target in the context of SUDs.

IBO, as well as other well-known nAChRs antagonists, like dextromethorphan and 18-methoxycoronaridine (18-MC), have low selectivity for the α3β4 subtype. Variable levels of attenuation of OWS have thus been observed (Muldoon et al., 2014). However, later studies using highly selective α3β4 antagonists, like α-conotoxin AuIB (AuIB), have confirmed the role of this target to attenuate the somatic signs of opioid withdrawal (Bajic et al., 2015).

Neurotrophic factors

Both IBO and NOR induce the expression of glial cell-derived neurotrophic factors (GDNF) and brain-derived neurotrophic factors (BDNF) in both cell cultures and in vivo rat studies (Marton et al., 2019; Carnicella et al., 2010). Neurotrophic factors (NTFs) are proteins that regulate neuronal survival and the differentiation and the migration of neuroprogenitor cells. In addition, they are reported to protect neurons from toxins and injury (Koskela et al., 2017).

BDNF binds to tropomyosin-related kinase B (TrkB), initiating downstream signaling via the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), phospholipase Cγ (PLCγ), and phosphoinositol 3-kinase (PI3K) pathways (Huang and Reichardt, 2003; Liran et al., 2020). BDNF and TrkB are widely expressed in the brain, especially in the cortex, hippocampus, and cerebellum (Hofer et al., 1990; Liran et al., 2020). GDNF is expressed in multiple brain regions, notably the striatum, thalamus, cortex, and hippocampus (Pochon et al., 1997; Liran et al., 2020). GDNF exerts its signaling effects through the receptor tyrosine kinase RET (Durbec et al., 1996; Liran et al., 2020).

The relationship between NTFs and SUDs is highly complex. Most of the effects of NTFs on the central nervous system (CNS) are still not well understood. According to a review conducted by Ghitza et al. (2010), both GDNF and BDNF can either facilitate or inhibit drug-taking behaviors. The outcome depends on various factors such as the specific type of drug, the brain region where NTFs are induced, the addiction phase, and the timing between NTF manipulations and behavioral assessments related to reward and relapse (Ghitza et al., 2010). For example, the administration of both BDNF and GDNF in the mesocorticolimbic system of rats has been shown to enhance cravings for cocaine and heroin (Airavaara et al., 2011). Conversely, when a GDNF infusion is administered in the VTA of rats, it exhibits a dose-dependent reduction in ethanol operant self-administration (Carnicella et al., 2008).

The roles of GDNF and BDNF are also not clear in regards to opioid dependence and withdrawal. While in some studies, heterozygous GDNF+/− mice displayed enhanced conditioned place

preference (CPP) for morphine (Niwa et al., 2007), others reported a similar CPP between GDNF+/− and wild-type mice (Koo et al., 2012). In studies examining heroin cravings, it was found that direct injections of GDNF into the NAc, but not the VTA, resulted in an increased extinction response following withdrawal.

BDNF has been identified as a negative modulator of morphine action (Koo et al., 2012). Chronic administration of morphine to mice has been found to suppress BDNF expression in the VTA. This allows for the enhancement of rewarding and locomotor responses to morphine by increasing the excitability of DA neurons. In this context, when BDNF is administered to VTA, morphine CPP is extinguished (Koskela et al., 2017). Moreover, it has been reported that morphine suppresses the binding of phospho-CREB (cAMP response element-binding protein) and NURR1 (nuclear receptor related-1) to Bdnf gene promoters in the VTA, resulting in the decreased expression of BDNF. Overexpression of NURR1 in the VTA decreased morphine CPP, whereas a local knockout of Bdnf halted this effect (Koskela et al., 2017; Koo et al., 2015). In contrast, repeated exposure to heroin in rats has been shown to elevate BDNF levels in the VTA. Furthermore, infusions of BDNF into the VTA induce a shift from a DA-dependent opiate reward system to a DA-independent one (Vargas-Pérez et al., 2009).

In conclusion, the role of GDNF and BDNF in opioid dependence and withdrawal remains largely unknown. IBO induces the gene expression of BDNF in the prefrontal cortex (PFC) and upregulates GDNF levels especially in the VTA (Marton et al., 2019). Given that the GDNF pathway has been proposed as a potential strategy for the treatment of SUDs, and both the PFC and the VTA are critical brain areas involved in this disorder, further research should elucidate IBO's potential NTF-related mechanisms in this context.

Significantly, when assessing the structural and functional plasticity of both IBO and NOR, it was found that NOR, rather than IBO, induces neural plasticity. NOR specifically increases dendritic arbor complexity with an EC50 value comparable to ketamine (Ly et al., 2018). Despite a weak binding affinity, this effect seems to be at least partially mediated by the 5-HT2A receptor, since ketanserin, a selective 5-HT2 serotonin receptor antagonist, blocked this effect (Ly et al., 2018).

Other targets of ibogaine

The targets that have been discussed in previous sections are not only those closely related to SUD treatment, but also those that IBO has shown greater binding affinity for. Besides these targets, IBO has also demonstrated minor to moderate affinity for various targets potentially associated with SUDs. Low-affinity binding interactions are not necessarily unproductive (Csermely et al., 2005). They should therefore not be overlooked when seeking a comprehensive understanding of IBO's anti-addictive effects.

ABC transporters

One potential mechanism underlying IBO's anti-addictive effects may be related to its inhibitory effects on P-glycoprotein (P-gp) and the breast cancer resistance protein (BCRP) (Tournier et al., 2010; Martins et al., 2022). This hypothesis has not yet been mentioned in the literature, but it deserves further attention.

Both P-gp and BCRP belong to the ATP-binding cassette (ABC) transporter subfamily. They are responsible for the drug efflux from the cell and are therefore highly involved in the processes of multidrug resistance (MDR) and tolerance. They are present in a myriad of structures, such as the blood–brain barrier (BBB), the blood–cerebrospinal fluid barrier, intestinal epithelial cells, the bile canaliculi membrane, and kidney tubules. Importantly, animals that have developed tolerance to drugs, including opioids, exhibit elevated levels of P-gp and BCRP (Mercer and Coop, 2011). This occurs because a greater number of P-gp and BCRP transporters are recruited to facilitate the efflux of the drugs. Inhibiting P-gp or BCRP would effectively impede the development of tolerance.

Dexamethasone, morphine, and methadone are recognized as P-gp substrates (DrugBank, 2020). Regarding methadone, in vivo studies performed with P-gp KO mice or rats exposed to P-gp inhibitors showed that brain concentrations of methadone were greater and its analgesic effect was higher when P-gp was absent or inhibited (Wang et al., 2004; Rodriguez et al., 2004). These findings are especially relevant considering the recent interest in IBO for methadone detoxification [NCT04003948]. This is because a decrease in tolerance may allow for effective dose tapering in a much faster manner than usual protocols.

IBO's potential ability to inhibit P-gp and BCRP could be associated with the reduced tolerance to drugs reported by preclinical studies (Bhargava and Cao, 1997; Sunder Sharma and Bhargava, 1998). This complements other IBO-related mechanisms that lower tolerance, such as the inhibition of β-arrestin-2 recruitment (Maillet et al., 2015). However, further studies are needed. The relevance of ABC transporters and BBB transport at this juncture is speculative.

Sigma receptors

Radioligand binding assays have shown that IBO has moderate affinity for sigma-2 (σ2) and a slight affinity for sigma-1 (σ1) receptors (Bowen et al., 1995; Mach et al., 1995). NOR shows less affinity for sigma receptors (Maillet et al., 2015). These receptors were initially described as subtypes of opioid receptors, but they are currently considered a distinct orphan class. There are a few known endogenous compounds for σ receptors (N,N-dimethyltryptamine or DMT, a powerful hallucinogenic compound, neuroactive steroids and choline) (Hidalgo-Jiménez and Bouso, 2022). The development of σ receptor ligands is currently a high interest area given their involvement in cancer, pain, neuropsychiatric disorders, and SUDs (Rousseaux and Greene, 2016).

It is not clear from the literature whether IBO acts as an agonist or an antagonist at these receptors. It has been challenging for researchers to define other σ receptor ligands as either agonists or antagonists (Sambo et al., 2018).

Although the affinity on the σ2 receptor has been linked to IBO's neurotoxic effects (Vilner et al., 1998), the modulation of this receptor can also play a role in IBO's anti-addictive effects. Preclinical research with cocaine, amphetamine (Matsumoto, 2009), and alcohol (Quadir et al., 2019) has demonstrated the recruitment of sigma receptors is necessary to establish SUDs. Accordingly, σ1 receptor antagonists are able to interrupt addictive behaviors in animal models (Matsumoto et al., 2002, 2008).

It is noteworthy that drugs like cocaine and methamphetamine interact primarily with the σ1 receptor (Matsumoto et al., 2003; Nguyen et al., 2005), whereas IBO has 43-fold selectivity for

the σ2 receptor (Bowen et al., 1995). Further research should define the exact role of IBO/NOR at σ sites and its putative implications for its anti-addictive effects.

Serotonin receptors

Although the affinity of IBO for serotonin receptors is weak (Ki > 10,000 nM for 5-HT2A, 5-HT2C, 5-HT1A; > 100,000 nM for 5-HT1B, 5-HT1D) (Ray, 2010), it might be enough to achieve relevant modifications or at least enhance the effects of IBO on other targets, as stated above (Csermely et al., 2005). The non-selective, low-binding affinity of IBO/NOR for multiple serotonin receptors would thus be especially relevant for the antidepressant effects exerted via SERT inhibition. It has been recently demonstrated that serotonin receptors (5-HT1A) are involved in some of IBO's effects in mice (González-Trujano et al., 2022). Preliminary evidence suggests that the low-binding affinities found in IBO/NOR may play a potential role, supporting the assertions derived from the polypharmacology paradigm.

Muscarinic receptors

It has been observed that both IBO and NOR bind to muscarinic receptors (M1, M2, and M3) at similar affinities (7.6-16 μM for M1 and 5.9-31 μM for M2) (Sweetnam et al., 1995; Ray, 2010; Glick et al., 1999). Indeed, its agonistic action on those receptors might be responsible for IBO-induced bradycardia (Glick et al., 1999).

Relatively little research has focused on the involvement of these receptors in the overall anti-addictive effects of IBO, yet muscarinic agonists reduce psychostimulant self-administration in mice (Thomsen et al., 2010). Therefore, it has been suggested that M1 agonists may become useful for treating SUDs, especially in the case of psychostimulants (Dencker et al., 2012). The involvement of muscarinic receptors in the anti-addictive effects of IBO therefore cannot be ruled out.

Potential synergistic effects

One of the main mechanisms through which synergy can be produced is the display of multi-target effects, since binding to distinct targets can produce stronger effects on certain complex systems (Ona and Bouso, 2021). The modulation of different targets by IBO/NOR (see Table 1 for the Ki of IBO in its main targets) can therefore potentially result in synergistic effects. Considering the previous discussion, there is speculation about potential synergistic effects that could contribute to IBO's anti-addictive properties. These effects should be appropriately evaluated through specifically designed studies.

Table 1. Binding affinities of ibogaine and noribogaine to the main targets related to SUDs. MOR = mu opioid receptor; KOR = kappa opioid receptor; DAT = dopamine transporter; SERT = serotonin transporter; NMDA = N-Methyl-d-aspartate; nAChR = nicotinic acetylcholine receptor; ABC transporters = ATP-binding cassette transporters; NTFs = neurotrophic factors; IBO = ibogaine; NOR = noribogaine.

Target	Affinity	Action	Reference
MOR	IBO: 2-3 μM; NOR: 0.68-13 μM	Weak antagonists	Antonio et al. 2013; Glick et al. 1999
KOR	IBO: 2.1-13.8 μM; NOR: 0.61-0.96 μM	Agonists	Pearl et al. 1996; Glick et al. 1999, Popik et al. 1999
DAT	IBO: 2-4.11 μM; NOR: 2.05-3.35 μM	Inhibitory	Sweetnam et al. 1995; Ray, 2010; Staley et al. 1996; Popik et al. 1999;
SERT	IBO: 0.59 μM; NOR: 0.04 μM	Inhibitory	Staley et al. 1996;
NMDA	IBO: 1-5.6 μM; NOR: 15-31.4 μM	Antagonists	Sweetnam et al. 1995; Glick et al. 1999; Popik et al. 1999
nAChR (α3β4 subtype)	IBO: 0.22 μM; NOR: 6.2 μM	Antagonists	Arias et al. 2010
ABC transporters	-	Inhibitory	Tournier et al. 2010
NTFs	-	Increased expression	Marton et al. 2019

The first area in which potential synergistic effects are produced by IBO/NOR is the modulation of the dopaminergic system. IBO/NOR may increase extracellular DA levels by DAT inhibition. However, indirect effects related to KOR agonism might counteract this effect since acute KOR agonism decreases DA levels. On the other hand, chronic activation (possibly induced by NOR) facilitates DA neurotransmission (Escobar et al., 2020). The available preclinical evidence suggests that DA efflux related to different drugs decreases rather than being potentiated (Szumlinski et al., 2000; Pearl et al., 1996). One additional pathway through which dopaminergic neurotransmission can be modulated is through IBO's reported NMDA antagonism. As with other NMDA antagonists, IBO/NOR would diminish the DA release associated with OWS as well as the acute reinforcing effects of drugs (Glass, 2011). The DA-related rewarding effects could therefore be attenuated, and the associated neuronal pathways could adapt to less pathological functioning.

Another potential synergy could occur in the case of decreased tolerance to drugs, especially opioids. The main mechanisms attributed to IBO's tolerance-decreasing effect are related to the inhibition of β-arrestin-2 recruitment for MOR and KOR agonists, as well as its agonist activity at KOR (Maillet et al., 2015). However, this decrease in tolerance might be enhanced by IBO/NOR's NMDA antagonism since NMDA receptor antagonists are known to block opioid tolerance (Inturrisi, 1997). Furthermore, the previously mentioned inhibition of P-gp and BCRP may

potentiate tolerance reduction. Consequently, the combined action of these mechanisms can lead to a sensitization to drugs, particularly opioids, which can attenuate the occurrence of withdrawal syndrome if the drug is still present in plasma concentrations. This effect also enables an effective tapering of the drug's dose during the detoxification processes.

IBO also modulates different targets that can reduce withdrawal symptoms. For instance, it has been demonstrated that NMDA antagonism attenuates OWS (Glass, 2011; Wang et al., 2005). Additionally, withdrawal from chronic exposure to MOR agonists is highly associated with reduced 5-HT levels, which would be partially counteracted by the SERT inhibition produced by IBO/NOR. Finally, the antagonist effect of IBO/NOR on nicotinic receptors could also attenuate withdrawal symptoms, since preclinical evidence suggests nicotinic antagonists attenuate OWS (Taraschenko et al., 2005; Hall et al., 2011). Closely linked to that would be an antidepressant effect obtained via different receptors. IBO/NOR inhibit SERT, are NMDA antagonists, and induce the expression of neurotrophic factors which are mechanisms individually associated with antidepressant effects.

Closing remarks

As previously described, both IBO and NOR display multi-target effects on several sites related to SUDs directly or indirectly. Most of the previous literature that tried to discuss the mechanisms of action underlying IBO's anti-addictive effects was focused on finding the "key target." This approach used either highly selective ligands or specific antagonists to either confirm or refute the relevance of those targets. This procedure is in line with the classical paradigm in drug design, focused on the development of "magic bullets." As emerging techniques such as "omics" (Caesar et al., 2021) and paradigms like polypharmacology (Hopkins, 2008) gain prominence, there is a need to adopt a more comprehensive perspective when studying IBO's anti-addictive effects. Studies using omics or complex approaches have not yet been performed with IBO. Instead of focusing on certain targets or receptors, omics techniques allow researchers to discover the entire molecular landscape affected by drugs. Several other examples in highly complex natural products research demonstrate groundbreaking results in recent literature (Liu et al., 2022; Wang et al., 2020; Xue et al., 2022).

IBO's anti-addictive effects could be better understood as resulting from its complex polypharmacology, which is able to modulate a high number of relevant targets, rather than simply modulating certain key targets. As Luccock stated, a symphony cannot be whistled, it takes an orchestra to play it (Pike and Krumm, 1954). The anti-addictive effects demonstrated by the multi-target effects of IBO and its metabolite should not be oversimplified. The polypharmacology paradigm offers us an opportunity to gain a deeper understanding of these complex effects. IBO/NOR serves as an illustrative example of how multiple, weak perturbations at various targets can produce a notable impact on the complex patterns involved in SUDs.

However, this complex approach does not prevent us from needing to perform more preclinical research to fully understand the pharmacology of IBO/NOR. The complex landscape of treating SUDs, where IBO exhibits its complex effects amidst neural adaptations to different drugs, should be replicated and carefully characterized in preclinical models using omics and other recently developed techniques. This would support the rational design of multi-target drugs that might be safer than IBO. Also, a detailed description of receptor binding and affinity, as well as the pharmacokinetics of IBO when using therapeutic doses, should be investigated.

In addition, human studies have demonstrated that IBO's psychoactive effects can play a role in the psychological impacts of SUDs and provide personal insights that can produce changes in behavior (Kohek et al., 2020; Rodriguez-Cano et al., 2022). Regrettably, there is limited research on the subjective experience elicited by IBO, especially with contemporary methods involving specific psychometric questionnaires or visual analogue scales (VAS). However, anecdotal reports have mentioned various subjective effects such as synesthesia, visions of spirit beings, cosmic experiences, or representations of the iboga plant (Brown et al., 2019; Kohek et al., 2020). Additionally, individuals commonly report reviewing their life and experiencing the retrieval of multiple past memories during IBO sessions (Brown et al., 2019; Schenberg et al., 2017). The intricate interplay between the pharmacological properties of IBO and its subjective effects underscores the complex nature of this compound.

Acknowledgements: This study was part of GO's PhD project for which he received the Industrial Doctorate public grant from AGAUR-GENCAT.

The authors wish to express their appreciation to Dr. Daniele Caprioli for his valuable insights regarding the conceptualization and writing of this manuscript.

References

Airavaara M, Pickens CL, Stern AL, Wihbey KA, Harvey BK, Bossert JM, Liu QR, Hoffer BJ and Shaham Y (2011) Endogenous GDNF in ventral tegmental area and nucleus accumbens does not play a role in the incubation of heroin craving. Addiction Biology 16: 261–272.

Ali SF, Newport GD, Slikker W Jr, Rothman RB, Baumann MH (1996) Neuroendocrine and neurochemical effects of acute ibogaine administration: a time course evaluation. Brain research, 737(1-2): 215–220.

Allen RM, Carelli RM, Dykstra LA, Suchey TL and Everett CV (2005) Effects of the competitive N-methyl-D-aspartate receptor antagonist, LY235959 [(-)-6-phosphonomethyl-deca-hydroisoquinoline-3-carboxylic acid], on responding for cocaine under both fixed and progressive ratio schedules of reinforcement. Journal of Pharmacology and Experimental Therapeutics 315: 449–457.

Alper KR, Stajić M and Gill JR (2012) Fatalities temporally associated with the ingestion of ibogaine. Journal of Forensic Science 57(2): 398–412.

Alper K, Bai R, Liu N, Fowler SJ, Huang XP, Priori SG and Ruan Y (2016) hERG Blockade by Iboga Alkaloids. Cardiovascular Toxicology 16(1): 14–22.

Antonio T, Childers SR, Rothman RB, Dersch CM, King C, Kuehne M, Bornmann WG, Eshleman AJ, Janowsky A, Simon ER, Reith MEA and Alper K (2013) Effect of Iboga alkaloids on μ-opioid receptor-coupled G protein activation. PLoS ONE 8(10): e77262.

Arias HR, Rosenberg A, Targowska-Duda KM, Feuerbach D, Yuan XJ, Jozwiak K, Moaddel R and Wainer IW (2010) Interaction of ibogaine with human α3β4-nicotinic acetylcholine receptors in different conformational states. International Journal of Biochemistry and Cell Biology 42(9): 1525–1535.

Bajic D, Soiza-Reilly M, Spalding AL, Berde CB and Commons KG (2015) Endogenous cholinergic neurotransmission contributes to behavioral sensitization to morphine. PLoS One 10(2): e0117601.

Baumann MH, Rothman RB, Pablo J and Mash DC (2001a) In vivo neurobiological effects of ibogaine and its Odesmethyl metabolite, 12-hydroxyibogaine (noribogaine) in rats. Journal of Pharmacology and Experimental Therapeutics 297: 531–539.

Baumann MH, Pablo J, Ali SF, Rothman RB and Mash DC (2001b) Comparative neuropharmacology of ibogaine and its O-desmethyl metabolite, noribogaine. The Alkaloids. Chemistry and Biology 56: 79–113.

Baumann MH, Rothman RB, Ali SF (1998) Neurochemical and Neuroendocrine Effects of Ibogaine in Rats: Comparison to MK-801. Annals of the New York Academy of Sciences, 844(1): 252–264.

Beerepoot P, Lam VM and Salahpour A (2016) Pharmacological chaperones of the dopamine transporter rescue dopamine transporter deficiency syndrome mutations in heterologous cells. Journal of Biological Chemistry 291(42): 22053–22062.

Belgers M, Leenaars M, Homberg JR, Ritskes-Hoitinga M, Schellekens AFA and Hooijmans CR (2016) Ibogaine and addiction in the animal model, a systematic review and meta-analysis. Translational Psychiatry 6(5): e826.

Bhargava HN and Cao YJ (1997) Effects of noribogaine on the development of tolerance to antinociceptive action of morphine in mice. Brain Research 771(2): 343–346.

Bhat S, Guthrie DA, Kasture A, El-Kasaby A, Cao J, Bonifazi A,... and Newman A (2021) Tropane-based ibogaine analog rescues folding-deficient serotonin and dopamine transporters. ACS Pharmacology and Translational Science 4(2): 503–516.

Bisaga A, Comer SD, Ward AS, Popik P, Kleber HD and Fischman MW (2001) The NMDA antagonist memantine attenuates the expression of opioid physical dependence in humans. Psychopharmacology (Berl) 157: 1–10.

Blat Y (2010) Non-competitive inhibition by active site binders. Chemical Biology and Drug Design 75(6): 535–540.

Bowen WD, Vilner BJ, Williams W, Bertha CM, Kuehne ME and Jacobson AE (1995) Ibogaine and its congeners are σ2 receptor-selective ligands with moderate affinity. European Journal of Pharmacology 279(1): R1-3.

Brown, T. K., Noller, G. E., & Denenberg, J. O. (2019). Ibogaine and Subjective Experience: Transformative States and Psychopharmacotherapy in the Treatment of Opioid Use Disorder. Journal of psychoactive drugs, 51(2), 155–165.

Brown TK, Alper K (2018) Treatment of opioid use disorder with ibogaine: detoxification and drug use outcomes. The American journal of drug and alcohol abuse, 44(1): 24–36.

Butelman ER, Yuferov V and Kreek MJ (2012) κ-opioid receptor/dynorphin system: genetic and pharmacotherapeutic implications for addiction. Trends in Neuroscience 35(10): 587–596.

Caesar LK, Montaser R, Keller NP and Kelleher NL (2021) Metabolomics and genomics in natural products research: complementary tools for targeting new chemical entities. Natural Product Reports 38: 2041–2065.

Cameron LP, Tombari RJ, Lu J, Pell AJ, Hurley ZQ, Ehinger Y,... and Olson DE (2021) A non-hallucinogenic psychedelic analogue with therapeutic potential. Nature 589(7842): 474–479.

Carnicella S, He DY, Yowell QV, Glick SD and Ron D (2010) Noribogaine, but not 18-MC, exhibits similar actions as ibogaine on GDNF expression and ethanol self-administration. Addiction Biology 15: 424–433.

Carnicella S, Kharazia V, Jeanblanc J, Janak PH and Ron D (2008) GDNF is a fast-acting potent inhibitor of alcohol consumption and relapse. Proceedings of the National Academy of Science USA 105: 8114–8119.

Chen K, Kokate TG, Donevan SD, Carroll I and Rogawski MA (1996) Ibogaine block of the NMDA receptor: In vitro and in vivo studies. Neuropharmacology 35(4): 423–431.

Codd EE (1995) High affinity ibogaine binding to a mu opioid agonist site. Life Sciences 57(20): 315–320.

Coleman JA, Yang D, Zhao Z, Wen PC, Yoshioka C, Tajkhorshid E and Gouaux E (2019) Serotonin transporter–ibogaine complexes illuminate mechanisms of inhibition and transport. Nature 569: 141–145.

Corkery JM (2018) Ibogaine as a treatment for substance misuse: Potential benefits and practical dangers. Progress in Brain Research 242: 217–257.

Csermely P, Ágoston V and Pongor S (2005) The efficiency of multi-target drugs: the network approach might help drug design. Trends in Pharmacology Science 26(4): 178–182.

Dencker D, Thomsen M, Wörtwein G, Weikop P, Cui Y, Jeon J,... and Fink-Jensen A (2012) Muscarinic acetylcholine receptor subtypes as potential drug targets for the treatment of schizophrenia, drug abuse, and Parkinson's disease. ACS Chemical Neuroscience 3(2): 80–89.

dos Santos RG, Bouso JC and Hallak JEC (2016) The antiaddictive effects of ibogaine: a systematic literature review of human studies. Journal of Psychedelic Studies 1(1): 20–28.

dos Santos RG, Crippa JAS, Machado-de-Sousa JP and Hallak JEC (2014) Salvinorin a and related compounds as therapeutic drugs for psychostimulant-related disorders. Current Drug Abuse Reviews 7(2): 128–132.

DrugBank (2020) P-glycoprotein substrates. Available in: <https://go.drugbank.com/categories/DBCAT002668>. Access in: 2022, December 22.

Durbec P, Marcos-Gutierrez CV, Kilkenny C, Grigoriou M, Wartiowaara K, Suvanto P,... and Pachnis V (1996) GDNF signalling through the Ret receptor tyrosine kinase. Nature 381: 789–793.

Escobar AP, Casanova JP, Andrés ME and Fuentealba JA (2020) Crosstalk between kappa opioid and dopamine systems in compulsive behaviors. Frontiers in Pharmacology 11: 57.

Faura R and Langlois A (2020) The future of iboga: Perspectives from Central Africa. Phase II Report 2020. https://www.iceers.org/iboga-community-engagement-initiative-phase-2-report/ Accessed March 25, 2022.

Gassaway MM, Jacques TL, Kruegel AC, Karpowicz RJ Jr, Li X, Li S, Myer Y, and Sames D (2016) Deconstructing the iboga alkaloid skeleton: potentiation of FGF2-induced glial cell line derived neurotrophic factor release by a novel compound. ACS Chemical Biology 11: 77−87.

Ghitza UE, Zhai H, Wu P, Airavaara M, Shaham Y and Lu L (2010) Role of BDNF and GDNF in drug reward and relapse: a review. Neuroscience and Biobehavioral Reviews 35: 157–171.

Glass MJ (2011) Opioid dependence and NMDA receptors. ILAR Journal 52(3): 342–351.

Glick SD, Maisonnueve IM, Hough LB, Kuehne ME and Bandarage UK (1999) (±)-18-Methoxycoronaridine: A novel iboga alkaloid congener having potential anti-addictive efficacy. CNS Drug Reviews 5(1): 27–42.

Glue P, Winter H, Garbe K, Jakobi H, Lyudin A, Lenagh-Glue Z and Hung CT (2015a) Influence of CYP2D6 activity on the pharmacokinetics and pharmacodynamics of a single 20 mg dose of ibogaine in healthy volunteers. Journal of Clinical Pharmacology 55(6): 680–687.

Glue P, Lockhart M, Lam F, Hung N, Hung CT and Friedhoff L (2015b) Ascending-dose study of noribogaine in healthy volunteers: pharmacokinetics, pharmacodynamics, safety, and tolerability. Journal of Clinical Pharmacology 55(2): 189–194.

González J, Prieto JP, Rodríguez P, Cavelli M, Benedetto L, Mondino A, Pazos M, Seoane G, Carrera I, Scorza C and Torterolo P (2018) Ibogaine Acute Administration in Rats Promotes Wakefulness, Long-Lasting REM Sleep Suppression, and a Distinctive Motor Profile. Frontiers in Pharmacology 9: 374.

González J, Cavelli M, Castro-Zaballa S, Mondino A, Tort ABL, Rubido N, Carrera I, and Torterolo P (2021) EEG Gamma Band Alterations and REM-like Traits Underpin the Acute Effect of the Atypical Psychedelic Ibogaine in the Rat. ACS pharmacology & translational science 4(2): 517–525.

González-Trujano ME, Krengel F, Reyes-Chilpa R, Villasana-Salazar B, González-Gómez JD, Santos-Valencia F,... and Martínez-Vargas D (2022) Tabernaemontana arborea and ibogaine induce paroxysmal EEG activity in freely moving mice: Involvement of serotonin 5-HT1A receptors. Neurotoxicology 89: 79–91.

Goodman A, Le Bourdonnec B and Dolle R (2007) Mu Opioid Receptor Antagonists: Recent Developments. ChemMedChem 2: 1552–1570.

Hall BJ, Pearson LS, Terry AV Jr and Buccafusco JJ (2011) The use-dependent, nicotinic antagonist BTMPS reduces the adverse consequences of morphine self-administration in rats in an abstinence model of drug seeking. Neuropharmacology 61(4): 798–806.

Hidalgo-Jiménez J and Bouso JC (2022) Significance of mammalian N, N-dimethyltryptamine (DMT): A 60-year-old debate. Journal of psychopharmacology 36(8): 905–919.

Hofer M, Pagliusi SR, Hohn A, Leibrock J and Barde YA (1990) Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. EMBO Journal 9: 2459–2464.

Hopkins AL (2008) Network pharmacology: the next paradigm in drug discovery. Nature Chemistry Biology 4(11): 682–90.

Huang EJ and Reichardt LF (2003) Trk receptors: roles in neuronal signal transduction. Annual Review of Biochemistry 72: 609–642.

Inturrisi CE (1997) Preclinical evidence for a role of glutamatergic systems in opioid tolerance and dependence. Seminars in Neuroscience 9: 110–119.

Kasture A, El-Kasaby A, Szöllosi D, Mazhar Asjad HM, Grimm A, Stockner T,... and Sucic S (2016) Functional rescue of a misfolded Drosophila melanogaster dopamine transporter mutant associated with a sleepless phenotype by pharmacological chaperones. Journal of Biological Chemistry 291(40): 20876–20890.

Kirby LG, Zeeb FD and Winstanley CA (2011) Contributions of serotonin in addiction vulnerability. Neuropharmacol 61(3): 421–432.

Knuijver T, Schellekens A, Belgers M, Donders R, van Oosteren T, Kramers K and Verkes R (2022) Safety of ibogaine administration in detoxification of opioid-dependent individuals: a descriptive open-label observational study. Addiction 117(1): 118–128.

Köch P, Frölich K, Walter M, Lang U and Dürsteler M (2022) A systematic literature review of clinical trials and therapeutic applications of ibogaine. Journal of Substance Abuse and Treatment 138: 108717.

Koenig X, Hilber K (2015) The anti-addiction drug ibogaine and the heart: a delicate relation. Molecules 20(2): 2208–2228.

Koenig X, Kovar M, Rubi L (2013) Anti-addiction drug ibogaine inhibits voltage-gated ionic currents: a study to assess the drug's cardiac ion channel profile. Toxicology and Applied Pharmacology 273: 259–268.

Kohek M, Ohren M, Hornby P, Alcázar-Córcoles MA, and Bouso JC (2020) The Ibogaine Experience: A Qualitative Study on the Acute Subjective Effects of Ibogaine. Anthropology of Consciousness 31(1): 91–119.

Koo JW, Mazei-Robison MS, Chaudhury D, Juarez B, LaPlant Q, Ferguson D,… and Nestler EJ (2012) BDNF is a negative modulator of morphine action. Science 338: 124–128.

Koo JW, Mazei-Robison M, LaPlant Q, Egervari G, Braunscheidel KM, Adank DN,… and Nestler EJ (2015) Epigenetic basis of opiate suppression of Bdnf gene expression in the ventral tegmental area. Nature Neuroscience 18: 415–422.

Koskela M, Bäck S, Voikar V, Richie CT, Domanskyi A, Harvey BK and Airavaara M (2017) Update of neurotrophic factors in neurobiology of addiction and future directions. Neurobiology of Disease 97: 189–200.

Kreek MJ, Borg L, Ducat E and Ray B (2011) Pharmacotherapy in the treatment of addiction: methadone. Journal of Addictive Diseases 29(2): 200–216.

Kyzar EJ, Nichols CD, Gainetdinov RR, Nichols DE and Kalueff AV (2017) Psychedelic drugs in biomedicine. Trends in Pharmacological Science 38: 992.

Lavaud C and Massiot G (2017) The iboga alkaloids. In: Falk A, Gibbons H, and Kobayashi J. (eds) Progress in the Chemistry of Organic Natural Products 105. Springer, Cham.

Liran M, Rahamim N, Ron D and Barak S (2020) Growth Factors and Alcohol Use Disorder. Cold Spring Harbor Perspectives in Medicine 10(12): a039271.

Liu S, Zhao F, Deng Y, Zeng Y, Yan B, Guo J and Gao Q (2022) Investigating the multi-target therapeutic mechanism of Guihuang formula on Chronic Prostatitis. Journal of Ethnopharmacology 294: 115386.

Lu J, Tjia M, Mullen B, Cao B, Lukasiewicz K, Shah-Morales S, Weiser S, Cameron LP, Olson DE, Chen L, Zuo Y (2021) An analog of psychedelics restores functional neural circuits disrupted by unpredictable stress. Molecular psychiatry 26(11): 6237–6252.

Lutfy K, Eitan S, Bryant CD, Yang YC, Saliminejad N, Walwyn W, Kieffer BL, Takeshima H, Carroll FI, Maidment NT and Evans CJ (2003) Buprenorphine-induced antinociception is mediated by μ-opioid receptors and compromised by concomitant activation of opioid receptor-like receptors. Journal of Neuroscience 23: 10331–10337.

Ly C, Greb AC, Cameron LP, Wong JM, Barragan EV, Wilson PC,… and Olson DE (2018) Psychedelics promote structural and functional neural plasticity. Cell Reports 23(11): 3170–3182.

Mach RH, Smith CR and Childers SR (1995) Ibogaine possesses a selective affinity for σ2 receptors. Life Sciences 57(4): 57–62.

Maillet EL, Milon N, Heghinian MD, Fishback J, Schürer SC, Garamszegi N, Mash DC (2015) Noribogaine is a G-protein biased κ-opioid receptor agonist. Neuropharmacology 99: 675–688.

Maisonneuve IM, Keller RW and Glick SD (1991) Interactions between ibogaine, a potential anti-addictive agent, and morphine: An in vivo microdialysis study. European Journal of Pharmacology 199: 35–42.

Malcolm BJ, Polanco M, Barsuglia JP (2018) Changes in Withdrawal and Craving Scores in Participants Undergoing Opioid Detoxification Utilizing Ibogaine. Journal of psychoactive drugs, 50(3): 256–265.

Mansvelder HD and McGehee DS (2002) Cellular and synaptic mechanisms of nicotine addiction. Journal of Neurobiology 53: 606–617.

Martins MLF, Heydari P, Li W, Martínez-Chávez A, Venekamp N, Lebre MC,... and Schinkel AH (2022) Drug transporters ABCB1 (P-gp) and OATP, but not drug-metabolizing enzyme CYP3A4, affect the pharmacokinetics of the psychoactive alkaloid ibogaine and its metabolites. Frontiers in Pharmacology 13: 855000.

Marton S, González B, Rodríguez-Bottero S, Miquel E, Martínez-Palma L, Pazos M,... and Carrera I (2019) Ibogaine administration modifies GDNF and BDNF expression in brain regions involved in mesocorticolimbic and nigral dopaminergic circuits. Frontiers in Pharmacology 10: 193.

Mash DC, Kovera CA, Pablo J, Tyndale R, Ervin FR, Kamlet JD and Hearn WL (2001) Ibogaine in the treatment of heroin withdrawal. Alkaloids: Chemistry and Biology 56, 155–171.

Mash DC, Duque L, Page B and Allen-Ferdinand K (2018) Ibogaine detoxification transitions opioid and cocaine abusers between dependence and abstinence: clinical observations and treatment outcomes. Frontiers in Pharmacology 9: 529.

Matsumoto RR (2009) Targeting sigma receptors: novel medication development for drug abuse and addiction. Expert Review in Clinical Pharmacology 2(4): 351–358.

Matsumoto RR, Liu Y, Lerner M, Howard EW and Brackett DJ (2003) Sigma receptors: potential medications development target for anti-cocaine agents. European Journal of Pharmacology 469: 1–12.

Matsumoto RR, McCracken KA, Pouw B, Zhang Y and Bowen WD (2002) Involvement of sigma receptors in the behavioral effects of cocaine: evidence from novel ligands and antisense oligodeoxynucleotides. Neuropharmacology 42(8): 1043–1055.

Matsumoto RR, Shaikh J, Wilson LL, Vedam S and Coop A (2008) Attenuation of methamphetamine-induced effects through the antagonism of sigma (sigma) receptors: evidence from in vivo and in vitro studies. European Neuropsychopharmacology 18(12): 871–881.

Mercer SL and Coop A (2011) Opioid analgesics and P-glycoprotein efflux transporters: A potential systems-level contribution to analgesic tolerance. Current Topics in Medicinal Chemistry 11(9): 1157–1164.

Muldoon PP, Jackson KJ, Perez E, Harenza JL, Molas S, Rais B,… and Damaj MI (2014) The α3β4* nicotinic ACh receptor subtype mediates physical dependence to morphine: mouse and human studies. British Journal of Pharmacology 171(16): 3845–3857.

Nguyen EC, McCracken KA, Pouw B and Matsumoto RR (2005) Involvement of sigma receptors in the actions of methamphetamine: receptor binding and behavioral studies. Neuropharmacology 49: 638–645.

Niwa M, Nitta A, Yamada K and Nabeshima T (2007) The roles of glial cell line-derived neurotrophic factor, tumor necrosis factor-alpha, and an inducer of these factors in drug dependence. Journal of Pharmacological Science 104: 116–121.

Noller GE, Frampton CM, Yazar-Klosinski B (2018) Ibogaine treatment outcomes for opioid dependence from a twelve-month follow-up observational study. The American Journal of Drug and Alcohol Abuse 44(1): 37–46.

Ona G, Sampedro F, Romero S, Valle M, Camacho V, Migliorelli C, Mañanas MÁ, Antonijoan RM, Puntes M, Coimbra J, Ballester MR, Garrido M, Riba J (2022) The Kappa Opioid Receptor and the Sleep of Reason: Cortico-Subcortical Imbalance Following Salvinorin-A. The International Journal of Neuropsychopharmacology 25(1): 54–63.

Ona G, Rocha JM, Bouso JC, Hallak JEC, Borràs T, Colomina MT, dos Santos RG (2021) The adverse events of ibogaine in humans: an updated systematic review of the literature (2015–2020). Psychopharmacology (Berl) 239(6): 1977-1987.

Ona G and Bouso JC (2021) Towards the use of whole natural products in psychedelic research and therapy: Synergy, multi-target profiles, and beyond. Frontiers in Natural Product Chemistry 8: 99–145.

Pablo JP and Mash DC (1998) Noribogaine stimulates naloxone-sensitive [35S]GTPgammaS binding. Neuroreport 9: 109–114.

Pearl SM, Maisonneuve IM and Glick SD (1996) Prior morphine exposure enhances ibogaine antagonism of morphine-induced dopamine release in rats. Neuropharmacol 1996;35:1779-1784.

Pike JA, Krumm JM (1954) Roadblocks to Faith. New York: Morehouse-Gorham Co. p. 110.

Pochon NA-M, Menoud A, Tseng JL, Zurn AD and Aebischer P (1997) Neuronal GDNF expression in the adult rat nervous system identified by in situ hybridization. European Journal of Neuroscience 9: 463–471.

Popik P (1996) Facilitation of memory retrieval by the “anti-addictive” alkaloid ibogaine. Life Sciences 59(24): 379–385.

Popik P, Layer RT, Skolnick P (1995) 100 years of ibogaine: neurochemical and pharmacological actions of a putative anti-addictive drug. Pharmacology Reviews 47(2): 235–253.

Popik P, Layer RT and Skolnick P (1994) The putative anti-addictive drug ibogaine is a competitive inhibitor of [–3H]MK-801 binding to the NMDA receptor complex. Psychopharmacology 114(4): 672–674.

Prior PL and Prior SL (2014) Ibogaine effect on cocaine craving and use in dependent patients - A double-blind, placebo-controlled pilot study. Jacobs Journal of Addiction and Therapy 1(1): 003.

Quadir SG, Cottone P and Sabino V (2019) Role of sigma receptors in alcohol addiction. Frontiers in Pharmacology 10: 687.

Ray TS (2010) Psychedelics and the human receptorome. PLoS ONE 5(2): e9019.

Reid MS, Hsu K, Souza KH, Broderick PS and Berger SP (1996) Neuropharmacological characterization of local ibogaine effects on dopamine release Journal of Neural Transmission 103: 967–985.

Repke DB, Artis DR, Nelson JT and Wong EHF (1994) Abbreviated Ibogaine Congeners. Synthesis and Reactions of Tropan-3-yl-2- and -3-indoles. Investigation of an Unusual Isomerization of 2-Substituted Indoles Using Computational and Spectroscopic Techniques. Journal of Organic Chemistry 59: 2164.

Rezayof A, Nazari-Serenjeh F, Zarrindast MR, Sepehri H and Delphi L (2007) Morphine-induced place preference: involvement of cholinergic receptors of the ventral tegmental area. European Journal of Pharmacology 562: 92–102.

Rodriguez-Cano B, Kohek M, Ona G, Alcázar-Córcoles MA, dos Santos RG, Hallak JEC and Bouso JC (2022) Underground ibogaine use for the treatment of substance use disorders: A qualitative analysis of subjective experiences. Drug and Alcohol Review. doi: 10.1111/dar.13587

Rodriguez P, Urbanavicius J, Prieto JP, Fabius S, Reyes AL, Havel V,... Carrera I (2020) A single administration of the atypical psychedelic ibogaine or its metabolite noribogaine induces an antidepressant-like effect in rats. ACS Chemical Neuroscience 11(11): 1661–1672.

Rodriguez M, Ortega I, Soengas I, Suarez E, Lukas JC and Calvo R (2004) Effect of P-glycoprotein inhibition on methadone analgesia and brain distribution in the rat. Journal of Pharmacy and Pharmacology 56: 367–374.

Roth BL, Baner K, Westkaemper R, Siebert D, Rice KC, Steinberg S, Ernsberger P, Rothman RB (2002) Salvinorin A: a potent naturally occurring nonnitrogenous kappa opioid selective agonist. Proc Natl Acad Sci U S A 99: 11934–11939.

Rousseaux CG and Greene SF (2016) Sigma receptors [σRs]: biology in normal and diseased states. Journal of Receptor Signal and Transduction 36: 4327–4388.

Sambo DO, Lebowitz JJ and Khoshbouei H (2018) The sigma-1 receptor as a regulator of dopamine neurotransmission: A potential therapeutic target for methamphetamine addiction. Pharmacology and Therapeutics 186: 152–167.

Schenberg EE, de Castro Comis MA, Alexandre JFM, Tófoli LF, Chaves BDR, da Silveira DX (2017) A phenomenological analysis of the subjective experience elicited by ibogaine in the context of a drug dependence treatment, Journal of Psychedelic Studies 1(2): 74-83.

Shelton KL and Balster RL (1997) Effects of gamma-aminobutyric acid agonists and N-methyl-D-aspartate antagonists on a multiple schedule of ethanol and saccharin self-administration in rats. Journal of Pharmacology and Experimental Therapeutics 280: 1250–1260.

Shi M, Wesseling S, Bouwmeester H and Rietjens IMCM (2021) A new approach methodology (NAM) for the prediction of (nor)ibogaine-induced cardiotoxicity in humans. ALTEX 38(4): 636–652.

Staley JK, Ouyang Q, Pablo J, Hearn WL, Flynn DD, Rothman RB, Rice KC and Mash Dc (1996) Pharmacological screen for activities of 12-hydroxyibogamine: a primary metabolite of the indole alkaloid ibogaine. Psychopharmacology (Berl) 127(1): 10–18.

Stein C (2016) Opioid receptors. Annual Review of Medicine 67: 433–451.

Sunder Sharma S and Bhargava HN (1998) Enhancement of morphine antinociception by ibogaine and noribogaine in morphine-tolerant mice. Pharmacology 57: 229–232.

Sweetnam PM, Lancaster J, Snowman A, Collins JL, Perschke S, Bauer C and Ferkany J (1995) Receptor binding profile suggests multiple mechanisms of action are responsible for ibogaine's putative anti-addictive activity. Psychopharmacology (Berl) 118(4): 369–376.

Szumlinski KK, Maisonneuve IM and Glick SD (2000) Differential effects of ibogaine on behavioural and dopamine sensitization to cocaine. European Journal of Pharmacology 398: 259–262.

Taraschenko OD, Panchal V, Maisonneuve IM and Glick SD (2005) Is antagonism of alpha3beta4 nicotinic receptors a strategy to reduce morphine dependence? European Journal of Pharmacology 513(3): 207–218.

Tejeda HA and Bonci A (2019) Dynorphin/kappa-opioid receptor control of dopamine dynamics: Implications for negative affective states and psychiatric disorders. Brain Research 1713: 91–101.

Thomsen M, Conn PJ, Lindsley C, Wess J, Boon JY, Fulton BS and Caine SB (2010) Attenuation of cocaine's reinforcing and discriminative stimulus effects via muscarinic M1 acetylcholine receptor stimulation Journal of Pharmacology and Experimental Therapeutics 332: 959–969.

Toll L, Berzetei-Gurske IP, Polgar WE, Brandt SR, Adapa ID, Rodriguez L,... and Auh JS (1998) Standard binding and functional assays related to medications development division testing for potential cocaine and opiate narcotic treatment medications. NIDA Research Monograph 178: 440–466.

Tournier N, Chevillard L, Megarbane B, Pirnay S, Scherrmann JM and Declèves X (2010) Interaction of drugs of abuse and maintenance treatments with human P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2). International Journal of Neuropsychopharmacology 13(7): 905–915.

Vargas-Perez H, Kee RT, Walton CH, Hansen DM, Razavi R, Clarke L,… and van der Kooy D (2009) Ventral tegmental area BDNF induces an opiate-dependent-like reward state in naive rats. Science 324: 1732–1734.

Vastag B (2005) Addiction research. Ibogaine therapy: a 'vast, uncontrolled experiment'. Science 308(5720): 345–346.

Vezina P and Stewart J (1984) Conditioning and place-specific sensitization of increases in activity induced by morphine in the VTA. Pharmacology, Biochemistry, and Behavior 20: 925–934.

Vilner BJ, Bandarage UK, Kuehne ME, Bertha CM and Bowen WD (1998) The neurotoxic effect of iboga alkaloids may be mediated by sigma-2 receptors. NIDA Research Monograph 178: 235.

Wang HL, Xiang XH, Guo Y, Wu WR, Cao DY, Wang HS and Zhao Y (2005) Ionotropic glutamatergic neurotransmission in the ventral tegmental area modulates deltaFosB expression in the nucleus accumbens and abstinence syndrome in morphine withdrawal rats. European Journal of Pharmacology 527: 94–104.

Wang JS, Ruan Y, Taylor RM, Donovan JL, Markowitz JS and DeVane CL (2004) Brain penetration of methadone (R)- and (S)-enantiomers is greatly increased by P-glycoprotein deficiency in the blood-brain barrier of Abcb1a gene knockout mice. Psychopharmacology 173: 132–138.

Wang KX, Gao Y, Lu C, Li Y, Zhou B, Qin X, Du G, Gao L, Guan D and Lu A (2020) Uncovering the Complexity Mechanism of Different Formulas Treatment for Rheumatoid Arthritis Based on a Novel Network Pharmacology Model. Frontiers in Pharmacology 11: 1035.

Wells GB, Lopez MC and Tanaka JC (1999) The effects of ibogaine on dopamine and serotonin transport in rat brain synaptosomes. Brain Research Bulletin 48(6): 641–647.

Xi ZX and Stein EA (2002) Blockade of ionotropic glutamatergic transmission in the ventral tegmental area reduces heroin reinforcement in rat. Psychopharmacology (Berl) 164: 144–150.

Xue DJ, Zhen Z, Wang KX, Zhao J, Gao Y, Chen Y,... and Huang T (2022) Uncovering the potential mechanism of Xue Fu Zhu Yu Decoction in the treatment of intracerebral hemorrhage. BMC Complementary Medicine and Therapies 22(1): 103.

Zahniser NR and Sorkin A (2004) Rapid regulation of the dopamine transporter: role in stimulant addiction? Neuropharmacology 47(1): 80–91.

Zhu H and Ho IK (1998) NMDA-R1 antisense oligonucleotide attenuates withdrawal signs from morphine. European Journal of Pharmacology 352: 151–156.

Publication 4

A transcriptomic analysis in mice following a single dose of ibogaine identifies new potential therapeutic targets

Judit Brull, Genís Ona, Lineth Alarcón-Franco, Maria Teresa Colomina

Translational Psychiatry, 2023; 14(1):41

https://doi.org/10.1038/s41398-024-02773-7

Study 4 overview:

What do we already know?

The action of ibogaine has been extensively investigated in preclinical models. However, no definitive conclusions have been drawn regarding the mechanisms of action at work when inducing anti-addictive effects.

What does this study add?

This study explores, by a transcriptomic analysis, short term changes in the gene expression in a core area for addiction and withdrawal symptomatology. We identified new targets that might be significant in the studuy of mechanisms of action of ibogaine and its potential as a terapheutic agent. Through this contemporary technique –transcriptomic analysis– we are able to identify a broad landscape of genes and pathways affected by ibogaine, instead of focusing on specific molecules. This is the first time that omics are used in the study of ibogaine, and thus provides an innovative approach highly needed in the field.

Highlights

Significant changes in gene expression in the mouse frontal cortex were observed.

Genes related to hormonal pathways and synaptogenesis exhibited increased expression levels following ibogaine.

The identification of gender differences underscores the importance of considering gender as a potential factor influencing IBO's effects, adding a new layer of understanding.

This study represents a noteworthy advancement in unraveling the molecular actions of ibogaine, shedding light on its influence on gene expression patterns.

Translational Psychiatry www.nature.com/tp

ARTICLE OPEN

Check for updates

A transcriptomic analysis in mice following a single dose of ibogaine identifies new potential therapeutic targets

Judit Biosca-Brull[1,2,3], Genis Ona[4,5], Lineth Alarcón-Franco[1,6] and Maria Teresa Colomina[1,2,3] ✉

Ibogaine (IBO) is an atypical psychedelic with a complex mechanism of action. To date, the mechanisms that may underlie its anti-addictive effects are still not defined. This study aims to identify changes in gene expression induced by a single oral dose of IBO in the cortex of mice by means of a transcriptomic analysis for the first time. Our results showed significant alterations in gene expression in mouse frontal cortex samples 4 h after a single oral dose of IBO. Specifically, genes involved in hormonal pathways and synaptogenesis exhibited upregulation, while genes associated with apoptotic processes and endosomal transports showed downregulation. The findings were further corroborated through quantitative polymerase chain reaction (qPCR) analysis. However, the validation of gene expression related to hormonal pathways did not entirely align with the transcriptomic analysis results, possibly due to the brain region from which tissue was collected. Sex differences were observed, with female mice displaying more pronounced alterations in gene expression after IBO treatment. High variability was observed across individual animals. However, this study represents a significant advancement in comprehending IBO's molecular actions. The findings highlight the influence of IBO on gene expression, particularly on hormonal pathways, synaptogenesis, apoptotic processes, and endosomal transports. The identification of sex differences underscores the importance of considering sex as a potential factor influencing IBO's effects. Further research to assess different time points after IBO exposure is warranted.

Translational Psychiatry (2024) 14:41 ; https://doi.org/10.1038/s41398-024-02773-7

INTRODUCTION

Substance use disorders (SUDs) constitute one of the biggest challenges for treatment and recovery. It is estimated that about 0.5 million annual deaths worldwide are attributed to drug use [1]. Apart from fatal outcomes, several health problems related to SUDs can be observed over the short- and long-term, including intoxication, misuse, heart disease, depression, and more [2]. Treating SUDs generally has poor adherence and high rates of relapse [3, 4] which makes innovative approaches highly necessary.

Ibogaine (IBO) is an alkaloid naturally found in the root bark of some plants belonging to the Apocynaceae family, including *Tabernanthe iboga*. For decades, users and activists have claimed that IBO has remarkable anti-addictive properties [5]. However, research on this substance is scarce which is most likely due to its undesired effects, such as hallucinogenic effects [6] and cardiovascular toxicity [7]. There are no published trials supporting these claims, although the first Phase II trial was launched in 2020 by our group [NCT04003948], and the final results will be published soon.

The available evidence suggesting IBO is an efficacious treatment for SUDs is mainly based on preclinical and observational/open-label research. Various studies have shown that IBO decreases morphine, cocaine, alcohol, and nicotine self-administration (SA) in rats [8]. Three studies reported no reductions in conditioned place preference (CPP) with IBO when rats were trained in CPP using amphetamine and morphine [9–11]. It's important to note that the CPP paradigm is typically utilized to evaluate Pavlovian conditioning, which involves automatic and involuntary responses. In contrast, SA tests encompass both Pavlovian and operant conditioning. The latter involves voluntary behaviors, and therefore, data from studies utilizing the SA paradigm are more translatable. In addition, it has been observed that IBO reduces naloxone-precipitated opioid withdrawal in rats [12–16]. Two reports [17, 18] indicate that naloxone-precipitated opioid withdrawal in rats remained unaffected by IBO, possibly due to the specific route of administration employed (subcutaneous). These findings suggest that the effects of IBO may have a central role influenced by first-pass metabolism. Indeed, O-demethylation through cytochrome P4502D6 (CYP2D6) converts IBO to noribogaine (NOR), its main metabolite, which has a higher volume of distribution and a longer half-life than the parent drug [19].

Observational research involving administering IBO to people with SUDs has shown promising results. For instance, Davis et al. [20] recruited a sample of people who underwent past IBO treatment and reported that 80% of them noted a drastic

[1]Universitat Rovira i Virgili, Research Group in Neurobehavior and Health (NEUROLAB), Tarragona, Spain. [2]Universitat Rovira i Virgili, Department of Psychology and Research Center for Behavior Assessment (CRAMC), Tarragona, Spain. [3]Universitat Rovira i Virgili, Center of Environmental, Food and Toxicological Technology (TECNATOX), Reus, Spain. [4]ICEERS–International Center for Ethnobotanical Education, Research, and Services, Barcelona, Spain. [5]Universitat Rovira i Virgili, Department of Anthropology, Philosophy and Social Work, Tarragona, Spain. [6]Grupo de Investigación Infetarre, Facultad de Medicina, Universidad Cooperativa de Colombia, Medellín, Colombia.
✉email: mariateresa.colomina@urv.cat

Received: 7 August 2023 Revised: 8 January 2024 Accepted: 10 January 2024
Published online: 19 January 2024

reduction in withdrawal symptoms. Fifty percent (50%) felt a reduction in opioid cravings and 30% did not use opioids again after the treatment. Other studies reported similar findings [21-24].

At least three open-label studies using IBO have been published [25-27]. Two of them highlighted enthusiastic results [26, 27]. However, they consist of a non-peer-reviewed chapter [27] and a commentary [26] mentioning non-published data with no specific details on methodology or reported outcomes. In addition, another publication by the same authors [28] references the commentary and claims there was no drug-related clinically relevant QT prolongation (which is data that is not reported anywhere). The researchers stated that the study population consisted of 191 patients, while the commentary reports 257 people. Due to these inconsistencies and the lack of published data, these results must be interpreted with caution. In fact, the third open-label trial [25] did not report such favorable results: 50% of patients reported QTcs above 500 ms and severe ataxia after IBO doses of 10 mg/kg.

The mechanisms through which IBO may exert its putative anti-addictive effects are still not fully understood. An early review summarized all the targets both IBO and NOR interact with [29]. However, more recent studies have suggested other potential targets/mechanisms [30-32], as well as sex-specific effects of IBO [33] which were hinted at in earlier preclinical studies [34]. A recent review collected all the available literature regarding the main targets of IBO/NOR in relation to the suggested benefits of SUD treatment [35]. These include affinity for both μ and κ opioid receptors, serotonin (SERT) and dopamine (DAT) transporters, N-methyl-D-aspartate (NMDA) and α3β4 nicotinic acetylcholine receptors, as well as increases in glial-derived (GDNF) and brain-derived (BDNF) neurotrophic factors, among others.

Due to the complex pattern of multi-target action, the scientific field would benefit from recently developed methodologies that provide a better understanding of the mechanisms of action of drugs, such as omics techniques. The entire molecular landscape affected by drugs can be revealed through these comprehensive techniques, instead of focusing on certain targets or receptors belonging to the G protein-coupled receptor (GPCR) family. This may identify previously unknown molecular players involved in drug response. For instance, some authors in the psychedelic research field claimed that the therapeutic effect of these substances can be attributable to modifications in the endocrine system [36]. This is because the hypothalamus possesses a notable concentration of 5-HT2A receptors, alongside other receptors implicated in the intricate workings of psychedelic substances. The administration of these drugs is correlated with the release of oxytocin and various other neuropeptides [37, 38] possibly modulating crucial aspects involved in psychopathology such as social cognition [39]. These potentially related—and still unexplored—mechanisms can be elucidated by exploring molecular changes in cells or tissues.

To date, few studies have introduced omics to the study of psychedelic drugs [40-44]. There are no published studies using these techniques with IBO. The aim of this study is to analyze the effects of a single IBO administration on gene expression in mice using transcriptomic analysis.

MATERIALS AND METHODS

Animals

Twelve eight-week-old C57BL/6J mice (six males and six females) were obtained from Charles River Laboratories (Barcelona, Spain). After one week of quarantine, male and female mice were assigned to the control (CNT) group or the IBO-treated group by simple randomization. The total number of animals for each group was 6, with three males and three females. Animals were maintained in a 12 h light/dark automatic cycle (the lights were on between 8 a.m. and 8 p.m.) with a controlled temperature (22 ± 2 °C) and humidity (50 ± 10%). Food (SAFE® A04 diet, Panlab, Barcelona, Spain) and water were administered *ad libitum*. All the experiments of this study were conducted in compliance with the Spanish Royal Decree 53/2013 on the protection of animals used in experiments and the European Communities Council Directive (86/609/EC) and were approved by the Animal Care and Use Committee at the Rovira i Virgili University (Catalonia, Spain).

Treatment and experimental design

Young mice were exposed to 60 mg/kg of IBO (12-Methoxyibogamine) provided by the International Center for Ethnobotanical Education, Research and Service (ICEERS) (Barcelona, Spain). The reported purity of IBO was 98.4% (±0.3%) as analyzed through Nuclear Magnetic Resonance (NMR) by Eurecat (Reus, Spain). IBO was dissolved in distilled water (the vehicle) and adjusted to administer the desired dose in 10 μL/g of body weight by gavage. The control group received the vehicle. The animals were exposed orally to mimic the administration of IBO in capsules in humans. In addition, the dose administered was between 40-80 mg/kg which is considered a medium dose and corresponds to the most frequent doses used in humans (10-25 mg/kg) corrected for body surface area [8]. In accordance with the results reported by Kubiliene et al. [45], the control and IBO-treated groups were euthanized by cervical dislocation 4 h after the oral administration, since this is the time when the peak concentration of IBO is observed in the brain. Brain samples were immediately removed, snap-frozen in liquid nitrogen, and stored at −80 °C until transcriptomic and gene expression analysis was done.

Transcriptomic analysis

Frontal cortex tissue was selected to study changes in gene expression due to its involvement in different aspects related to drug dependence, such as reinforcement response to drugs during intoxication, activation during craving, and deactivation during withdrawal, as well as a generalized dysfunction in drug-dependent individuals [46]. Samples were sent to the Center for Omic Science (COS) (Reus, Spain) for RNA sequencing. RNA was extracted using the Purelink RNA mini kit from Invitrogen (Walthman, MA, USA) and quantified by a Qubit 2.0 Fluorometer (ThermoFisher Scientifics, Waltham, MA, USA). Then, the quality of the RNA was assessed using the Agilent TapeStation team and the Agilent RNA ScreeTape Assay (Agilent, Santa Clara, CA, USA). The sequencing libraries were created from 0.75 μg of RNA samples using the Illumina Stranded mRNA Prep (Illumina, San Diego, CA, USA) and quantified by microfluidic electrophoresis using Agilent's TapeStation equipment and the Agilent DNA High Sensitivity ScreenTape kit (Agilent, Santa Clara, CA, USA). The length and concentration were determined in each sample. Finally, pools with a concentration of 750 pM were created. These pool sequencing libraries were done using NextSeq200 equipment from Illumina (San Diego, CA, USA).

The obtained gene database was screened for outliers which were then eliminated. Table 1 shows the total number of animals used.

Gene expression analysis

The complementary RNA (cDNA) from frontal cortex tissues was synthesized from 1 mg of RNA samples using a Maxima First Strand cDNA Kit for RT-qPCR (ThermoFisher Scientific, Waltham, MA, USA). Then we performed the real-time polymerase chain reaction (qPCR) analysis with the Maxima SYBR Green/ROX qPCR Master Mix (2X) Kit (ThermoFisher Scientific, Waltham, MA, USA) and the Rotor-Gene Q Real-time Q cycler (Qiagen Inc., Hilden, Germany) to evaluate the gene expression of oxytocin (*Oxt*), arginine vasopressin (*Avp*), cerebellin 4 (*Cbln4*) and 2 (*Cbln2*) precursors and ubiquitin-specific peptidase 35 (*Usp35*). Duplicates of each RNA sample were included in the qPCR. We used the Rotor-Gene Q Real-Time PCR 2.0

Table 1. Animals used in this study.

Treatment	CNT		IBO	
Type of analysis	Omics	Gene expression	Omics	Gene expression
Males	3	3	3	3*
Females	2	3	3	3*

The asterisk indicates that one sample in *Oxt* and *Avp* genes was excluded because of expression values are more than 200.

Table 2. DESeq2 results of differentially expressed genes comparing CNT and IBO-treated groups.

Gene	Log2FC	*p* adj.	Effect size Cohen's d	Effect size
Oxt	5.2268	0.0162	0.8050	Large
Avp	4.7158	0.0448	0.9300	Large
Cbln4	0.7727	0.0162	0.7880	Medium
Cbln2	0.3975	0.0010	0.4020	Small
Usp35	-0.2846	0.0224	0.7070	Medium
Ap5b1	-0.3880	0.0010	0.8140	Large
Gm34306	-2.8117	0.0356	2.2880	Large

Green log2FC indicates significant upregulated genes, while red log2FC indicates significant downregulated genes at *p* adj. < 0.05. Orange and yellow effect sizes indicate large and medium effects, respectively, whereas no color indicates a small effect according to Cohen's *d*.
FC fold change.

software (Qiagen Inc., Hilden, Germany) to calculate the cycle threshold (Ct). Each sample was normalized to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) (ΔCt) and standardized to the male control group (ΔΔCt) average to assess the relative gene expression levels in accordance with the 2-ΔΔCt method [47]. The primer sequences used for qPCR were as follows: *Oxt* (forward: 5′-TGGCTTACTGGCTCTGACCT-3′; reverse: 5′-GGCAGGTAGTTCTCCTCCTG-3′) [48], *Avp* (forward: 5′-CAGGATGCTCAACACTACGC-3′; reverse: 5′-CAGAATCCACGGACTCCCG-3′) [48], *Cbln4* (forward: 5′-GCACCGAGGAAAGGAATCTA-3′; reverse: 5′-TGCAGAGATGACTGGTTTTCC-3′) [49], *Cbln2* (forward: 5′-TGACCCTCAGATGGATTGCAC-3′; reverse: 5′-CTGCTGGGCTCTTGCTTTAAGC-3′) [50], *Usp35* (forward: 5′-TGCCATTAGCAGGATGATTGA-3′; reverse: 5′-AGCGAAACCTCGATCAAGATG-3′) [51] and the reference gene *Gapdh* (forward: 5′-ACAACTTTGGCATTGTGGAA-3′; reverse: 5′-AGCGAAACCTCGATCAAGATG-3′) [52].

Statistical analysis

The sample size was calculated according to pharmacokinetic studies [45]. The data obtained was mapped against a reference genome using the alignment program HISAT2 2.2.1, while the annotation and quantification of the aligned reads were performed using StringTie 2.1.4. To investigate the changes in gene expression profiles induced by IBO treatment, we utilized R 4.3.0 and its specific package DESeq 1.40.1 to calculate the fold change (FC) values of each gene relative to the CNT group. Statistical analysis excluded genes that have less than five counts in each treated sample. The threshold for identifying significant differences was set at *p* adj. < 0.05. In addition, we calculated the effect size using Cohen's *d*. Values above 0.8 indicate a large effect, values between 0.50 and 0.79 indicate a medium effect and values between 0.21 and 0.49 indicate small effects, while values below 0.20 indicate no effect [53].

Gene expression analysis was performed using SPSS 28.0 software (IBM Corp. Chicago, IL, USA). The homogeneity of variance was evaluated by the Levene test. A two-way analysis of variance (ANOVA) was conducted to assess significant differences between sex or treatment and their interactions. All the data are presented as the mean ± S.E.M, and statistical significance was set at $p < 0.05$.

RESULTS

Screening for gene expression alterations

The first analysis compared the CNT and IBO-treated groups, regardless of sex. Table 2 demonstrates that in the total number of evaluated genes, seven were differentially expressed. Specifically, four genes showed a significant increase in expression after IBO administration (oxytocin (*Oxt*), vasopressin (*Avp*), cerebellin (*Cbln*) 2 and 4 precursors). Ubiquitin-specific peptidase 35 adaptor (*Usp35*), adaptor-related protein complex 5, beta 1 subunit (*Ap5b1*), and the predicted gene *Gm34306* showed a significant decrease (Table 2). Cohen's d showed a large effect in all significant genes, except for *Cbln4* and *Usp35* which showed a medium effect, and *Cbln2* with a small effect.

The differences in gene expression changed when we evaluated males and females separately. In males, eight of the total number of evaluated genes were differentially expressed (Table 3), whereas there were 28 genes that showed expression alterations in females (Table 4). Male mice treated with IBO presented an upregulation of *Gm51898*, *Cbln4*, and interleukin 1 (IL1) receptor antagonists (*Il1rn*). Conversely, two predicted genes (*Gm36884* and *Gm6334)* were downregulated, as well as phospholipase A2 (*Pla2g4b*), and one of their inhibitors (*Pinlyp*) (Table 3). All the genes showed Cohen's *d* values greater than 0.8, indicating a large effect.

Out of the 28 genes analyzed in female mice, 18 were found to be upregulated and 10 were downregulated. In particular, female mice treated with IBO showed an increase in the expression of neuronal pentraxin 2 (*Nptx2*), gamma-aminobutyric acid receptor-associated protein (*Gabarap11*), tumor necrosis factor receptor superfamily member 25 (*Tnfrsf25*), cyclin-dependent kinase inhibitor 1 A (*Cdkn1a*), pleckstrin homology like domain family A member 1 (*Phlda1*), early growth response 4 (*Egr4*), solute carrier family 25 member 25 (*Slc25a25*), mitochondrial calcium uniporter (*Mcu*), small integral membrane protein 3 (Smim3), SLAM family member 7 (*Slamf7*), dual specificity phosphatase 5 (*Dusp5*), DNAJ heat shock protein family (*Hsp40*) member C21 (*Dnajc21*), and R-spondin1 (*Rspo1*). Other genes such as tumor suppressor coiled-coil domain containing 154 (*Ccdc154*), the dehydrogenase/reductase 2 (*Dhrs2*), SH2 domain containing 1B1 (*Sh2d1b1*), eomesodermin (*Eomes*), CD19 antigen (*Cd19*) and adenomatous polyposis coli (*Apcdd1*) were decreased. In addition, we found uncharacterized and predicted genes that were also differentially expressed in IBO-treated females. The genes that showed over-expression were *LOC118567915, 4930447N08Rik, Gm44505, Gm30298*, and *Gm41448. Gm39659, Gm40399, Gm32029*, and *Gm6937* were downregulated (Table 4). Cohen's *d* values were greater than 0.8 in all significant genes, except for *Dhrs2* and *Apcdd1* which showed medium and small effects, respectively.

Validation of gene expression alterations

Based on the observed alterations in gene expression after 4 h of IBO administration, we aimed to validate the obtained results by comparing the CNT and IBO-treated groups using qPCR. For this purpose, we assessed the gene expression listed in Table 2, excluding Ap5b1 and the predicted gene Gm4306.

A two-way ANOVA (sex and treatment) analysis of the variance showed significant effects of sex in both *Cbln4* and *Cbln2* genes (*Cbln4* [$F_{1,11} = 15.777$, $p = 0.004$] and *Cbln2* [$F_{1,11} = 8.904$, $p = 0.017$]). Females showed less expression compared to males in both cases (Fig. 1C, D). Treatment effects were only observed on

Table 3. DESeq2 results of differentially expressed genes comparing male CNT and IBO-treated groups.

Gene	Log2FC	*p* adj.	Effect size Cohen's d	Effect size
Gm51898	6.1708	0.0312	3.3490	Large
Il1rn	6.1087	0.0312	1.6260	Large
Cbln4	0.8880	0.0276	0.8200	Large
Ucn	-1.5304	0.0411	1.5280	Large
Gm36884	-5.8499	0.0276	2.4950	Large
Pinlyp	-6.3404	0.0276	3.2170	Large
Gm6334	-7.3776	0.0005	1.6940	Large
Pla2g4b	-21.5209	0.0005	0.8170	Large

Green log2FC indicates significant upregulated genes, while red log2FC indicates significant downregulated genes at *p* adj. < 0.05. Orange effect size indicates a large effect according to Cohen's *d*.
FC fold change.

Table 4. DESeq2 results of differentially expressed genes comparing female CNT and IBO-treated groups.

Gene	Log2FC	*p* adj.	Effect size Cohen's d	Effect size
Gm44505	20.9289	0.0045	0.8700	Large
4930447N08Rik	7.2089	0.0032	6.1250	Large
Slamf7	6.2804	0.0309	3.9110	Large
Gm30298	2.8380	0.0028	5.0850	Large
LOC118567915	1.2873	0.0411	3.4960	Large
Gm41448	0.9969	0.0060	3.301	Large
Tnfrsf25	0.7807	0.0003	1.8210	Large
Dusp5	0.6973	0.0450	1.6730	Large
Egr4	0.6847	0.0208	2.0400	Large
Rspo1	0.5732	0.0465	2.0110	Large
Cdkn1a	0.4510	0.0317	1.5900	Large
Nptx2	0.4110	1.0072	1.7000	Large
Dnajc21	0.3816	0.0001	1.4340	Large
Smim3	0.3381	0.0110	1.3790	Large
Phlda1	0.2786	0.0407	1.3890	Large
Slc25a25	0.2269	0.0208	1.2000	Large
Mcu	0.1948	0.0208	1.1120	Large
Gabarapl1	0.0971	0.0001	0.8180	Large
Apcdd1	-0.2524	0.0016	0.1080	No effect
Eomes	-2.1563	0.0001	7.8390	Large
Sh2d1b1	-5.6031	0.0250	4.0010	Large
Gm39659	-5.6644	0.0407	2.4280	Large
Gm40399	-5.7609	0.0208	3.000	Large
Ccdc154	-5.8608	0.0110	6.3340	Large
Cd19	-6.0236	0.0233	2.2000	Large
Gm32029	-6.2098	0.0131	2.2730	Large
Dhrs2	-6.3902	0.0034	0.6210	Medium
Gm6937	-8.3869	9.0167	5.0000	Large

Green log2FC indicates significant upregulated genes, while red log2FC indicates significant downregulated genes at *p* adj. < 0.05. Orange and yellow effect sizes indicate large and medium effect, respectively, whereas no color indicates no effect according to Cohen's *d*.
FC fold change.

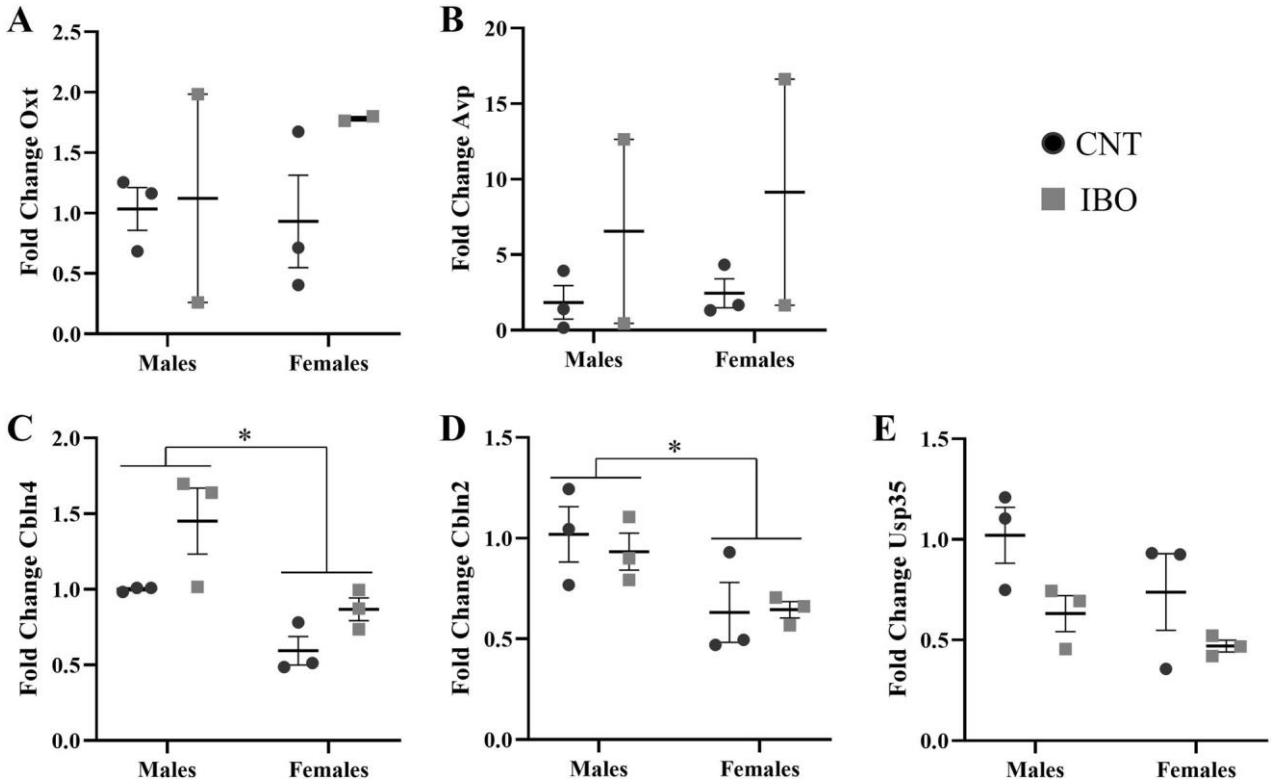


Fig. 1 Frontal cortex gene expression determined by qPCR. *Oxt* (A), *Avp* (B), *Cbln4* (C), *Cbln2* (D) and *Usp35* (E). The symbol * indicates differences between sexes at $p < 0.05$.

two genes: *Cbln4* ([$F_{1,11} = 8.483$, $p = 0.020$]) (Fig. 2A) showed an upregulation in IBO-treated subjects and *Usp35* ([$F_{1,11} = 6.698$, $p = 0.032$]) (Fig. 2B) showed a downregulation in IBO-treated subjects. No significant effects were observed in the rest of the analyzed genes (Fig. 1). It is important to mention that two outliers, one in males and one in females, were excluded from the statistical analyses for the group of IBO-treated mice in relation to *Oxt* and *Avp* expression. These outliers exhibited significantly higher expression levels, hundreds of times greater than the mean expression level of the group.

DISCUSSION

This study aimed to identify changes in gene expression in the frontal cortex in mice 4 h after a single oral dose of IBO. To date, this is the first time that transcriptomics has been used to study IBO's mechanisms of action. Following the transcriptomic analysis, it was observed that genes associated with hormonal pathways and synaptogenesis were upregulated by IBO. Conversely, genes involved in apoptotic processes and endosomal transports showed downregulation. Validation of gene expression through qPCR confirmed the observed results, except for the genes related to hormonal pathways.

Due to the limited understanding of IBO's mechanisms of action, there is a significant need to use new techniques to identify new targets and potential mechanisms of action and since transcriptomic is an exploratory approach by nature, there was a high degree of variability among different samples. However, certain patterns can be observed. First, a general difference in sex was clearly observed. Males showed changes in eight genes when comparing CNT and IBO conditions, whereas females had modifications in 28 genes. This might be attributed to IBO's greater bioavailability in females, as reported in preclinical studies [33, 34]. However, further research should confirm these findings persist for longer periods to explain the long-term effects of IBO.

Both *Oxt* and *Avp* genes were upregulated, indicating their involvement in hormonal pathways as they encode oxytocin and vasopressin, respectively. While the potential involvement of the neuroendocrine system in therapeutic outcomes has been explored in the context of other psychedelics [38, 54–56], there is currently no evidence of this in relation to IBO. In contrast with classic psychedelics, the potential effect of IBO in the neuroendocrine system would not be mediated by the stimulation of 5-HT2A receptors, as it does not bind to that receptor. Indeed, the only study assessing the neuroendocrine effects of IBO found an absence of effect on cortisol levels [57]. Studies have reported that LSD [37, 38], MDMA [58], and mescaline [37] can increase oxytocin levels. This increase in oxytocin may be directly associated with the prosocial effects [59–61] and promotion of neuroplasticity [37] observed with psychedelics. In a recent study, IBO was shown to reinstate social reward learning for more than 4 weeks after an acute administration [62], so a putative mechanism could be the promoting effect of *Oxt*.

Vasopressin has also been associated with prosocial effects [59, 63, 64]. Additionally, low levels of this hormone have been associated with depression or psychotic disorders [65, 66]. In regards to substance use disorders, it has been observed that the central administration of vasopressin blocks amphetamine-induced conditioned place preference in rats [67]. It is believed that the septum/vasopressin system modulates the release of neurotransmitters in the reward system [68]. IBO's possible modulation of both *Oxt* and *Avp* may have direct implications for understanding its anti-addictive effects. This is particularly relevant given the recent advancements in our understanding of the roles oxytocin and vasopressin may have in substance use disorders [64]. However, the validation analysis with qPCR could not confirm the overexpression of *Oxt* or *Avp* in the obtained samples. This might be due to the high variability found between subjects and differences between sexes. Based on these, future studies should include brain areas such as the hypothalamus where the expression of *Oxt* [69] or *Avp* [67] is high.

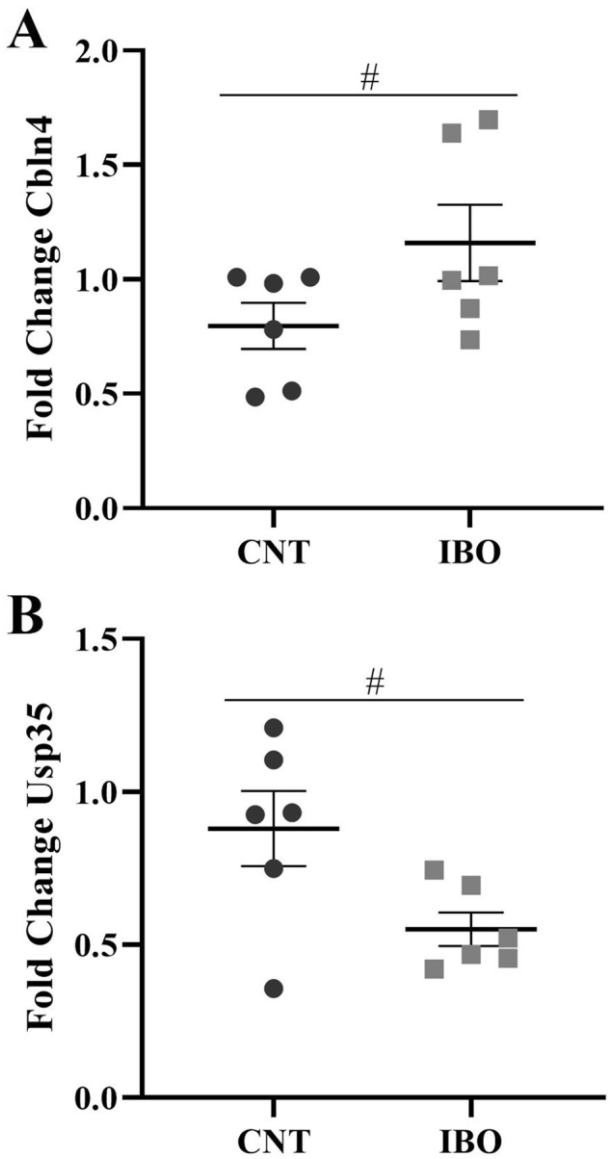


Fig. 2 Frontal cortex gene expression determined by qPCR with treatment differences. *Cbln4* (A) and *Usp35* (B). The symbol # indicates differences between treatments at $p < 0.05$.

Cbln2 and *Cbln4*, cerebellins belonging to the C1q and tumor necrosis factor, have a strong association with synaptogenesis [70, 71] and also showed increased expression in transcriptomic analysis. Their upregulation suggests that IBO is inducing cellular-level neuroplasticity. To date, the main mechanism by which IBO induces neuroplasticity has been restricted to both glial- and brain-derived neurotrophic factors [32, 72]. However, a prior study has reported that it is actually NOR, the principal metabolite of IBO, and not IBO itself that induces neuroplasticity [73]. It is worth noting that a significant increase in these cerebellins, particularly *Cbln4*, was observed following IBO administration in males but not females. Future studies using larger samples should explore these potential differences by sex in depth. The overexpression of cerebellins reported in transcriptomic analysis was confirmed by qPCR. Furthermore, there was an observed increase in the *Nptx2* gene, which was also related to the synaptogenesis of excitatory neurons and related to AMPA receptor synapse clustering in IBO-treated females.

The ubiquitin-specific peptidase 35 adaptor (*Usp35*) gene was downregulated after IBO administration in both males and females. This gene is associated with apoptotic processes, although its specific role is not yet clear. While some studies suggest that *Usp35* is a tumor suppressor [51, 74], others point to an upregulation of *Usp35* in ovarian cancer [75]. Four different isoforms of *Usp35* have been identified so far [76]. It is therefore possible that different pathways modulating specific isoforms lead to distinct effects. The downexpression of *Usp35* reported in transcriptomics analysis was confirmed by qPCR. Nevertheless, other genes related to apoptotic processes or cell growth were also found to be upregulated by IBO in females (*Tnfrsf25*, *Cdkn1a*, and *Phlad1*) [77, 78], while those negatively regulating these processes were downregulated (*Ccdc154* and *Dhr2*) [79, 80].

The gene *Ap5b1* was also downregulated in the IBO group. This gene is associated with endosomal transport. It is challenging to suggest specific implications of this gene's downregulation. Similarly, there were several other genes affected by IBO for which specific functions are not yet known because they were predicted or uncharacterized (e.g., *Gm34306*, *Gm51898*, *Gm44505*, among others), or are related to complex systems such as the immune and inflammatory system (e.g., *Il1rn*, *Eomes*, *Sh2d1b1*, *Cd19*, *Egr4*) or calcium ion channels (*Smim3*, *Slc25a25*, *Mcu*). These effects on the immune and inflammatory systems open new therapeutic implications.

The main limitation of this study was the high variability observed in the transcriptomic analyses which highlights the need for a larger sample, especially to better explore differences observed between sexes. Additionally, another limitation was the collection of only one measurement at +4 h post-IBO administration. Future studies should investigate changes in gene expression at various time points, including long-term assessments to better understand IBO's sustained effects. This approach is crucial given the numerous reports of long-term behavioral changes in observational research. While the long-lasting action of NOR, ibogaine's metabolite, has been suggested as the potential cause of these effects [81], it is essential to consider the possibility of gene expression modifications by both IBO and NOR. Further investigations should address these limitations and explore the complex interplay between gene expression changes and the behavioral outcomes of IBO and its metabolite NOR.

In conclusion, this study represents a significant step forward in understanding the molecular mechanisms underlying the effects of IBO. Through the application of transcriptomic analysis, we have identified notable changes in gene expression following a single dose of IBO in mice. Our findings reveal that the genes involved in hormonal pathways and synaptogenesis were upregulated by IBO. Conversely, the genes associated with apoptotic processes and endosomal transports were downregulated. These results were further validated through quantitative polymerase chain reaction (qPCR). It's important to note that the validation of gene expression pertaining to hormonal pathways did not completely corroborate the findings of the transcriptomic analysis. In addition, we also observed general sex differences, with females showing more alterations in gene expression after IBO treatment. Overall, this study expands our knowledge of IBO's molecular actions and underscores the potential of omics techniques in investigating the effects of psychedelic drugs. Further research is warranted to study the contribution of each of the identified genes at different time points to establish acute and long-term effects after IBO treatment, specifically for those pathways involved in neuromodulation. The precise mechanisms through which IBO modulates gene expression are especially relevant to identifying new therapeutic applications.

DATA AVAILABILITY

The data that support the findings of this study and the R script analyses are available from the corresponding author upon reasonable request. Read counts of RNA sequencing are available at https://doi.org/10.34810/data912.

REFERENCES

1. World Health Organization. Drugs (psychoactive). WHO;2023. https://www.who.int/health-topics/drugs-psychoactive#tab=tab_2.
2. Schulte MT, Substance Y-IH. Use and associated health conditions throughout the lifespan. Physiol Behav. 2019;176:139-48.
3. Fleury M-J, Djouini A, Huỳnh C, Tremblay J, Ferland F, Ménard J-M, et al. Remission from substance use disorders: a systematic review and meta-analysis. Drug Alcohol Depend. 2016;168:293-306.
4. Lappan SN, Brown AW, Hendricks PS. Dropout rates of in-person psychosocial substance use disorder treatments: a systematic review and meta-analysis. Addiction. 2020;115:201-17.
5. Oaklander M. Inside ibogaine, one of the most promising and perilous psychedelics for addiction. Time. 2021. https://time.com/5951772/ibogaine-drug-treatment-addiction/.
6. Rodríguez-Cano BJ, Kohek M, Ona G, Alcázar-Córcoles MÁ, dos Santos RG, Hallak JEC, et al. Underground ibogaine use for the treatment of substance use disorders: a qualitative analysis of subjective experiences. Drug Alcohol Rev. 2023;42:401-14.
7. Koenig X, Hilber K. The anti-addiction drug ibogaine and the heart: a delicate relation. Molecules. 2015;20:2208-28.
8. Belgers M, Leenaars M, Homberg JR, Ritskes-Hoitinga M, Schellekens AFA, Hooijmans CR. Ibogaine and addiction in the animal model, a systematic review and meta-analysis. Transl Psychiatry. 2016;6:e826-e826.
9. Luxton T, Parker LA, Siegel S. Ibogaine fails to interrupt the expression of a previously established one-trial morphine place preference. Prog Neuropsychopharmacol Biol Psychiatry. 1996;20:857-72.
10. Moroz I, Parker LA, Siegel S. Ibogaine interferes with the establishment of amphetamine place preference learning. Exp Clin Psychopharmacol. 1997;5:119-22.
11. Parker LA, Siegel S, Luxton T. Ibogaine attenuates morphine-induced conditioned place preference. Exp Clin Psychopharmacol. 1995;3:344-8.
12. Cappendijk SLT, Fekkes D, Dzoljic MR. The inhibitory effect of norharman on morphine withdrawal syndrome in rats: Comparison with ibogaine. Behav Brain Res. 1994;65:117-9.
13. Dzoljic ED, Kaplan CD, Dzoljic MR. Effect of ibogaine on naloxone-precipitated withdrawal syndrome in chronic morphine-dependent rats. Arch Int Pharmacodyn Ther. 1988;294:64-70.
14. Glick SD, Kuehne ME, Raucci J, Wilson TE, Larson D, Keller RW, et al. Effects of iboga alkaloids on morphine and cocaine self-administration in rats: relationship to tremorigenic effects and to effects on dopamine release in nucleus accumbens and striatum. Brain Res. 1994;657:14-22.
15. Leal MB, Michelin K, Souza DO, Elisabetsky E. Ibogaine attenuation of morphine withdrawal in mice: role of glutamate N-methyl-d-aspartate receptors. Prog Neuropsychopharmacol Biol Psychiatry. 2003;27:781-5.
16. Parker LA, Burton P, McDonald RV, Kim JA, Siegel S. Ibogaine interferes with motivational and somatic effects of naloxone-precipitated withdrawal from acutely administered morphine. Prog Neuropsychopharmacol Biol Psychiatry. 2002;26:293-7.
17. Francés B, Gout R, Cros J, Zajac J. Effects of ibogaine on naloxone-precipitated withdrawal in morphine-dependent mice. Fundam Clin Pharm. 1992;6:327-32.
18. Sharpe LG, Jaffe JH. Ibogaine fails to reduce naloxone-precipitated withdrawal in the morphine-dependent rat. Neuroreport. 1990;1:17-19.
19. Glue P, Winter H, Garbe K, Jakobi H, Lyudin A, Lenagh-Glue Z, et al. Influence of CYP2D6 activity on the pharmacokinetics and pharmacodynamics of a single 20 mg dose of ibogaine in healthy volunteers. J Clin Pharmacol. 2015;55:680-7.
20. Davis AK, Barsuglia JP, Windham-Herman A-M, Lynch M, Polanco M. Subjective effectiveness of ibogaine treatment for problematic opioid consumption: Short- and long-term outcomes and current psychological functioning. J Psychedelic Stud. 2017;1:65-73.
21. Brown TK, Alper K. Treatment of opioid use disorder with ibogaine: detoxification and drug use outcomes. Am J Drug Alcohol Abus. 2018;44:24-36.
22. Davis AK, Averill LA, Sepeda ND, Barsuglia JP, Amoroso T. Psychedelic treatment for trauma-related psychological and cognitive impairment among us special operations forces veterans. Chronic Stress. 2020;4:247054702093956.
23. Heink A, Katsikas S, Lange-Altman T. Examination of the phenomenology of the ibogaine treatment experience: role of altered states of consciousness and psychedelic experiences. J Psychoact Drugs. 2017;49:201-8.
24. Malcolm BJ, Polanco M, Barsuglia JP. Changes in withdrawal and craving scores in participants undergoing opioid detoxification utilizing ibogaine. J Psychoact Drugs. 2018;50:256-65.
25. Knuijver T, Schellekens A, Belgers M, Donders R, Oosteren T, Kramers K, et al. Safety of ibogaine administration in detoxification of opioid-dependent individuals: a descriptive open-label observational study. Addiction. 2022;117:118-28.
26. C. Mash D. Breaking the cycle of opioid use disorder with Ibogaine. Am J Drug Alcohol Abus. 2018;44:1-3.
27. Mash DC, Kovera CA, Pablo J, Tyndale R, Ervin FR, Kamlet JD, et al. Chapter 8 Ibogaine in the treatment of heroin withdrawal. In: Elsevier (ed). *The Alkaloids: Chemistry and Biology*. 2001. p. 155-71.
28. Luz M, Mash DC. Evaluating the toxicity and therapeutic potential of ibogaine in the treatment of chronic opioid abuse. Expert Opin Drug Metab Toxicol. 2021;17:1019-22.
29. Popik P, Layer RT, Skolnick P. 100 years of ibogaine: neurochemical and pharmacological actions of a putative anti-addictive drug. Pharm Rev. 1995;47:235-53.
30. Coleman JA, Yang D, Zhao Z, Wen P-C, Yoshioka C, Tajkhorshid E, et al. Serotonin transporter-ibogaine complexes illuminate mechanisms of inhibition and transport. Nature. 2019;569:141-5.
31. González J, Cavelli M, Castro-Zaballa S, Mondino A, Tort ABL, Rubido N, et al. EEG gamma band alterations and REM-like traits underpin the acute effect of the atypical psychedelic ibogaine in the rat. ACS Pharm Transl Sci. 2021;4:517-25.
32. Marton S, González B, Rodríguez-Bottero S, Miquel E, Martínez-Palma L, Pazos M, et al. Ibogaine administration modifies GDNF and BDNF expression in brain regions involved in mesocorticolimbic and nigral dopaminergic circuits. Front Pharm. 2019;10:1-13.
33. Tatalović N, Vidonja Uzelac T, Mijović M, Koželj G, Nikolić-Kokić A, Oreščanin Dušić Z, et al. Ibogaine has sex-specific plasma bioavailability, histopathological and redox/antioxidant effects in rat liver and kidneys: a study on females. Life. 2021;12:16.
34. Pearl SM, Hough LB, Boyd DL, Glick SD. Sex differences in ibogaine antagonism of morphine-induced locomotor activity and in ibogaine brain levels and metabolism. Pharm Biochem Behav. 1997;57:809-15.
35. Ona G, Reverte I, Rossi GN. Main targets of ibogaine and noribogaine associated with its putative anti-addictive effects: A mechanistic overview. J Psychopharmacol. 2023;37:1190-200.
36. Schneider G. Automating drug discovery. Nat Rev Drug Discov. 2018;17:97-113.
37. Ley L, Holze F, Arikci D, Becker AM, Straumann I, Klaiber A, et al. Comparative acute effects of mescaline, lysergic acid diethylamide, and psilocybin in a randomized, double-blind, placebo-controlled cross-over study in healthy participants. Neuropsychopharmacology. 2023;48:1659-67.
38. Schmid Y, Enzler F, Gasser P, Grouzmann E, Preller KH, Vollenweider FX, et al. Acute effects of lysergic acid diethylamide in healthy subjects. Biol Psychiatry. 2015;78:544-53.
39. MacDonald K, MacDonald TM, Brüne M, Lamb K, Wilson MP, Golshan S, et al. Oxytocin and psychotherapy: a pilot study of its physiological, behavioral and subjective effects in males with depression. Psychoneuroendocrinology. 2013;38:2831-43.
40. Donovan LL, Johansen JV, Ros NF, Jaberi E, Linnet K, Johansen SS, et al. Effects of a single dose of psilocybin on behaviour, brain 5-HT2A receptor occupancy and gene expression in the pig. Eur Neuropsychopharmacol. 2021;42:1-11.
41. Katchborian-Neto A, Santos WT, Nicácio KJ, Corrêa JOA, Murgu M, Martins TMM, et al. Neuroprotective potential of Ayahuasca and untargeted metabolomics analyses: applicability to Parkinson's disease. J Ethnopharmacol. 2020;255:112743.
42. Martin DA, Marona-Lewicka D, Nichols DE, Nichols CD. Chronic LSD alters gene expression profiles in the mPFC relevant to schizophrenia. Neuropharmacology. 2014;83:1-8.
43. Nichols C, Sanders-Bush E. A single dose of lysergic acid diethylamide influences gene expression patterns within the mammalian brain. Neuropsychopharmacology. 2002;26:634-42.
44. Oommen AM, Roberts KJ, Joshi L, Cunningham S. Transcriptomic analysis of glycosylation and neuroregulatory pathways in rodent models in response to psychedelic molecules. Int J Mol Sci. 2023;24:1200.
45. Kubiliene A, Sveikata A, Zevzikovas A, Sadauskiene I, Ivanov L. Investigation into pharmacokinetic properties of active alkaloid ibogaine and its metabolite noribogaine. Acta Pol Pharma Drug Res. 2017;74:1591-7.
46. Goldstein RZ, Volkow ND. Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry. 200;159:1642-52.
47. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT Method. Methods. 2001;25:402-8.
48. Stohn JP, Martinez ME, Zafer M, López-Espíndola D, Keyes LM, Hernandez A. Increased aggression and lack of maternal behavior in Dio3-deficient mice are associated with abnormalities in oxytocin and vasopressin systems. Genes Brain Behav. 2018;17:23-35.
49. Bradford ST, Hiramatsu R, Maddugoda MP, Bernard P, Chaboissier MC, Sinclair A, et al. The cerebellin 4 precursor gene is a direct target of SRY and SOX9 in mice. Biol Reprod. 2009;80:1178-88.
50. Shibata M, Pattabiraman K, Muchnik SK, Kaur N, Morozov YM, Cheng X, et al. Hominini-specific regulation of CBLN2 increases prefrontal spinogenesis. Nature. 2021;598:489-94.
51. Liu C, Wang L, Chen W, Zhao S, Yin C, Lin Y, et al. USP35 activated by miR let-7a inhibits cell proliferation and NF-κB activation through stabilization of ABIN-2. Oncotarget. 2015;6:27891-906.

52. Yao Q, Chen L, Liang Y, Sui L, Guo L, Zhou J, et al. Blastomere removal from cleavage-stage mouse embryos alters placental function, which is associated with placental oxidative stress and inflammation. Sci Rep. 2016;6:25023.
53. Lenhard W, Lenhard A. Computation of different effect sizes like d, f, r and transformation of different effect sizes: Psychometrica. https://www.psychometrica.de/effect_size.html (accessed 3 Nov 2023).
54. Collu R, Letarte J, Leboeuf G, Ducharme JR. Endocrine effects of chronic administration of psychoactive drugs to prepuberal male rats. I: Δ9-tetrahydrocannabinol. Life Sci. 1975;16:533-42.
55. Meltzer HY, Fessler RG, Simonovic M, Doherty J, Fang VS. Lysergic acid diethylamide: Evidence for stimulation of pituitary dopamine receptors. Psychopharmacol (Berl). 1977;54:39-44.
56. Schindler EAD, Wallace RM, Sloshower JA, D'Souza DC. Neuroendocrine associations underlying the persistent therapeutic effects of classic serotonergic psychedelics. Front Pharm. 2018;9:1-16.
57. Cachat J, Kyzar EJ, Collins C, Gaikwad S, Green J, Roth A, et al. Unique and potent effects of acute ibogaine on zebrafish: the developing utility of novel aquatic models for hallucinogenic drug research. Behav Brain Res. 2013;236:258-69.
58. Kirkpatrick MG, Francis SM, Lee R, de Wit H, Jacob S. Plasma oxytocin concentrations following MDMA or intranasal oxytocin in humans. Psychoneuroendocrinology. 2014;46:23-31.
59. Bachner-Melman R, Ebstein RP. The role of oxytocin and vasopressin in emotional and social behaviors. In: Handbook of clinical neurology. Handb Clin Neurol. 2014;124:53-68.
60. Preller KH, Vollenweider FX. Modulation of social cognition via hallucinogens and "Entactogens". Front Psychiatry. 2019;10:1-13.
61. Striepens N, Kendrick KM, Maier W, Hurlemann R. Prosocial effects of oxytocin and clinical evidence for its therapeutic potential. Front Neuroendocrinol. 2011;32:426-50.
62. Nardou R, Sawyer E, Song YJ, Wilkinson M, Padovan-Hernandez Y, de Deus JL, et al. Psychedelics reopen the social reward learning critical period. Nature. 2023;618:790-8.
63. Ramos L, Hicks C, Kevin R, Caminer A, Narlawar R, Kassiou M, et al. Acute prosocial effects of oxytocin and vasopressin when given alone or in combination with 3,4-methylenedioxymethamphetamine in rats: involvement of the V1A receptor. Neuropsychopharmacology. 2013;38:2249-59.
64. Wronikowska-Denysiuk O, Mrozek W, Budzyńska B. The role of oxytocin and vasopressin in drug-induced reward–implications for social and non-social factors. Biomolecules. 2023;13:405.
65. Abramova O, Zorkina Y, Ushakova V, Zubkov E, Morozova A, Chekhonin V. The role of oxytocin and vasopressin dysfunction in cognitive impairment and mental disorders. Neuropeptides. 2020;83:102079.
66. Rubin LH, Carter CS, Bishop JR, Pournajafi-Nazarloo H, Drogos LL, Hill SK, et al. Reduced levels of vasopressin and reduced behavioral modulation of oxytocin in psychotic disorders. Schizophr Bull. 2014;40:1374-84.
67. Gárate-Pérez MF, Méndez A, Bahamondes C, Sanhueza C, Guzmán F, Reyes-Parada M, et al. Vasopressin in the lateral septum decreases conditioned place preference to amphetamine and nucleus accumbens dopamine release. Addiction Biol. 2021;26:1-10.
68. Raggenbass M. Overview of cellular electrophysiological actions of vasopressin. Eur J Pharm. 2008;583:243-54.
69. Gould BR, Zingg HH. Mapping oxytocin receptor gene expression in the mouse brain and mammary gland using an oxytocin receptor-LacZ reporter mouse. Neuroscience. 2003;122:155-67.
70. Matsuda K, Yuzaki M. Cbln family proteins promote synapse formation by regulating distinct neurexin signaling pathways in various brain regions. Eur J Neurosci. 2011;33:1447-61.
71. Seigneur E, Südhof TC. Cerebellins are differentially expressed in selective subsets of neurons throughout the brain. J Comp Neurol. 2017;525:3286-311.
72. He D-Y, McGough NNH, Ravindranathan A, Jeanblanc J, Logrip ML, Phamluong K, et al. Glial cell line-derived neurotrophic factor mediates the desirable actions of the anti-addiction drug ibogaine against alcohol consumption. J Neurosci. 2005;25:619-28.
73. Ly C, Greb AC, Cameron LP, Wong JM, Barragan EV, Wilson PC, et al. Psychedelics promote structural and functional neural plasticity. Cell Rep. 2018;23:3170-82.
74. Wang W, Wang M, Xiao Y, Wang Y, Ma L, Guo L, et al. USP35 mitigates endoplasmic reticulum stress-induced apoptosis by stabilizing RRBP1 in non-small cell lung cancer. Mol Oncol. 2022;16:1572-90.
75. Zhang J, Chen Y, Chen X, Zhang W, Zhao L, Weng L, et al. Deubiquitinase USP35 restrains STING-mediated interferon signaling in ovarian cancer. Cell Death Differ. 2021;28:139-55.
76. Cao J, Wu D, Wu G, Wang Y, Ren T, Wang Y, et al. USP35, regulated by estrogen and AKT, promotes breast tumorigenesis by stabilizing and enhancing transcriptional activity of estrogen receptor α. Cell Death Dis. 2021;12:619.
77. Dutto I, Tillhon M, Cazzalini O, Stivala LA, Prosperi E. Biology of the cell cycle inhibitor p21CDKN1A: molecular mechanisms and relevance in chemical toxicology. Arch Toxicol. 2015;86:155-78.
78. Peng H, Wang J, Song X, Huang J, Hua H, Wang F, et al. PHLDA1 suppresses TLR4-TRiggered Proinflammatory Cytokine Production by Interaction with Tollip. Front Immunol. 2022;13:1-13.
79. Liao W, Zhao R, Lu L, Zhang R, Zou J, Xu T, et al. Overexpression of a novel osteopetrosis-related gene CCDC154 suppresses cell proliferation by inducing G 2 /M arrest. Cell Cycle. 2012;11:3270-9.
80. Li Z, Tan Y, Li X, Quan J, Bode AM, Cao Y, et al. DHRS2 inhibits cell growth and metastasis in ovarian cancer by downregulation of CHKα to disrupt choline metabolism. Cell Death Dis. 2022;13:845.
81. Baumann MH, Pablo J, Ali SF, Rothman RB, Mash DC. Chapter 5 Comparative neuropharmacology of ibogaine and its O-desmethyl metabolite, noribogaine. In: Elsevier (ed). The Alkaloids Chemistry and Biology. 2001, p. 79-113.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Celeste de Paolo, Esperanza Chernichero, and Juan Valencia for their technical support in caring for the animals. We are also grateful to Lorena Garcia, Dr. Nerea Abasolo, and Dr. Adrià Cereto of the Transcriptomics facility of the Center for Omic Sciences (COS) Joint Unit of the Universitat Rovira i Virgili-Eurecat for their contribution to the transcriptomic analysis. This study was part of the PhD project of GO, for which he received the Industrial Doctorate public grant from AGAUR-GENCAT (2020 DI 025).

AUTHOR CONTRIBUTIONS

MTC conceived and supervised the study. JBB and LAF performed the experimental part with animals. JBB performed the statistical and gene expression analysis and with GO drafted the manuscript. All authors discussed the results and provided feedback on the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Maria Teresa Colomina.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

DISCUSSION

5.Discussion

Throughout the studies included in this thesis, we have updated the data on adverse ibogaine events and classified them into different categories based on their nature, affected systems, and duration. The findings show how using ibogaine entails certain risks, especially in non-controlled settings, when combined with other substances, when its purity is unknown, and when doses exceed the potential efficacious amount.

We have provided clinical data from a Phase-II clinical trial in which ibogaine was administered to patients in methadone maintenance programs. It was observed that a low dose (100 mg) of ibogaine effectively reduced methadone tolerance, allowing 17 out of 20 patients to halve their daily methadone dose within a week of the dosing session. Ibogaine (100 mg) was also found to be safe and well-tolerated. Moreover, these low doses of ibogaine induced metabolic changes, suggesting certain benefits in terms of energetic efficiency, potentially reversing the well-known effects of chronic opioid administration.

We have also summarized and updated the pharmacological information about ibogaine, detailing in a single manuscript all its mechanisms potentially associated with its anti-addictive potential. This publication clarifies that ibogaine exerts its action through multiple neurotransmitter systems and targets, indicating that complex approaches are needed when assessing its mechanisms.

Lastly, we used an animal model to thoroughly examine the mechanisms of action of ibogaine, conducting the first-ever transcriptomic analysis of cortical tissue of mice exposed to ibogaine. This study identified hormonal and signaling pathways involved in neuromodulation, which are potentially related to ibogaine's effects.

In this section, the results of the four studies will be discussed in an integrated manner to contextualize the new knowledge provided and highlight its limitations. At the time of writing, international interest in ibogaine is skyrocketing, with the first pharmaceutical company initiating trials. Thus, special attention will be given to future perspectives in ibogaine research.

5.1. General Discussion

Two significant reasons for the notable lack of clinical research on ibogaine are safety concerns regarding its use and a limited understanding of its mechanisms of action regarding its putative anti-addictive effects. Thus, the exact way in which ibogaine works remains unknown, and its safety profile has led to reluctance in its use. These factors may have contributed to the lack of interest in this substance within the medical community despite its potential benefits for SUDs. This thesis addresses both the safety profile and mechanisms of action of ibogaine in detail, providing new data for further research and clinical considerations.

The safety profile of ibogaine is one of the main concerns that hinders its further development as a commercialized drug. For this reason, we conducted a systematic review of adverse events reported after using this substance. While two previous reviews (Alper et al., 2012; Koenig & Hilber, 2015) covered adverse events up to 2015, our review includes data published between 2015 and 2020.

The main findings showed that high doses of ibogaine administered in informal settings are associated with severe symptoms such as seizures, arrhythmia, vomiting, and diarrhea. In contrast, when ibogaine is administered in clinically controlled settings, it is reasonably safe, with non-serious adverse events such as hallucinations/visual alterations and mild cardiovascular, motor, and gastrointestinal alterations. However, the doses administered in these clinical settings were low (20 mg of ibogaine or 60, 120, and 180 mg of noribogaine). An open-label trial published after our review (Knuijver et al., 2022) reported a poorer safety profile with much higher doses (10 mg/kg), where over 50% of patients showed QT prolongations above 500 ms. The sample consisted of individuals on opioid substitution treatment who were converted to a short-acting opioid (morphine) eight days before the ibogaine session, receiving their last dose of morphine four hours before ibogaine administration. The methods used in this study were highly criticized by other authors (Luz & Mash, 2022). For instance, metoclopramide, an inhibitor of CYP2D6, the main enzyme responsible for ibogaine metabolism, was administered just before the experimental session. Thus, traces of morphine and therapeutic doses of metoclopramide in circulation could have limited ibogaine metabolism, leading to serious adverse events.

A recently published article concluded that the dose of ibogaine and the activity of the CYP2D6 enzyme are the most relevant factors to consider regarding safety (Michele & Sophie, 2023). In addition, the authors noted that the affinity of both ibogaine and noribogaine for the sigma-2 receptor may lead to neurotoxic effects, as this receptor modulates calcium ion channels, cellular calcium levels, and mitochondrial function. Thus, calcium ion channel function abnormalities can impact cellular processes related to apoptosis or calcium regulation (Michele & Sophie, 2023).

Given the acceptable safety profile reported with very low doses (20 mg) (Glue et al. 2015b) and the poor safety profile with high doses (700 mg, assuming a weight of 70 kg; Knuijver et al., 2022), it was necessary to establish the therapeutic and the maximum tolerated dose (MTD) of ibogaine. To determine which doses are safe and efficacious, a Phase-II, randomized, double-blind clinical trial was designed, administering 100-600 mg of ibogaine to methadone-dependent subjects (NCT04003948). In the context of this thesis, the results from the first administration (100 mg ibogaine) to 20 participants were reported. As the data from the first administrations (100 mg each) in both groups were taken, the nature of this study is better conceptualized as an open- label trial.

The sample consisted of 17 males and three females. The participants' methadone doses ranged from 20 to 100 mg, with a mean of 46.1 mg. One of the key findings was that a 100 mg dose of ibogaine demonstrated a good safety and tolerability profile, with no participant showing a QT interval reaching 500 ms within 24 hours after administration and no serious adverse events reported. However, one of the patients had to be excluded after completing the first session because their QTc increased to 484 ms. The cardiologist determined that even when this value was not clinically significant, the possible QT progression in further sessions if the patient was included in the ascending-dose arm would not be safe, so it was concluded that it was not safe for this patient to continue in the study. The occurrence of such a case in a small sample of 20 participants suggests that ibogaine may be particularly dangerous for certain individuals, for whom even low doses could potentially result in fatal outcomes. This highlights the need of an appropriate and careful monitoring when administering ibogaine.

Notably, the 100 mg dose of ibogaine, considered low compared to doses typically used in informal settings (15-20 mg/kg; Davis et al., 2017), allowed 17 out of 20 participants to reduce their daily methadone dose by half and maintain this dose during the following week without withdrawal symptoms. In addition, methadone use was interrupted for a mean of 18.03 hours. In contrast, even a brief interruption of these doses (a few hours) is typically associated with the appearance of severe withdrawal syndrome due to a 50% decrease in plasma levels following a single missed dose (Bart, 2013; Verebely & Kutt, 1975).

Previously published literature, based on case reports or observational studies, has indicated that ibogaine can produce complete cessation of drug craving for extended periods, even after a single large dose (Alper et al., 2001; Camlin et al., 2018; Davis et al., 2017; Mash et al., 2001,2018). In the present study, it is important to note that participants were not actively struggling with an SUD but were dependent on methadone, which could not be interrupted. Therefore, the protocol used in the study should be conceptualized as a model for detoxification purposes rather than treating an active SUD involving craving and compulsive substance use.

These results are promising from both clinical and economic standpoints. Typically, patients in methadone maintenance programs remain enrolled for several years (Gutiérrez-Cáceres et al., 2019). Even when some patients attempt to taper their methadone dose gradually, the process can take years and is not always successful (Amato et al., 2013). Thus, methadone programs represent a significant cost for public health systems. The use of ibogaine could offer a rapid and effective way of detoxifying patients from methadone.

These findings highlight the efficacy and safety of a low dose of 100 mg of ibogaine. Higher doses are known to produce intense psychoactive effects (Kohek et al., 2020). However, in this study, participants reported mainly feelings of relaxation when assessed using the VAS. Informal facilitators suggest that higher doses of ibogaine can induce hallucinogenic effects that may lead to transformative experiences, offering insights into personal issues related to patterns of substance abuse among their clients (Rodríguez-Cano et al., 2023). This hypothesis aligns with the belief that powerful psychological effects are necessary for achieving the therapeutic benefits of psychedelics, a view shared by various authors regarding the use of classical psychedelics such as psilocybin or LSD (Yaden & Griffiths, 2020). While we cannot discard this possibility, our data indicate that low doses of ibogaine, even without prominent psychological effects, effectively reduce withdrawal symptoms during detoxification.

To better understand the potential mechanisms of action associated with the effects of low-dose ibogaine and offer new perspectives, we utilized review strategies and state-of-the-art omics techniques. This comprehensive approach enabled us to explore a wide range of targets rather than focusing on specific ones (Paananen & Fortino, 2020).

In our clinical study, plasma samples were collected from 13 participants before and after ibogaine administration and analyzed using a metabolomic approach to identify potential differences in biomarkers attributable to ibogaine. The results indicated that aqueous metabolites were significantly more altered than lipid-based metabolites. Notably, levels of 2-oxo isocaproate and 2-hydroxy butyrate increased post-ibogaine administration in participants taking low daily doses of methadone. In contrast, in the group taking high daily doses of methadone, valine, phenylalanine, and lactate levels were most affected by ibogaine treatment, with increased valine and phenylalanine levels and decreased lactate levels. These metabolites are crucial for energy metabolism, which is implicated in OUD, as suggested by some studies (Chen et al., 2007).

Consistent with our findings, a previously published report by Paskulin et al. (2006) also identified alterations in energy metabolism in the brain following ibogaine administration, albeit more consistently. The discrepancies and heterogeneity in our results could be attributed to the lower ibogaine dosage used or the differences between participants with or without drug dependence. These findings are preliminary and offer initial insights into the complex effects of ibogaine. Future research should further investigate the impact of ibogaine on various biomarkers to comprehensively elucidate both its safety and efficacy.

To continue clarifying the mechanisms of action of ibogaine, a narrative review, and a preclinical study were conducted as part of this thesis to offer mechanistic explanations of the results obtained in the clinical setting.

The currently available knowledge regarding the mechanisms of action of ibogaine was compiled and presented in an accessible format. The most comprehensive review before this was published 25 years ago by Popik (1995). Since then, significant advancements have been made in both *in vitro* and *in vivo* research. Additionally, we have gained a deeper understanding of the various targets implicated in SUDs that are also influenced by ibogaine. Thus, the review provided a significant and necessary update on the mechanisms of action of ibogaine.

The key findings of the review included the evident need to distinguish between the binding profiles of ibogaine and its main metabolite, noribogaine. The longer half-life of noribogaine suggests its potential importance in achieving sustained detoxification and reducing craving and withdrawal symptoms (Glue et al., 2015a). The key differences include: ibogaine shows greater affinity for MOR, whereas noribogaine favors KOR; noribogaine is approximately 10 times stronger as a SERT inhibitor than ibogaine; ibogaine has greater affinity for the NMDA and sigma receptors than noribogaine; and ibogaine does not modify neural plasticity indicators whereas noribogaine does (Ly et al., 2018).

Throughout the review, certain areas were identified as needing further research. These include understanding the different dynamics of opioid receptor binding by both ibogaine and its metabolite; exploring the eventual effects of the interaction between ibogaine and DAT; determining the exact mechanism by which ibogaine induces psychoactive effects; investigating the role of the release of neurotrophic factors, ABC transporters inhibition, and interactions with muscarinic, serotonergic, and sigma receptors in the overall anti-addictive effects of ibogaine; and exploring the potential synergistic effects arising from interactions with multiple targets.

Given this information, we note that the reduction of methadone tolerance by ibogaine observed in the clinical trial may involve various receptor systems, including NMDA (Inturrisi, 1997), opioid receptors (Maillet et al., 2015), or the inhibition of both P-gp and BCRP. In addition, the observed reduction in OWS severity could be mediated by the binding of ibogaine to NMDA receptors (Zhu & Ho, 1998), α3β4 nicotinic receptors (Muldoon et al., 2014), or its inhibitory effect on SERT (Kirby et al., 2011). The aversive-like feelings expressed by some subjects toward substances other than methadone (e.g., coffee, tobacco, or alcohol) may be mediated by the action of ibogaine as a KOR agonist (Maillet et al., 2015).

To further expand our understanding of the mechanisms of action of ibogaine and propose new potential targets, a preclinical study was included in this doctoral thesis. Indeed, much of the existing research on ibogaine is based on animal models (Belgers et al., 2016). The earliest studies, dating back over 120 years, involved testing ibogaine on dogs, rabbits, guinea pigs, and frogs. These studies described its effects as similar to alcohol, inducing hallucinatory states and causing death at high doses (Phisalix, 1901). Subsequent research identified cardiovascular risks associated with ibogaine (primarily manifesting as decreases in heartrate and blood pressure), findings that were later confirmed in further preclinical studies (Belgers et al., 2016; Binienda et al., 1998; Schneider & Rinehart, 1957).

Our study with mice involved a transcriptomic analysis of the cortical brain tissue from animals acutely exposed to ibogaine 4 hours previously. The results of this analysis revealed an up-regulation of genes associated with hormonal pathways (*Oxt*, encoding oxytocin; *Avp*, encoding vasopressin) and synaptogenesis (*Cbln2* and *Cbln4*) and a down-regulation of genes involved in apoptotic processes (*Usp35*) and endosomal transport (Ap5b1).

To validate these findings, we employed real-time quantitative polymerase chain reaction (RT-qPCR), a technique that accurately quantifies gene expression levels in a sample. While transcriptomic analyses provide a broad overview of gene expression levels across thousands of genes, offering a high-throughput approach to understanding gene expression, this technique can occasionally produce false positives or noise that may obscure low-abundance transcripts (Everaert et al., 2017; Hughes, 2009).

The RT-qPCR technique enables precise quantification of gene expression levels for specific genes of interest, thereby confirming the results obtained from high-throughput methods and ensuring data accuracy and reliability. Notably, some authors have argued against the necessity of using qPCR as a validation technique. For a comprehensive discussion of this topic, see Coenye (2021).

The RT-qPCR performed in our study validated all the findings except for the enhanced expression of genes related to hormonal pathways. This may be due to high sample variability and significant differences between sexes. Specifically, males showed changes in eight genes when comparing ibogaine-exposed and control conditions, whereas females showed changes in 28 genes. This could be linked to the previously reported enhanced bioavailability of ibogaine in females (Pearl et al., 1997; Tatalović et al., 2021).

Future studies should consider collecting tissue from other brain regions, particularly the hypothalamus and nucleus accumbens to validate further the alteration of genes associated with hormonal pathways. Recent research with mice demonstrated that a single administration of ibogaine reinstated a critical period for social reward learning for more than four weeks, longer than similar effects induced by LSD, MDMA, and others (Nardou et al., 2023). Interestingly, this reinstatement of the critical period was accompanied by the restoration of oxytocin-mediated long-term depression in the nucleus accumbens, highlighting the acute and long-term effects of ibogaine on oxytocin levels, which warrants further investigation. Of particular interest for future research is the distinction between hyperplasticity (a general increase in synaptic activity or connectivity) and metaplasticity (the brain's ability to modify how it changes, affecting the potential for future synaptic alterations). The findings of this study suggest that psychedelic drugs, including ibogaine, impact metaplasticity rather than hyperplasticity (Nardou et al., 2023).

The observed up-regulation of *Cbln* genes also suggests that ibogaine increases brain plasticity. Combined with the findings of the narrative review on the mechanisms of action of ibogaine included in this thesis, this study suggests that changes in brain plasticity could underpin the potential therapeutic effects of ibogaine. This hypothesis is supported by the behavioral changes observed in the clinical study, where participants not only reduced methadone use but also showed reductions in tobacco, alcohol, or coffee consumption. Future investigations should explore further modifications in genes related to apoptotic processes or endosomal transport, potentially creating new hypotheses regarding the mechanisms of action of ibogaine.

Taken together, the findings from this thesis provide compelling evidence on the efficacy and safety of ibogaine. Our clinical trial demonstrated that a single low-dose administration of ibogaine improved the metabolic state in a sample of opioid-dependent individuals, diminished subjective withdrawal symptoms, and facilitated a temporary reduction in daily methadone doses. Regarding the cardiotoxic effects, it is clear that this substance has certain risks and that it needs a close cardiovascular monitoring, as noted by the QT prolongation of one of the subjects. With this monitoring and adjusted low doses, we can expect their use safe enough in clinical settings. This information is useful for designing new clinical studies aimed at refining doses to achieve safe therapeutic levels. Moreover, it is worth noting that a single dose has demonstrated the ability to induce significant biological changes observed in both clinical and preclinical studies.

Overall, ibogaine shows promise as a neuromodulator in the CNS with a potentially broader field of applications. For instance, a study in which ibogaine was used for the treatment of traumatic brain injury was recently published (Cherian et al., 2024), highlighting the need for continued research.

5.2. Limitations and Future Perspectives

The studies described in this thesis have noteworthy limitations meriting consideration. The data presented here originate from preliminary investigations, marking the inaugural application of transcriptomic analysis in preclinical ibogaine research. Furthermore, this work is the first to administer ibogaine within a controlled clinical setting. Regarding the former, it is imperative to underscore the necessity for future studies involving diverse cerebral regions and larger sample sizes to confirm the roles of hormonal and neural plasticity pathways in the therapeutic properties of ibogaine.

From a clinical standpoint, this research constitutes an initial glimpse into a broader clinical trial that remains in progress. Comprehensive data from all treatment sessions and participants will enable a full evaluation of the safety and efficacy profiles across varying low and moderate doses of ibogaine, shedding further light on its therapeutic potential.

Only three women were included in the clinical study, consistent with the gender distribution of participants enrolled in methadone maintenance programs. This imbalance highlights the need to study the effects of ibogaine specifically in women, given that they tend to present longer QT intervals, and preliminary evidence suggests that females have enhanced ibogaine bioavailability. Notably, results from the preclinical study indicated more gene alterations in females than males following acute ibogaine exposure, suggesting potential sex differences in its safety profile.

At the time of composing this text, the pharmaceutical firm DemeRx has concluded its Phase I clinical trial involving ibogaine [ClinicalTrials.gov ID NCT05029401], although the findings remain unpublished. Meanwhile, DemeRx is conducting an ongoing Phase II trial in the United Kingdom. As a pharmaceutical entity, DemeRx has expressed a near-term goal of commercializing ibogaine. Therefore, it is anticipated that upon disseminating this thesis, ibogaine will attract increased attention and generate heightened interest within the scientific community. This is expected to lead to a surge in research efforts dedicated to studying this compound. We hope the empirical insights presented in this thesis will serve as a foundational resource to inform and guide these future investigations, facilitating the development of appropriate clinical strategies for administering ibogaine.

CONCLUSIONS

6. Conclusions

The main hypothesis of this thesis was that low doses of ibogaine, when administered in a controlled clinical setting, are safe and effective in reducing drug tolerance and the severity of opioid withdrawal syndrome. Additionally, it was also hypothesized that multiple targets are involved in supporting the anti-addictive effects of ibogaine. Based on these hypotheses, the results of this thesis have prompted the following conclusions.

Clinical studies

1- A systematic review indicates that ibogaine use is potentially associated with serious adverse events, primarily involving cardiovascular function. QT prolongation was the most commonly reported complication, persisting up to seven days.

2- Findings from a systematic review highlight that serious adverse events associated with ibogaine are primarily related to cardiovascular, gastrointestinal, and neurological alterations. Some of these effects lasted more than 24 hours post-ibogaine use. Most cases were reported in non-medical settings, lacking precise information on dosage or the presence of other drugs/ medications, thereby challenging the scientific validity of these reports.

3- Results from a Phase-II clinical study demonstrated that a single oral dose of 100 mg ibogaine administered to patients in a methadone maintenance program effectively reduced opioid withdrawal syndrome. Specifically, patients were able to abstain from daily methadone use for an average of 18.03 hours.

2- In a Phase-II clinical study, 17 patients on a methadone maintenance program who received a single oral dose of 100 mg ibogaine showed a reduction in their methadone dose, halving it for a minimum duration of one week.

4- The administration of ibogaine at 100 mg was not associated with serious adverse events and did not cause clinically significant alterations in cardiovascular function.

5- Results obtained in a Phase II clinical study showed that the administration of 100 mg of ibogaine causes slight subjective psychoactive effects, which correspond mainly to feelings of relaxation.

6- The metabolomic analysis concluded that certain metabolites —valine, phenylalanine, and lactate— can be considered biomarkers of the overall action of ibogaine. Notably, subjects taking daily high doses of methadone showed marked differences in these substances, suggesting a reversal effect on the modulatory influence on energy metabolism caused by the chronic use of opioids.

Mechanistic and preclinical studies

7- According to a narrative review, ibogaine acts as a multi-target drug, potentially exerting its anti-addictive effects through various mechanisms, including modulation of opioid and glutamate receptors, dopamine and serotonin transporters, or the release of neurotrophic factors.

8- Findings from a preclinical study in mice revealed that four hours after acute ibogaine exposure, there was an up-regulation of genes involved in hormonal pathways (genes encoding oxytocin and vasopressin) and synaptogenesis (*Cbln4* and *Cbln2*). Conversely, genes associated with apoptotic processes (*Usp35*) and endosomal transport (Ap5b1) showed downregulation.

9. Four hours after acute exposure to ibogaine, significant sex differences were observed, with females displaying alterations in 28 genes compared to eight in males.

6.1. General Conclusion

The mechanisms of action of ibogaine potentially involve multiple targets requiring further investigation, particularly those associated with hormonal pathways and neural plasticity, as highlighted in the preclinical study. In a study with humans, a 100 mg dose of ibogaine demonstrated significant efficacy in alleviating symptoms of opioid withdrawal syndrome following methadone abstinence, allowing the methadone dosage to be halved over a week without serious adverse events or clinically significant psychoactive effects. These findings suggest that ibogaine holds promise as a treatment for methadone and other opioid dependencies. Given the available (and sufficient) data on the mechanisms of action and safety of this substance, further research is warranted to fully explore its therapeutic potential.

REFERENCES

7. References

Aharonovich, E., Hasin, D. S., Brooks, A. C., Liu, X., Bisaga, A., & Nunes, E. V. (2006). Cognitive deficits predict low treatment retention in cocaine dependent patients. Drug and alcohol dependence, 81(3), 313–322. https://doi.org/10.1016/j.drugalcdep.2005.08.003

Akbarali, H. I., Inkisar, A., & Dewey, W. L. (2014). Site and mechanism of morphine tolerance in the gastrointestinal tract. Neurogastroenterology and motility, 26(10), 1361–1367. https://doi.org/10.1111/nmo.12443

Alper, K. R., Stajić, M., & Gill, J. R. (2012). Fatalities temporally associated with the ingestion of ibogaine. Journal of forensic sciences, 57(2), 398–412. https://doi.org/10.1111/j.1556-4029.2011.02008.x

Alper, K. R., Lotsof, H. S., & Kaplan, C. D. (2008). The ibogaine medical subculture. Journal of Ethnopharmacology, 115(1), 9–24. https://doi.org/10.1016/j.jep.2007.08.034

Alper, K. R., & Lotsof, H. S. (2007). The use of ibogaine in the treatment of addictions. In: Winkelman, W. J., Roberts, T. B. (Eds.). *Psychedelic medicine*. Praeger.

Alper K. R. (2001). Ibogaine: a review. The Alkaloids. Chemistry and biology, 56, 1–38. https://doi.org/10.1016/s0099-9598(01)56005-8

Alper, K. R., Beal, D., & Kaplan, C. D. (2001). A contemporary history of ibogaine in the United States and Europe. The Alkaloids. Chemistry and biology, 56, 249–281. https://doi.org/10.1016/s0099-9598(01)56018-6

Alper, K. R., Lotsof, H. S., Frenken, G. M., Luciano, D. J., & Bastiaans, J. (1999). Treatment of acute opioid withdrawal with ibogaine. The American journal on addictions, 8(3), 234–242. https://doi.org/10.1080/105504999305848

Amato, L., Davoli, M., Minozzi, S., Ferroni, E., Ali, R., & Ferri, M. (2013). Methadone at tapered doses for the management of opioid withdrawal. The Cochrane database of systematic reviews, 2013(2), CD003409. https://doi.org/10.1002/14651858.CD003409.pub4

American Psychiatric Association (APA). (2022). Diagnostic and statistical manual of mental disorders (5th ed., text rev.). https://doi.org/10.1176/appi.books.9780890425787

Antonio, T., Childers, S. R., Rothman, R. B., Dersch, C. M., King, C., Kuehne, M., Bornmann, W. G., Eshleman, A. J., Janowsky, A., Simon, E. R., Reith, M. E., & Alper, K. (2013). Effect of Iboga alkaloids on μ-opioid receptor-coupled G protein activation. PloS one, 8(10), e77262. https://doi.org/10.1371/journal.pone.0077262

Baldacchino, A., Armanyous, M., Balfour, D. J., Humphris, G., & Matthews, K. (2017). Neuropsychological functioning and chronic methadone use: A systematic review and meta-analysis. Neuroscience and biobehavioral reviews, 73, 23–38. https://doi.org/10.1016/j.neubiorev.2016.11.008

Bart, G. (2013). Methadone population pharmacokinetics: toward understanding the dose-response relationship in the treatment of opiate addiction. PhD dissertation. University of Minnesota.

Baumann, M. H., Rothman, R. B., Pablo, J. P., & Mash, D. C. (2001). In vivo neurobiological effects of ibogaine and its O-desmethyl metabolite, 12-hydroxyibogamine (noribogaine), in rats. The Journal of pharmacology and experimental therapeutics, 297(2), 531–539.

Belgers, M., Leenaars, M., Homberg, J. R., Ritskes-Hoitinga, M., Schellekens, A. F., & Hooijmans,

C. R. (2016). Ibogaine and addiction in the animal model, a systematic review and meta-analysis. Translational psychiatry, 6(5), e826. https://doi.org/10.1038/tp.2016.71

Bell, J., Burrell, T., Indig, D., & Gilmour, S. (2006). Cycling in and out of treatment; participation in methadone treatment in NSW, 1990-2002. Drug and alcohol dependence, 81(1), 55–61. https://doi.org/10.1016/j.drugalcdep.2005.05.010

Belzak, L., & Halverson, J. (2018). The opioid crisis in Canada: a national perspective. La crise des opioïdes au Canada : une perspective nationale. Health promotion and chronic disease prevention in Canada: research, policy and practice, 38(6), 224–233. https://doi.org/10.24095/hpcdp.38.6.02

Binienda, Z., Beaudoin, M. A., Thorn, B. T., Prapurna, D. R., Johnson, J. R., Fogle, C. M., Slikker, W., Jr, & Ali, S. F. (1998). Alteration of electroencephalogram and monoamine concentrations in rat brain following ibogaine treatment. Annals of the New York Academy of Sciences, 844, 265–273.

Bodnar, R. J. (2013). Peptides Endogenous opiates and behavior: 2012. Peptides, 50(12), 55–

95. https://doi.org/10.1016/j.peptides.2013.10.001

Bohn, L. M., Lefkowitz, R. J., & Caron, M. G. (2002). Differential mechanisms of morphine antinociceptive tolerance revealed in (beta)arrestin-2 knock-out mice. The Journal of neuroscience : the official journal of the Society for Neuroscience, 22(23), 10494–10500. https://doi.org/10.1523/JNEUROSCI.22-23-10494.2002

Brondani, M., & Park, P. E. (2011). Methadone and oral health--a brief review. Journal of dental hygiene : JDH, 85(2), 92–98.

Brown, T. K., & Alper, K. (2018). Treatment of opioid use disorder with ibogaine: detoxification and drug use outcomes. The American journal of drug and alcohol abuse, 44(1), 24–36. https://doi.org/10.1080/00952990.2017.1320802

Brown T. K. (2013). Ibogaine in the treatment of substance dependence. Current drug abuse reviews, 6(1), 3–16. https://doi.org/10.2174/15672050113109990001

Brownstein, M. J. (1993). A brief history of opiates, opioid peptides, and opioid receptors. Proceedings of the National Academy of Sciences, 90(12), 5391–5393. https://doi.org/10.1073/pnas.90.12.5391

Brugal, M. T., Domingo-Salvany, A., Puig, R., Barrio, G., García de Olalla, P., & de la Fuente, L. (2005). Evaluating the impact of methadone maintenance programmes on mortality due to overdose and aids in a cohort of heroin users in Spain. Addiction (Abingdon, England), 100(7), 981–989. https://doi.org/10.1111/j.1360-0443.2005.01089.x

Calderon S. N. (2011). Nonpeptidic delta (delta) opioid agonists and antagonists of the diarylmethylpiperazine class: what have we learned? Topics in current chemistry, 299, 121–

140. https://doi.org/10.1007/128_2010_83

Calvey, T. (2019). Human Self-Domestication and the Extended Evolutionary Synthesis of Addiction: How Humans Evolved a Unique Vulnerability. Neuroscience, 419, 100–107. https://doi.org/10.1016/j.neuroscience.2019.09.013

Camlin, T. J., Eulert, D., Thomas Horvath, A., Bucky, S. F., Barsuglia, J. P., & Polanco, M. (2018). A phenomenological investigation into the lived experience of ibogaine and its potential to treat opioid use disorders. Journal of Psychedelic Studies, 2(1), 24-35. https://doi.org/10.1556/2054.2018.004

Cao, J. L., Vialou, V. F., Lobo, M. K., Robison, A. J., Neve, R. L., Cooper, D. C., Nestler, E. J., & Han, M. H. (2010). Essential role of the cAMP-cAMP response-element binding protein pathway in opiate-induced homeostatic adaptations of locus coeruleus neurons. Proceedings of the National Academy of Sciences of the United States of America, 107(39), 17011–17016. https://doi.org/10.1073/pnas.1010077107

Cappendijk, S. L., & Dzoljic, M. R. (1993). Inhibitory effects of ibogaine on cocaine self-administration in rats. European journal of pharmacology, 241(2-3), 261–265. https://doi.org/10.1016/0014-2999(93)90212-z

Carnicella, S., Amamoto, R., & Ron, D. (2009). Excessive alcohol consumption is blocked by glial cell line-derived neurotrophic factor. Alcohol (Fayetteville, N.Y.), 43(1), 35–43. https://doi.org/10.1016/j.alcohol.2008.12.001

Centers for Disease Control and Prevention (CDC). 2023. Understanding the opioid overdose epidemic. Available online in: https://www.cdc.gov/opioids/basics/epidemic.html#:~:text=The%20first%20wave%20began%20with,overdose%20deaths%20involving%20heroin4.

Chen, X. L., Lu, G., Gong, Y. X., Zhao, L. C., Chen, J., Chi, Z. Q., Yang, Y. M., Chen, Z., Li, Q. L., &

Liu, J. G. (2007). Expression changes of hippocampal energy metabolism enzymes contribute to behavioural abnormalities during chronic morphine treatment. Cell research, 17(8), 689–700. https://doi.org/10.1038/cr.2007.63

Cherian, K. N., Keynan, J. N., Anker, L., Faerman, A., Brown, R. E., Shamma, A., Keynan, O.,

Coetzee, J. P., Batail, J. M., Phillips, A., Bassano, N. J., Sahlem, G. L., Inzunza, J., Millar, T., Dickinson, J., Rolle, C. E., Keller, J., Adamson, M., Kratter, I. H., & Williams, N. R. (2024). Magnesium-ibogaine therapy in veterans with traumatic brain injuries. Nature medicine, 30(2), 373–381. https://doi.org/10.1038/s41591-023-02705-w

Christie, M. J. (2009). Cellular neuroadaptations to chronic opioids: tolerance, withdrawal and addiction. British Journal of Pharmacology, 154(2), 384–396. https://doi.org/10.1038/bjp.2008.100

Chunhua, C., Chunhua, X., Megumi, S., & Renyu, L. (2014). Kappa Opioid Receptor Agonist and Brain Ischemia. Translational perioperative and pain medicine, 1(2), 27–34.

Coenye, T. (2021). Do results obtained with RNA-sequencing require independent verification?. Biofilm, 3, 100043. https://doi.org/10.1016/j.bioflm.2021.100043

Crocq, M. A. (2007). Historical and cultural aspects of man's relationship with addictive drugs. Dialogues in clinical neuroscience, 9(4), 355–361. https://doi.org/10.31887/DCNS.2007.9.4/macrocq

Cruz, O. S. (2015). Nonproblematic Illegal Drug Use: Drug Use Management Strategies in a Portuguese Sample. Journal of Drug Issues, 45(2), 133-150. https://doi.org/10.1177/0022042614559842

Dávila, G., & Navarro, J. F. (1998). Efectos conductuales de la metadona. Revista Española de Drogodependencias, 23(2), 109–128.

Davis, A. K., Barsuglia, J. P., Windham-Herman, A. M., Lynch, M., & Polanco, M. (2017). Subjective effectiveness of ibogaine treatment for problematic opioid consumption: Short- and long-term outcomes and current psychological functioning. Journal of psychedelic studies, 1(2), 65–73. https://doi.org/10.1556/2054.01.2017.009

Department of Justice (DOJ). (2020, November 24). *Opioid Manufacturer Purdue Pharma Pleads Guilty to Fraud and Kickback Conspiracies* [Press Release]. https://www.justice.gov/opa/pr/opioid-manufacturer-purdue-pharma-pleads-guilty-fraud-and-kickback-conspiracies

Dole, V. P., & Nyswander, M. E. (1967). Heroin addiction--a metabolic disease. Archives of internal medicine, 120(1), 19–24.

dos Santos, R. G., Bouso, J. C., & Hallak, J. E. C. (2017). The antiaddictive effects of ibogaine: A systematic literature review of human studies. Journal of Psychedelic Studies, 1(1), 20-28. https://doi.org/10.1556/2054.01.2016.001

Drummer, O. H., Opeskin, K., Syrjanen, M., & Cordner, S. M. (1992). Methadone toxicity causing death in ten subjects starting on a methadone maintenance program. The American Journal of Forensic Medicine and Pathology, 13(4), 346–350. https://doi.org/10.1097/00000433-199212000-00017

Dworkin, S. I., Gleeson, S., Meloni, D., Koves, T. R., & Martin, T. J. (1995). Effects of ibogaine on responding maintained by food, cocaine and heroin reinforcement in rats.

Psychopharmacology, 117(3), 257–261. https://doi.org/10.1007/BF02246099

Dzoljic, E. D., Kaplan, C. D., & Dzoljic, M. R. (1988). Effect of ibogaine on naloxone-precipitated withdrawal syndrome in chronic morphine-dependent rats. Archives internationales de pharmacodynamie et de therapie, 294, 64–70.

Everaert, C., Luypaert, M., Maag, J. L. V., Cheng, Q. X., Dinger, M. E., Hellemans, J., & Mestdagh, P. (2017). Benchmarking of RNA-sequencing analysis workflows using whole-transcriptome RT-qPCR expression data. Scientific reports, 7(1), 1559. https://doi.org/10.1038/s41598-017-01617-3

Everitt, B. J., & Wolf, M. E. (2002). Psychomotor stimulant addiction: a neural systems perspective. The Journal of neuroscience: the official journal of the Society for Neuroscience, 22(9), 3312–3320. https://doi.org/10.1523/JNEUROSCI.22-09-03312.2002

Farré, M., Mas, A., Torrens, M., Moreno, V., & Camí, J. (2002). Retention rate and illicit opioid use during methadone maintenance interventions: a meta-analysis. Drug and alcohol dependence, 65(3), 283–290. https://doi.org/10.1016/s0376-8716(01)00171-5

Farrell, M. (1994). Opiate withdrawal. Addiction, 89(11), 1471–1475. https://doi.org/10.1111/j.1360-0443.1994.tb03745.x

Feng, Y., He, X., Yang, Y., Chao, D., H. Lazarus, L., & Xia, Y. (2012). Current Research on Opioid Receptor Function. Current Drug Targets, 13(2), 230–246. https://doi.org/10.2174/138945012799201612

Fonseca, F. (2010). Pharmacogenomic study of opioid addicts in methadone treatment. Tesis doctoral. Universitat Pompeu Fabra, Barcelona, Spain.

Foster, D. J., Somogyi, A. A., & Bochner, F. (1999). Methadone N-demethylation in human liver microsomes: lack of stereoselectivity and involvement of CYP3A4. British journal of clinical pharmacology, 47(4), 403–412. https://doi.org/10.1046/j.1365-2125.1999.00921.x

Francés, B., Gout, R., Cros, J., & Zajac, J. M. (1992). Effects of ibogaine on naloxone-precipitated withdrawal in morphine-dependent mice. Fundamental & clinical pharmacology, 6(8-9), 327–332. https://doi.org/10.1111/j.1472-8206.1992.tb00127.x

Fristad, I., Berggreen, E., & Haug, S. R. (2006). Delta (δ) opioid receptors in small and medium-sized trigeminal neurons supporting the dental pulp of rats. Archives of Oral Biology, 51(4), 273–

281. https://doi.org/10.1016/j.archoralbio.2005.08.007

Fuente, L., Brugal, T. M., Domingo-Salvany, A., Bravo, M. J., Neira-León, M., & Barrio, G. (2006). Más de treinta años de drogas ilegales en España: una amarga historia con algunos consejos para el futuro. Revista Española de Salud Pública, 80(5), 505-520. http://scielo.isciii.es/scielo.php?script=sci_arttext&pid=S1135-57272006000500009&lng=es&tlng=es

Gamella, J. F. (1994). The Spread of Intravenous Drug Use and AIDS in a Neighborhood in Spain. Medical Anthropology Quarterly, 8(2), 131-160.

https://doi.org/10.1525/maq.1994.8.2.02a00010

Gibson, D. R., Flynn, N. M., & McCarthy, J. J. (1999). Effectiveness of methadone treatment in reducing HIV risk behavior and HIV seroconversion among injecting drug users. AIDS (London, England), 13(14), 1807–1818. https://doi.org/10.1097/00002030-199910010-00002

Glick, S. D., Kuehne, M. E., Raucci, J., Wilson, T. E., Larson, D., Keller, R. W., Jr, & Carlson, J. N. (1994). Effects of iboga alkaloids on morphine and cocaine self-administration in rats: relationship to tremorigenic effects and to effects on dopamine release in nucleus accumbens and striatum. Brain research, 657(1-2), 14–22. https://doi.org/10.1016/0006-8993(94)90948-2

Glick, S. D., Rossman, K., Rao, N. C., Maisonneuve, I. M., & Carlson, J. N. (1992). Effects of ibogaine on acute signs of morphine withdrawal in rats: independence from tremor. Neuropharmacology, 31(5), 497–500. https://doi.org/10.1016/0028-3908(92)90089-8

Glick, S. D., Rossman, K., Steindorf, S., Maisonneuve, I. M., & Carlson, J. N. (1991). Effects and aftereffects of ibogaine on morphine self-administration in rats. European journal of pharmacology, 195(3), 341–345. https://doi.org/10.1016/0014-2999(91)90474-5

Glue, P., Lockhart, M., Lam, F., Hung, N., Hung, C. T., & Friedhoff, L. (2015a). Ascending-dose study of noribogaine in healthy volunteers: pharmacokinetics, pharmacodynamics, safety, and tolerability. Journal of clinical pharmacology, 55(2), 189–194. https://doi.org/10.1002/jcph.404

Glue, P., Winter, H., Garbe, K., Jakobi, H., Lyudin, A., Lenagh-Glue, Z., & Hung, C. T. (2015b). Influence of CYP2D6 activity on the pharmacokinetics and pharmacodynamics of a single 20 mg

dose of ibogaine in healthy volunteers. Journal of clinical pharmacology, 55(6), 680–687. https://doi.org/10.1002/jcph.471

Goutarel, R., Gollnhofer, O., & Sillans, R. (1993). Pharmacodynamics and therapeutic applications of iboga and ibogaine. Psychedelic Monographs and Essays, 6, 70–111.

Graham, D. L., Edwards, S., Bachtell, R. K., DiLeone, R. J., Rios, M., & Self, D. W. (2007). Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. Nature neuroscience, 10(8), 1029–1037. https://doi.org/10.1038/nn1929

Grissinger, M. (2011). Keeping patients safe from methadone overdoses. P & T : a peer-reviewed journal for formulary management, 36(8), 462–466.

Gutiérrez-Cáceres, S., Pedraz-Marcos, A., & Serrano-Gallardo, P. (2019). Envejecer en un programa de mantenimiento con metadona. Una perspectiva desde el marco de los determinantes sociales de la salud. Revista Española de Salud Pública, 93, e201908048.

Harrison, C. (2013). Trial watch: opioid receptor blocker shows promise in Phase II depression trial. Nature Reviews. Drug Discovery, 12(6), 415. https://doi.org/10.1038/nrd4028

He, D. Y., & Ron, D. (2006). Autoregulation of glial cell line-derived neurotrophic factor expression: implications for the long-lasting actions of the anti-addiction drug, Ibogaine. FASEB journal: official publication of the Federation of American Societies for Experimental Biology, 20(13), 2420–2422. https://doi.org/10.1096/fj.06-6394fje

He, D. Y., McGough, N. N., Ravindranathan, A., Jeanblanc, J., Logrip, M. L., Phamluong, K., Janak,

P. H., & Ron, D. (2005). Glial cell line-derived neurotrophic factor mediates the desirable actions of the anti-addiction drug ibogaine against alcohol consumption. The Journal of neuroscience : the official journal of the Society for Neuroscience, 25(3), 619–628. https://doi.org/10.1523/JNEUROSCI.3959-04.2005

Henstra, M., Wong, L., Chahbouni, A., Swart, N., Allaart, C., & Sombogaard, F. (2017). Toxicokinetics of ibogaine and noribogaine in a patient with prolonged multiple cardiac arrhythmias after ingestion of internet purchased ibogaine. Clinical toxicology (Philadelphia, Pa.), 55(6), 600–602. https://doi.org/10.1080/15563650.2017.1287372

Holmstrand, J., Anggård, E., & Gunne, L. M. (1978). Methadone maintenance: plasma levels and therapeutic outcome. Clinical pharmacology and therapeutics, 23(2), 175–180. https://doi.org/10.1002/cpt1978232175

Horns, W. H., Rado, M., & Goldstein, A. (1975). Plasma levels and symptom complaints in patients maintained on daily dosage of methadone hydrochloride. Clinical pharmacology and therapeutics, 17(6), 636–649. https://doi.org/10.1002/cpt1975176636

Hu, X., Huang, F., Szymusiak, M., Liu, Y., & Wang, Z. J. (2015). Curcumin attenuates opioid tolerance and dependence by inhibiting Ca2+/calmodulin-dependent protein kinase II α activity. The Journal of pharmacology and experimental therapeutics, 352(3), 420–428. https://doi.org/10.1124/jpet.114.219303

Hughes T. R. (2009). 'Validation' in genome-scale research. Journal of biology, 8(1), 3. https://doi.org/10.1186/jbiol104

Humphreys, K., Shover, C. L., Andrews, C. M., Bohnert, A. S. B., Brandeau, M. L., Caulkins, J. P., Chen, J. H., Cuéllar, M. F., Hurd, Y. L., Juurlink, D. N., Koh, H. K., Krebs, E. E., Lembke, A.,

Mackey, S. C., Larrimore Ouellette, L., Suffoletto, B., & Timko, C. (2022). Responding to the opioid crisis in North America and beyond: recommendations of the Stanford-Lancet Commission. Lancet (London, England), 399(10324), 555–604. https://doi.org/10.1016/S0140-6736(21)02252-2

Inturrisi C. E. (2005). Pharmacology of methadone and its isomers. Minerva anestesiologica, 71(7-8), 435–437.

Inturrisi, C. E. (1997). Preclinical evidence for a role of glutamatergic systems in opioid tolerance and dependence. Seminars in neuroscience, 9, 110–119. https://doi.org/10.1006/smns.1997.0111

Isbell, H. (1977). The search for a nonaddicting analgesic: has it been worth it? Clin Pharmacol Ther 22(4), 377-384.

Isbell, H., & Wikler, A. (1947). Tolerance and addiction liability of 6-dimethylamino-4-4-diphenylheptanone-3 (methadon). Journal of the American Medical Association, 135(14), 888–894. https://doi.org/10.1001/jama.1947.02890140008003

Jentsch, J. D., Olausson, P., De La Garza, R., 2nd, & Taylor, J. R. (2002). Impairments of reversal learning and response perseveration after repeated, intermittent cocaine administrations to monkeys. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 26(2), 183–190. https://doi.org/10.1016/S0893-133X(01)00355-4

Jones, C. M., Logan, J., Gladden, R. M., & Bohm, M. K. (2015). Vital Signs: Demographic and Substance Use Trends Among Heroin Users - United States, 2002-2013. MMWR. Morbidity and mortality weekly report, 64(26), 719–725.

Jutkiewicz, E. M., Baladi, M. G., Folk, J. E., Rice, K. C., & Woods, J. H. (2006). The convulsive and electroencephalographic changes produced by nonpeptidic delta-opioid agonists in rats: comparison with pentylenetetrazol. The Journal of Pharmacology and Experimental Therapeutics, 317(3), 1337–48. https://doi.org/10.1124/jpet.105.095810

Kalivas, P. W., & O'Brien, C. (2008). Drug addiction as a pathology of staged neuroplasticity. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology, 33(1), 166–180. https://doi.org/10.1038/sj.npp.1301564

Keefe, P. R. (2021). *El imperio del dolor: La historia secreta de la dinastía Sackler.* Reservoir Books.

Kirby, L. G., Zeeb, F. D., & Winstanley, C. A. (2011). Contributions of serotonin in addiction vulnerability. Neuropharmacology, 61(3), 421–432. https://doi.org/10.1016/j.neuropharm.2011.03.022

Kivell, B. M., Ewald, A. W., & Prisinzano, T. E. (2014). Salvinorin A analogs and other κ-opioid receptor compounds as treatments for cocaine abuse. Advances in pharmacology (San Diego, Calif.), 69, 481–511. https://doi.org/10.1016/B978-0-12-420118-7.00012-3

Knuijver, T., Ter Heine, R., Schellekens, A. F. A., Heydari, P., Lucas, L., Westra, S., Belgers, M., van Oosteren, T., Verkes, R. J., & Kramers, C. (2024). The pharmacokinetics and pharmacodynamics of ibogaine in opioid use disorder patients. Journal of psychopharmacology (Oxford, England), 38(5), 481–488. https://doi.org/10.1177/02698811241237873

Knuijver, T., Schellekens, A., Belgers, M., Donders, R., van Oosteren, T., Kramers, K., & Verkes,

R. (2022). Safety of ibogaine administration in detoxification of opioid-dependent individuals: a

descriptive open-label observational study. Addiction (Abingdon, England), 117(1), 118–128. https://doi.org/10.1111/add.15448

Koenig, X., & Hilber, K. (2015). The anti-addiction drug ibogaine and the heart: a delicate relation. Molecules (Basel, Switzerland), 20(2), 2208–2228. https://doi.org/10.3390/molecules20022208

Kohek, M., Ohren, M., Hornby, P., Alcázar-Córcoles, M. Á. & Bouso, J. C. (2020). The Ibogaine Experience: A Qualitative Study on the Acute Subjective Effects of Ibogaine. Anthropology of Consciousness, 31, 91–119. https://doi.org/10.1111/anoc.12119

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 35(1), 217–238. https://doi.org/10.1038/npp.2009.110

Koskela, M., Piepponen, T. P., Lindahl, M., Harvey, B. K., Andressoo, J. O., Võikar, V., & Airavaara, M. (2021). The overexpression of GDNF in nucleus accumbens suppresses alcohol-seeking behavior in group-housed C57Bl/6J female mice. Journal of biomedical science, 28(1),

87. https://doi.org/10.1186/s12929-021-00782-y

Kosten, T. R., & Baxter, L. E. (2019). Effective management of opioid withdrawal symptoms: A gateway to opioid dependence treatment. The American journal on addictions, 28(2), 55–62. https://doi.org/10.1111/ajad.12862

Kosten, T. R., & O'Connor, P. G. (2003). Management of drug and alcohol withdrawal. The New England journal of medicine, 348(18), 1786–1795. https://doi.org/10.1056/NEJMra020617

Lewis, R. G., Florio, E., Punzo, D., & Borrelli, E. (2021). The Brain's Reward System in Health and Disease. Advances in experimental medicine and biology, 1344, 57–69. https://doi.org/10.1007/978-3-030-81147-1_4

Luz, M., & Mash, D. C. (2022). Reply to Knuijver et al. on the 'Safety of ibogaine administration in detoxification of opioid-dependent individuals: a descriptive open-label observational study'. Addiction (Abingdon, England), 117(3), 835–836. https://doi.org/10.1111/add.15694

Ly, C., Greb, A. C., Cameron, L. P., Wong, J. M., Barragan, E. V., Wilson, P. C., Burbach, K. F., Soltanzadeh Zarandi, S., Sood, A., Paddy, M. R., Duim, W. C., Dennis, M. Y., McAllister, A. K., Ori-McKenney, K. M., Gray, J. A., & Olson, D. E. (2018). Psychedelics Promote Structural and Functional Neural Plasticity. Cell reports, 23(11), 3170–3182. https://doi.org/10.1016/j.celrep.2018.05.022

Lyden, J., & Binswanger, I. A. (2019). The United States opioid epidemic. Seminars in perinatology, 43(3), 123–131. https://doi.org/10.1053/j.semperi.2019.01.001

Maciulaitis, R., Kontrimaviciute, V., Bressolle, F. M., & Briedis, V. (2008). Ibogaine, an anti-addictive drug: pharmacology and time to go further in development. A narrative review. Human & experimental toxicology, 27(3), 181–194. https://doi.org/10.1177/0960327107087802

Madison, D. V., & Nicoll, R. A. (1988). Enkephalin hyperpolarizes interneurones in the rat hippocampus. The Journal of physiology, 398, 123–130. https://doi.org/10.1113/jphysiol.1988.sp017033

Maillet, E. L., Milon, N., Heghinian, M. D., Fishback, J., Schürer, S. C., Garamszegi, N., & Mash,

D. C. (2015). Noribogaine is a G-protein biased κ-opioid receptor agonist. Neuropharmacology, 99, 675–688. https://doi.org/10.1016/j.neuropharm.2015.08.032

Martin, W. R. (1979). History and development of mixed opioid agonists, partial agonists and antagonists. British Journal of Clinical Pharmacology, 7(S3), 273S–279S. https://doi.org/10.1111/j.1365-2125.1979.tb04700.x

Martínez-Oró, D. P. (2019). Opioides en España. Ni repunte de heroína ni crisis de opioides a la americana. https://pnsd.sanidad.gob.es/noticiasEventos/actualidad/2019_Actualidadpublica/pdf/20191211_Opioides_en_Espana_Ni_repunte_heroina_ni_crisis_americana.pdf

Martínez-Oró, D. P., & Pallarés, J. (2013). Riesgos, daños y placeres. In D. P. Martínez-Oró & J. Pallarés (Eds), *De riesgos y placeres* (pp. 23-36). Editorial Milenio.

Martins, S. S., Sampson, L., Cerdá, M., & Galea, S. (2015). Worldwide Prevalence and Trends in Unintentional Drug Overdose: A Systematic Review of the Literature. American journal of public health, 105(11), e29–e49. https://doi.org/10.2105/AJPH.2015.302843

Marton, S., González, B., Rodríguez-Bottero, S., Miquel, E., Martínez-Palma, L., Pazos, M., Prieto, J. P., Rodríguez, P., Sames, D., Seoane, G., Scorza, C., Cassina, P., & Carrera, I. (2019). Ibogaine Administration Modifies GDNF and BDNF Expression in Brain Regions Involved in Mesocorticolimbic and Nigral Dopaminergic Circuits. Frontiers in pharmacology, 10, 193. https://doi.org/10.3389/fphar.2019.00193

Mash D. C. (2023). IUPHAR - invited review - Ibogaine - A legacy within the current renaissance of psychedelic therapy. Pharmacological research, 190, 106620. https://doi.org/10.1016/j.phrs.2022.106620

Mash, D. C., Duque, L., Page, B., & Allen-Ferdinand, K. (2018). Ibogaine Detoxification Transitions Opioid and Cocaine Abusers Between Dependence and Abstinence: Clinical Observations and Treatment Outcomes. Frontiers in pharmacology, 9, 529. https://doi.org/10.3389/fphar.2018.00529

Mash, D. C. (2010). Ibogaine therapy for substance abuse disorders. In: Brizer, D., Castaneda,

R. (Eds.). *Clinical addiction psychiatry*. Cambridge University Press.

Mash, D. C., Kovera, C. A., Pablo, J., Tyndale, R., Ervin, F. R., Kamlet, J. D., & Hearn, W. L. (2001). Ibogaine in the treatment of heroin withdrawal. The Alkaloids. Chemistry and biology, 56, 155–

171. https://doi.org/10.1016/s0099-9598(01)56012-5

Mash, D. C., Kovera, C. A., Buck, B. E., Norenberg, M. D., Shapshak, P., Hearn, W. L., & Sanchez-Ramos, J. (1998). Medication development of ibogaine as a pharmacotherapy for drug dependence. Annals of the New York Academy of Sciences, 844, 274–292.

Matsui, A., Jarvie, B. C., Robinson, B. G., Hentges, S. T., & Williams, J. T. (2014). Separate GABA afferents to dopamine neurons mediate acute action of opioids, development of tolerance, and expression of withdrawal. Neuron, 82(6), 1346–1356. https://doi.org/10.1016/j.neuron.2014.04.030

Mattick, R. P., Breen, C., Kimber, J., & Davoli, M. (2014). Buprenorphine maintenance versus placebo or methadone maintenance for opioid dependence. The Cochrane database of systematic reviews, 2014(2), CD002207. https://doi.org/10.1002/14651858.CD002207.pub4

McFarland, K., & Kalivas, P. W. (2001). The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. The Journal of neuroscience: the official journal of the Society for Neuroscience, 21(21), 8655–8663. https://doi.org/10.1523/JNEUROSCI.21-21-08655.2001

Melis, M., Spiga, S., & Diana, M. (2005). The dopamine hypothesis of drug addiction: hypodopaminergic state. International review of neurobiology, 63, 101–154. https://doi.org/10.1016/S0074-7742(05)63005-X

Mercadante, S., & Bruera, E. (2018). Methadone as a First-Line Opioid in Cancer Pain Management: A Systematic Review. Journal of pain and symptom management, 55(3), 998–1003. https://doi.org/10.1016/j.jpainsymman.2017.10.017

Mercer, S. L., & Coop, A. (2011). Opioid analgesics and P-glycoprotein efflux transporters: a potential systems-level contribution to analgesic tolerance. Current topics in medicinal chemistry, 11(9), 1157–1164. https://doi.org/10.2174/156802611795371288

Michele, B. M. M., & Sophie, A. A. (2023). A review of the mechanisms involved in the neuroprotection and neurotoxicity of Iboga alkaloids. Pharmacological research – Natural products, 1, 100006. https://doi.org/10.1016/j.prenap.2023.100006

Morgan J. P. (1985). American opiophobia: customary underutilization of opioid analgesics. Advances in alcohol & substance abuse, 5(1-2), 163–173. https://doi.org/10.1300/J251v05n01_11

Muldoon, P. P., Jackson, K. J., Perez, E., Harenza, J. L., Molas, S., Rais, B., Anwar, H., Zaveri, N. T., Maldonado, R., Maskos, U., McIntosh, J. M., Dierssen, M., Miles, M. F., Chen, X., De Biasi, M., & Damaj, M. I. (2014). The α3β4* nicotinic ACh receptor subtype mediates physical dependence to morphine: mouse and human studies. British journal of pharmacology, 171(16), 3845–3857. https://doi.org/10.1111/bph.12741

Mutolo, D., Bongianni, F., Einum, J., Dubuc, R., & Pantaleo, T. (2007). Opioid-induced depression in the lamprey respiratory network. Neuroscience, 150(3), 720–729. https://doi.org/10.1016/j.neuroscience.2007.09.028

Nagase, H., & Saitoh, A. (2020). Research and development of κ opioid receptor agonists and δ opioid receptor agonists. Pharmacology & therapeutics, 205, 107427.

Nagase, H., Wakita, H., Kawai, K., Endo, T., & Matsumoto, O. (1992). Toray, Isoquinoline derivatives and uses thereof. JP04275288.

Nardou, R., Sawyer, E., Song, Y. J., Wilkinson, M., Padovan-Hernandez, Y., de Deus, J. L., Wright, N., Lama, C., Faltin, S., Goff, L. A., Stein-O'Brien, G. L., & Dölen, G. (2023). Psychedelics reopen the social reward learning critical period. Nature, 618(7966), 790–798. https://doi.org/10.1038/s41586-023-06204-3

Nicholson, T., Duncan, D., & White, J. (2002). Is recreational drug use normal? Journal of Substance Use, 7, 116-123. https://doi.org/10.1080/14659890210150162

Noller, G. E., Frampton, C. M., & Yazar-Klosinski, B. (2018). Ibogaine treatment outcomes for opioid dependence from a twelve-month follow-up observational study. The American journal of drug and alcohol abuse, 44(1), 37–46. https://doi.org/10.1080/00952990.2017.1310218

Observatorio Español de las Drogas y las Adicciones (OEDA). Informe 2022. Alcohol, tabaco y drogas ilegales en España. Madrid: Ministerio de Sanidad. Delegación del Gobierno para el Plan Nacional sobre Drogas.

Olds, J., & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. Journal of comparative and physiological psychology, 47(6), 419–427. https://doi.org/10.1037/h0058775

Ona, G., Sampedro, F., Romero, S., Valle, M., Camacho, V., Migliorelli, C., Mañanas, M. Á., Antonijoan, R. M., Puntes, M., Coimbra, J., Ballester, M. R., Garrido, M., & Riba, J. (2022). The Kappa Opioid Receptor and the Sleep of Reason: Cortico-Subcortical Imbalance Following Salvinorin-A. The international journal of neuropsychopharmacology, 25(1), 54–63. https://doi.org/10.1093/ijnp/pyab063

Ornell, F., Hansen, F., Schuch, F. B., Pezzini Rebelatto, F., Tavares, A. L., Scherer, J. N., Valerio, A. G., Pechansky, F., Paim Kessler, F. H., & von Diemen, L. (2018). Brain-derived neurotrophic factor in substance use disorders: A systematic review and meta-analysis. Drug and alcohol dependence, 193, 91–103. https://doi.org/10.1016/j.drugalcdep.2018.08.036

Paananen, J., & Fortino, V. (2020). An omics perspective on drug target discovery platforms. Briefings in bioinformatics, 21(6), 1937–1953. https://doi.org/10.1093/bib/bbz122

Paskulin, R., Jamnik, P., Zivin, M., Raspor, P., & Strukelj, B. (2006). Ibogaine affects brain energy metabolism. European journal of pharmacology, 552(1-3), 11–14. https://doi.org/10.1016/j.ejphar.2006.09.008

Pathan, H., & Williams, J. (2012). Basic opioid pharmacology: an update. British journal of pain, 6(1), 11–16. https://doi.org/10.1177/2049463712438493

Pearl, S. M., Hough, L. B., Boyd, D. L., & Glick, S. D. (1997). Sex differences in ibogaine antagonism of morphine-induced locomotor activity and in ibogaine brain levels and metabolism. Pharmacology, biochemistry, and behavior, 57(4), 809–815. https://doi.org/10.1016/s0091-3057(96)00383-8

Pedrero-Pérez, E. J. (2015). ¿Qué es y qué no es la adicción? Evidencia científica disponible. REICA. https://doi.org/10.13140/RG.2.1.2217.5208

Pfeiffer, A., Brantl, V., & Herz, A. (1986). Psychotomimesis Mediated by k opioid receptors. Science, 233, 774–776. https://doi.org/10.1126/science.3016896

Phisalix, M. C. (1901). Action Physiologique de l'ibogaïne. Comptes rendus des séances de la Société de biologie, 53, 1077–1081.

Piercey, M. F., Lahti, R. A., Schroeder, L. A., Einspahr, F. J., & Barsuhn, C. (1982). U-50488H, a pure kappa receptor agonist with spinal analgesic loci in the mouse. Life sciences, 31(12-13), 1197–1200. https://doi.org/10.1016/0024-3205(82)90341-1

Popik, P., Layer, R. T., & Skolnick, P. (1995). 100 years of ibogaine: neurochemical and pharmacological actions of a putative anti-addictive drug. Pharmacological reviews, 47(2), 235–253.

Popik, P., Layer, R. T., Fossom, L. H., Benveniste, M., Geter-Douglass, B., Witkin, J. M., & Skolnick, P. (1995). NMDA antagonist properties of the putative antiaddictive drug, ibogaine. The Journal of pharmacology and experimental therapeutics, 275(2), 753–760.

Porter, J., & Jick, H. (1980). Addiction rare in patients treated with narcotics. The New England journal of medicine, 302(2), 123. https://doi.org/10.1056/nejm198001103020221

Rezvani, A. H., Overstreet, D. H., & Lee, Y. W. (1995). Attenuation of alcohol intake by ibogaine in three strains of alcohol-preferring rats. Pharmacology, biochemistry, and behavior, 52(3), 615–620. https://doi.org/10.1016/0091-3057(95)00152-m

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain research. Brain research reviews, 18(3), 247–291. https://doi.org/10.1016/0165-0173(93)90013-p

Rodriguez, M., Ortega, I., Soengas, I., Suarez, E., Lukas, J. C., & Calvo, R. (2004). Effect of P-glycoprotein inhibition on methadone analgesia and brain distribution in the rat. The Journal of pharmacy and pharmacology, 56(3), 367–374. https://doi.org/10.1211/0022357022782

Rodríguez-Cano, B. J., Kohek, M., Ona, G., Alcázar-Córcoles, M. Á., Dos Santos, R. G., Hallak, J.

E. C., & Bouso, J. C. (2023). Underground ibogaine use for the treatment of substance use disorders: A qualitative analysis of subjective experiences. Drug and alcohol review, 42(2), 401–414. https://doi.org/10.1111/dar.13587

Schneider, J., & Rinehart, R. (1957). Analysis of the cardiovascular action of ibogaine hydrochloride. Archives Internationales de Pharmacodynamie et de therapie, 110, 92–102.

Schoenbaum, G., Saddoris, M. P., Ramus, S. J., Shaham, Y., & Setlow, B. (2004). Cocaine-experienced rats exhibit learning deficits in a task sensitive to orbitofrontal cortex lesions. The European journal of neuroscience, 19(7), 1997–2002. https://doi.org/10.1111/j.1460-9568.2004.03274.x

Schuckit M. A. (2016). Treatment of Opioid-Use Disorders. The New England journal of medicine, 375(4), 357–368. https://doi.org/10.1056/NEJMra1604339

Schultz W. (2007). Multiple dopamine functions at different time courses. Annual review of neuroscience, 30, 259–288. https://doi.org/10.1146/annurev.neuro.28.061604.135722

Sershen, H., & Lajtha, A. (1994). Ibogaine reduces preference for cocaine consumption in C57BL/6By mice. Pharmacology, biochemistry, and behavior, 47(1), 13–19. https://doi.org/10.1016/0091-3057(94)90105-8

Sertürner, F. W. (1817). Über das Morphium, eine neue salzfähige Grundlage, und die Mekonsäure, als Hauptbestandteile des Opiums. Annalen der Physik, 25(1), 56-90.

Shaham, Y., Shalev, U., Lu, L., de Wit, H., & Stewart, J. (2003). The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology, 168(1-2), 3–20. https://doi.org/10.1007/s00213-002-1224-x

Sharpe, L. G., & Jaffe, J. H. (1990). Ibogaine fails to reduce naloxone-precipitated withdrawal in the morphine-dependent rat. Neuroreport, 1(1), 17–19. https://doi.org/10.1097/00001756-199009000-00005

Shirazi, M. (2011). *Resalehe afyunieh*. Corrected by Choopani R, Panahee V, Sadeghpour O. Tehran: Traditional Iranian Medicine Press.

Siegel, R. K. (2005). *Intoxication: The Universal Drive for Mind-Altering Substances.* Inner Traditions - Bear & Company.

Silverman S. M. (2009). Opioid induced hyperalgesia: clinical implications for the pain practitioner. Pain physician, 12(3), 679–684.

Sim S. K. (1973). Methadone. Canadian Medical Association journal, 109(7), 615–619.

Spreen, L. A., Dittmar, E. N., Quirk, K. C., & Smith, M. A. (2022). Buprenorphine initiation strategies for opioid use disorder and pain management: A systematic review. Pharmacotherapy, 42(5), 411–427. https://doi.org/10.1002/phar.2676

Strang, J., McCambridge, J., Best, D., Beswick, T., Bearn, J., Rees, S., & Gossop, M. (2003). Loss of tolerance and overdose mortality after inpatient opiate detoxification: follow up study. BMJ (Clinical research ed.), 326(7396), 959–960. https://doi.org/10.1136/bmj.326.7396.959

Tatalović, N., Vidonja Uzelac, T., Mijović, M., Koželj, G., Nikolić-Kokić, A., Oreščanin Dušić, Z., Bresjanac, M., & Blagojević, D. (2021). Ibogaine Has Sex-Specific Plasma Bioavailability, Histopathological and Redox/Antioxidant Effects in Rat Liver and Kidneys: A Study on Females. Life (Basel, Switzerland), 12(1), 16. https://doi.org/10.3390/life12010016

Taylor, G. T., & Manzella, F. (2016). Kappa Opioids, Salvinorin A and Major Depressive Disorder. Current Neuropharmacology, 14(2), 165–176. https://doi.org/10.2174/1570159x13666150727220944

Torregrossa, M. M., Jutkiewicz, E. M., Mosberg, H. I., Balboni, G., Watson, S. J., & Woods, J. H. (2006). Peptidic delta opioid receptor agonists produce antidepressant-like effects in the forced swim test and regulate BDNF mRNA expression in rats. Brain Research, 1069(1), 172–181. https://doi.org/10.1016/j.brainres.2005.11.005

Torres-Lockhart, K. E., Lu, T. Y., Weimer, M. B., Stein, M. R., & Cunningham, C. O. (2022). Clinical Management of Opioid Withdrawal. Addiction (Abingdon, England), 117(9), 2540–2550. https://doi.org/10.1111/add.15818

Tournier, N., Chevillard, L., Megarbane, B., Pirnay, S., Scherrmann, J. M., & Declèves, X. (2010). Interaction of drugs of abuse and maintenance treatments with human P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2). The international journal of neuropsychopharmacology, 13(7), 905–915. https://doi.org/10.1017/S1461145709990848

Trescot, A. M., Datta, S., Lee, M., & Hansen, H. (2008). Opioid pharmacology. Pain physician, 11(2 Suppl), S133–S153.

United States Patent Office (1957). Tabernanthine, ibogaine containing analgesic compositions. Available online in: https://patentimages.storage.googleapis.com/ba/89/87/82bbfbe3ffd993/US2817623.pdf

Verebely, K., & Kutt, H. (1975). Methadone plasma levels in maintenance patients: the effect of dose omission. Research communications in chemical pathology and pharmacology, 11(3), 373–386.

Victorri-Vigneau, C., Verstuyft, C., Bouquié, R., Laforgue, E. J., Hardouin, J. B., Leboucher, J., Le Geay, B., Dano, C., Challet-Bouju, G., & Grall-Bronnec, M. (2019). Relevance of CYP2B6 and CYP2D6 genotypes to methadone pharmacokinetics and response in the OPAL study. British journal of clinical pharmacology, 85(7), 1538–1543. https://doi.org/10.1111/bcp.13936

Volkow, N. D., Michaelides, M., & Baler, R. (2019). The Neuroscience of Drug Reward and Addiction. Physiological reviews, 99(4), 2115–2140. https://doi.org/10.1152/physrev.00014.2018

Volkow, N. D., & Morales, M. (2015). The Brain on Drugs: From Reward to Addiction. Cell, 162(4), 712–725. https://doi.org/10.1016/j.cell.2015.07.046

Walsh, S. L., Strain, E. C., Abreu, M. E., & Bigelow, G. E. (2001). Enadoline, a selective kappa opioid agonist: Comparison with butorphanol and hydromorphone in humans.

Psychopharmacology, 157(2), 151–162. https://doi.org/10.1007/s002130100788

Wang, J. S., Ruan, Y., Taylor, R. M., Donovan, J. L., Markowitz, J. S., & DeVane, C. L. (2004). Brain penetration of methadone (R)- and (S)-enantiomers is greatly increased by P-glycoprotein deficiency in the blood-brain barrier of Abcb1a gene knockout mice.

Psychopharmacology, 173(1-2), 132–138. https://doi.org/10.1007/s00213-003-1718-1

Wasko, M. J., Witt-Enderby, P. A., & Surratt, C. K. (2018). DARK Classics in Chemical Neuroscience: Ibogaine. ACS chemical neuroscience, 9(10), 2475–2483. https://doi.org/10.1021/acschemneuro.8b00294

Weiss AJ, Heslin KC, Barrett ML, Rwaida Izar, and Arlene S. Bierman, Opioid-Related Inpatient Stays and Emergency Department Visits Among Patients Aged 65 Years and Older, 2010 and 2015. 2018 Sep 18. In: Healthcare Cost and Utilization Project (HCUP) Statistical Briefs [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2006 Feb-. Statistical Brief #244. Available from: https://www.ncbi.nlm.nih.gov/books/NBK534106/

Wilkins, C., dos Santos, R.G., Solá, J., Aixalà, M., Cura, P., Moreno, E., Alcázar-Córcoles, M. A., Hallak, J. E. C., & Bouso, J.C. (2017). Detoxification from methadone using low, repeated, and increasing doses of ibogaine: A case report. Journal of Psychedelic Studies, 1(1), 29-34. https://doi.org/10.1556/2054.01.2017.005

Yaden, D. B., & Griffiths, R. R. (2020). The subjective effects of psychedelics are necessary for their enduring therapeutic effects. ACS Pharmacology & Translational Science, 4(2), 568–572. https://doi.org/10.1021/acsptsci.0c00194

Zadina, J. E., Hackler, L., Ge, L.-J., & Kastin, A. J. (1997). A potent and selective endogenous agonist for the μ-opiate receptor. Nature. https://doi.org/10.1038/386499a0

Zhang, J. J., Song, C. G., Dai, J. M., Li, L., Yang, X. M., & Chen, Z. N. (2022). Mechanism of opioid addiction and its intervention therapy: Focusing on the reward circuitry and mu-opioid receptor. MedComm, 3(3), e148. https://doi.org/10.1002/mco2.148

Zhu, H., & Ho, I. K. (1998). NMDA-R1 antisense oligonucleotide attenuates withdrawal signs from morphine. European journal of pharmacology, 352(2-3), 151–156. https://doi.org/10.1016/s0014-2999(98)00367-7

Zieglgänsberger, W., French, E. D., Siggins, G. R., & Bloom, F. E. (1979). Opioid peptides may excite hippocampal pyramidal neurons by inhibiting adjacent inhibitory interneurons. Science (New York, N.Y.), 205(4404), 415–417. https://doi.org/10.1126/science.451610

Zubaran, C., Shoaib, M., Stolerman, I. P., Pablo, J., & Mash, D. C. (1999). Noribogaine generalization to the ibogaine stimulus: correlation with noribogaine concentration in rat brain. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology, 21(1), 119–126. https://doi.org/10.1016/S0893-133X(99)00003-2