# Behavioral Pharmacology of New Psychoactive Substance (NPS): Cannabinoids

Snehal Namdeo*, Chandrika Ahirwal

Faculty of Pharmacy Bharti Vishwavidyalaya, Durg – 491001, Chhattisgarh, India

**Abstract:**

The rapid emergence of New Psychoactive substance (NPS) represents a significant challenge for public health, law enforcement, and regulatory bodies worldwide. New Psychoactive Substance (NPS) are synthetic substance designed to mimic the effects of traditional illicit drugs, such as cocaine, cannabis, stimulants, or ecstasy. While circumventing legal controls. Despite their increasing prevalence, these substances are often understudied and their behavioral pharmacology remain poorly understood. This thesis explores the behavioral pharmacology of NPS focusing on their mechanism of action, their effects on behavior and their associated risk with their use. Key classes of NPS, including synthetic cannabinoids, cathinones, hallucinogen and stimulants, are examined in terms of their neuropharmacological interaction and their impact on mood, cognition, and motor behavior. Animal models and human case studies are reviewed to better understand the addictive potential, neurotoxicity, and psychiatric disturbance linked to NPS use, in addition, the thesis proposes harm reduction strategies for minimizing their ADR. Inform public health strategies to address the growing global threat by this substance. Synthetic cannabinoids (SCs) are a diverse class of new psychoactive substances (NPS) that act as potent agonists of the cannabinoid receptors CB1 and CB2, often with significantly higher efficacy than Δ9-tetrahydrocannabinol (Δ9-THC), the primary psychoactive compound in cannabis. First emerging in the early 2000s as "legal highs" in herbal incense blends such as *Spice* or *K2*, synthetic cannabinoids have since evolved into hundreds of structurally diverse compounds designed to evade drug legislation.

**Keywords:** CBD; Cannabinoids; THC; CB1; CB2 receptor; Anxiety; Ocomotor, Reward system

## 1. Introduction

In recent years, the rise of new psychoactive substance (NPS) has posed significant challenges to public health and safety across the globe, NPS are a diverse group of synthetic compounds designed to mimic the effects of traditional illicit drugs such as cannabis, cannabinoids, stimulant, and opioids. [1] Unlike established drugs of abuse, NPS are often created by modifying the chemical structure of existing substance, which allows them to evade regulation and remain undetected in drug testing. [2] As a result, NPS can rapidly spread within communities, often leading to increased instances of misuse, toxicity, and overdose. These substances are frequently marketed as “legal highs” or alternatives to illicit drugs, yet their safety profile remains largely unknown. [3-4] Behavioral pharmacology, the study of how substances affect behavior through interaction with brain and nervous system, play a critical role in understanding the dangers and potential therapeutic uses of NPS. [5] Most NPS are designed to target specific neurotransmitter system, including serotonin, dopamine, and gamma aminobutyric acid (GABA), which regulate mood, cognition, motor function, and perception. Due to the rapid development and variety of these substances, research on their behavioral effects is still in its infancy, and many of their neuropharmacological properties remain poorly understood. [6-8] The importance of investigating the behavioral pharmacology of NPS cannot be overstated. NPS have been associated with a range of psychological and physical effects, including euphoria, increased stimulation, hallucination, and, in some cases, severe agitation, psychosis, or even death. [9] The unpredictable nature of these substance, coupled with their novel chemical compositions, complicates medical and social responses to their use. Behavioral studies, especially in animal models, provide valuable insights into the potential risk associated with NPS use, including their addictive properties, toxicity, and long-term effects on mental and physical health. [10-12] Over the past decade, the rapid increase in the availability and use of these substance, often marketed as “legal” or designer’s drugs”, has led to rising concerns regarding their safety, misuse, and long-term consequences. Despite their widespread use, there is a notable gap in scientific understanding of the behavioral pharmacology of NPS, particularly their effects on behavior, cognition and emotional regulation. [13] NPS are increasingly available on the illicit drug, market, often as alternatives to well-known controlled substance like cocaine, cannabis, etc. their appeal lies in their perceived legality, accessibility, and the lack of standardized drug testing methods. [14] As a result, users may be unaware of the risk these substances pose, including ADRs, toxicity, psychosis, and death. Many NPS have not been thoroughly studied, and their effects on human behavioral outcomes of these substance can vary widely depending on their chemical structure, the specific receptor system they target, and individual user factors. [16-18] While some NPS may mimic the effects of traditional drugs, other have unique and poorly understood behavioral consequence. By studying this substance in preclinical models, we can gain important insights into their potential impact on motor activity, cognition, mood regulation, and addictive behaviors. [19] Many NPS have been linked to increased rated of abuse and dependence, particularly among younger populations. By analyzing the mechanisms by which NPS influence behavior, we can determine whether these substances produce reinforcement or tolerance similar to other addictive drugs, such as cocaine. [20] This research can help public health authorities

develop targeted strategies for prevention of NPS addiction. This thesis aims to explore the behavioral effects of NPS, focusing on their impact on motor activity, cognition, and emotional regulation, by examining both preclinical clinical researches, the study will attempt to unravel the complex pharmacological mechanisms underlying the behavioral response to this substance, additionally, this work will highlight the implication for public health, particularly regarding the identification, regulation, and treatment pf NPS reacted issues. [21-24]

**New Psychoactive Substances (NPS)**

NPS is New Psychoactive Substances (NPS) are a broad and diverse group of substances designed to mimic the effects of traditional illicit drugs, such as cannabis, cocaine, ecstasy, and heroin, but with chemical structures that differ from those of known controlled substances. [25] These substances are often referred to as "designer drugs" because they are chemically modified to avoid detection by existing drug laws. NPS can have effects on the central nervous system, and they can alter mood, perception, consciousness, and behaviour in ways similar to established drugs of abuse. Many NPS are synthetic substances, often created in laboratories. They may involve modifying the structure of existing drugs or combining chemical compounds to produce effects similar to those of banned substances. [26-27]

**Synthetic Cannabinoids**

Chemical Structure: These substances are chemically similar to THC, the active compound in cannabis, but they often have modified structures to enhance potency or evade existing drug laws. Effects: They can produce effects similar to those of cannabis, such as relaxation, euphoria, and altered perception. However, they can also lead to more severe side effects like agitation, paranoia, and hallucinations. Examples: JWH-018, JWH-073, UR-144, XLR-11. [28-30]

**Synthetic Cathinones**

Chemical Structure: These substances are related to amphetamines and cathinones (found in the khat plant), and they mimic the effects of stimulants like cocaine, methamphetamine, and MDMA (ecstasy). Effects: They produce stimulant effects such as increased energy, alertness, euphoria, and enhanced sociability. However, they can also cause dangerous side effects like agitation, violent behaviour, seizures, and cardiovascular issues. Examples: Mephedrone, Methylone, MDPV (Methylenedioxy pyrovalerone), Alpha-PVP. [32-34]

**Phenethylamines**

Chemical Structure: Phenethylamines are a broad class of chemicals that are structurally related to amphetamines and produce effects similar to hallucinogens, stimulants, or both. Effects: Depending on the substance, phenethylamines can produce stimulant effects (increased energy and euphoria) or hallucinogenic effects (altered perceptions and mood). Some phenethylamines can have dangerous side effects, including agitation and psychosis. Examples: 2C-B, 2C-I, MDA (3,4-methylenedioxyamphetamine), DOM (2,5-Dimethoxy-4-methylamphetamine). [35-37]

**Synthetic Opioids**

Chemical Structure: These substances are synthetic compounds designed to mimic the effects of natural opioids like heroin and morphine, or semi-synthetic opioids like fentanyl. Effects: They produce effects

similar to opioids, such as pain relief, euphoria, and relaxation, but can be much more potent and carry a high risk of overdose, especially due to their potency. Examples: Fentanyl (and its analogues, e.g., Alfentanil), U-47700, W-18, MT-45. [38-40]

**Tryptamines**

Chemical Structure: Tryptamines are a class of compounds that are chemically similar to serotonin. Some of these substances have hallucinogenic properties, producing effects similar to LSD, psilocybin (magic mushrooms), or DMT. Effects: They can cause altered perceptions of reality, hallucinations, and changes in sensory experiences. Some may also have stimulant properties. Examples: 5-MeO-DMT, DMT (Dimethyltryptamine), AMT (Alpha-methyltryptamine), MIPT (N-Methyl-N-isopropyl tryptamine). [41-43]

**Dissociative**

Chemical Structure: Dissociative are substances that cause feelings of detachment from reality and the body. They are chemically similar to substances like PCP and ketamine. Effects: These substances can cause dissociation, hallucinations, numbing, and distorted perceptions of time and space. They can also have serious side effects, including agitation, psychosis, and memory loss. Examples: Methoxetamine (MXE), 3-MeO-PCP, Delphinidin. [44-46]

**Deliriants**

Chemical Structure: Deliriants are substances that induce delirium, confusion, and memory loss. These compounds are typically less common in the NPS category but still emerge as part of the designer drug market. Effects: Deliriants can cause confusion, agitation, hallucinations, and loss of motor coordination. The effects can be highly dangerous due to the confusion and risk of injury. Examples: Benzyl tetrahydropyrrole, Scopolamine. [47-48]

**Other Psychoactive Substances**

Chemical Structure: There are numerous other substances that do not fall neatly into the categories above but still exhibit psychoactive effects. Effects: These can range from stimulants to hallucinogens, sedatives, or a mixture of effects, often with dangerous side effects or unknown long-term consequences. Examples: Piperazines (e.g., BZP - Benzylpiperazine), Premazepams (a benzodiazepine-like substance). [49-50]

**Benzodiazepine Analogues**

Chemical Structure: These NPS mimic the effects of benzodiazepines, which are typically prescribed for anxiety, sleep disorders, and muscle relaxation. Effects: They produce sedative, anxiolytic (anxiety-reducing), and hypnotic effects, similar to traditional benzodiazepines, but they can vary in potency and safety. Examples: Etizolam, Flutrimazole, Clonazepam. [51-54]


**2. Cannabinoids – Natural vs. Synthetic**

Cannabinoids are a class of chemical compounds that interact primarily with the body's endocannabinoid system (ECS), a complex cell-signaling network involved in regulating physiological processes such as pain sensation, mood, appetite, memory, and immune response. Cannabinoids can be broadly categorized into three main types: Phyto cannabinoids (plant-derived), endocannabinoids (endogenously produced), and synthetic cannabinoids (lab-designed). [55]

**Natural Cannabinoids**

**Phyto cannabinoids**

Phytocannabinoids are derived from *Cannabis sativa* and related species. The two most well-known and studied phytocannabinoids are: Δ9-Tetrahydrocannabinol (Δ9-THC) – The principal psychoactive compound, which acts as a partial agonist at cannabinoid receptors CB1 and CB2. Cannabidiol (CBD) – A non-psychoactive cannabinoid with low affinity for cannabinoid receptors but known to modulate various receptor systems including TRPV1 and 5-HT1A. [56] Phyto cannabinoids are typically characterized by moderate receptor efficacy and relatively mild psychoactive effects. Their pharmacokinetic profiles are influenced by factors such as lipophilicity, metabolism by hepatic cytochrome P450 enzymes, and extensive first-pass metabolism. [57]

**Endocannabinoids**

These are endogenous ligands produced by the body, including: Anandamide (AEA), 2-Arachidonoylglycerol (2-AG) Endocannabinoids are synthesized on-demand from membrane lipids and act as retrograde neurotransmitters, modulating neurotransmitter release in a tightly regulated manner. They are quickly degraded by enzymes such as fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). [58]

**Synthetic Cannabinoids**

Synthetic cannabinoids (SCs) are man-made compounds that mimic the effects of Δ9-THC by acting as agonists at CB1 and/or CB2 receptors. Initially developed for research and therapeutic purposes, they have since been misused in recreational contexts under names like Spice, K2, and herbal incense. Full

Agonists at CB1/CB2: Unlike Δ9-THC (a partial agonist), most SCs are full agonists with much higher receptor efficacy, leading to more intense and often unpredictable effects. Higher Potency: Some SCs are up to 100 times more potent than Δ9-THC, increasing the risk of overdose, severe psychiatric reactions, and death. Structural Diversity: SCs include many subfamilies (e.g., naphthoylindoles like JWH-018, indazole carboxamides like AB-FUBINACA), designed to bypass legal restrictions. [59]

**3. Mechanism of Action of Cannabinoid Receptors**

Cannabinoid receptors are a class of G-protein-coupled receptors (GPCRs) that mediate the physiological effects of endogenous and exogenous cannabinoids. The two primary cannabinoid receptors identified to date are CB1 and CB2, each exhibiting distinct tissue distributions and functional roles. [60]

**CB1 Receptor**

The CB1 receptor is predominantly expressed in the central nervous system (CNS), including brain regions such as the hippocampus, cerebellum, and basal ganglia. Activation of CB1 receptors modulates neurotransmitter release, influencing processes such as memory, motor coordination, pain perception, and appetite regulation. Upon binding of a cannabinoid ligand (e.g., $\Delta^9$-tetrahydrocannabinol or anandamide), the CB1 receptor activates inhibitory Gi/o proteins, leading to: Inhibition of adenylyl cyclase, resulting in decreased levels of cyclic adenosine monophosphate (cAMP). Inhibition of voltage-gated calcium channels, reducing presynaptic neurotransmitter release. Activation of inwardly rectifying potassium channels, causing neuronal hyperpolarization and reduced excitability. These intracellular events culminate in a decrease in excitatory neurotransmission, particularly in pathways involving glutamate and GABA.

**CB2 Receptor**

The CB2 receptor is primarily expressed in immune cells and peripheral tissues. It plays a significant role in immune modulation and inflammation. Similar to CB1, CB2 receptor activation inhibits adenylyl cyclase and modulates ion channels through Gi/o protein signaling, but it typically lacks psychoactive effects due to its limited expression in the CNS. CB2 receptor activation leads to: Suppression of inflammatory cytokine release. Modulation of immune cell migration and function. Potential analgesic and anti-inflammatory effects, especially in peripheral neuropathic pain models.

**Endogenous and Exogenous Ligands**

The main endogenous ligands for cannabinoid receptors, known as endocannabinoids, include anandamide (AEA) and 2-arachidonoylglycerol (2-AG). These are synthesized on demand and rapidly degraded by enzymes such as fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively. Exogenous ligands include phytocannabinoids like $\Delta^9$-tetrahydrocannabinol (THC), a partial agonist at both CB1 and CB2, and cannabidiol (CBD), which exhibits low affinity for both receptors but modulates their activity indirectly through multiple pathways.

**Key Behavioral Effects of Cannabinoids**

**Psychostimulant Sensitization and Novelty-Seeking Behavior**

Research has shown that the CB1 receptor is involved in the behavioral sensitization to nicotine, particularly in rats with high novelty-seeking traits. In these animals, administration of the CB1

antagonist AM251 reversed locomotor sensitization to nicotine and normalized elevated hippocampal serotonin (5-HT) levels, suggesting a role for CB1 in modulating responses to psychostimulants.

**Modulation of Food and Alcohol Consumption**

CB2 receptors, traditionally associated with immune system modulation, have been implicated in the regulation of food and alcohol intake. Studies indicate that activation of CB2 receptors can influence consumption behaviors, with potential implications for understanding addiction and eating disorders.

**Differential Roles of CB1 and CB2 Receptors in Psychostimulant Responses**

The modulation of psychostimulant effects by cannabinoids involves both CB1 and CB2 receptors. Activation of CB2 receptors and inhibition of CB1 receptors have been shown to reduce the behavioral and molecular effects induced by psychostimulants. This suggests that targeting CB2 receptors may offer therapeutic potential for treating addiction, with fewer psychiatric side effects compared to CB1 receptor antagonists. [61]

**4. Emerging Concepts in Cannabinoid Pharmacology**

The "entourage effect" refers to the hypothesis that various compounds in cannabis, beyond cannabinoids like THC and CBD, interact synergistically to modulate the plant's overall psychoactive effects. This concept has implications for understanding the full spectrum of cannabis's behavioral effects and for developing more effective therapeutic applications.

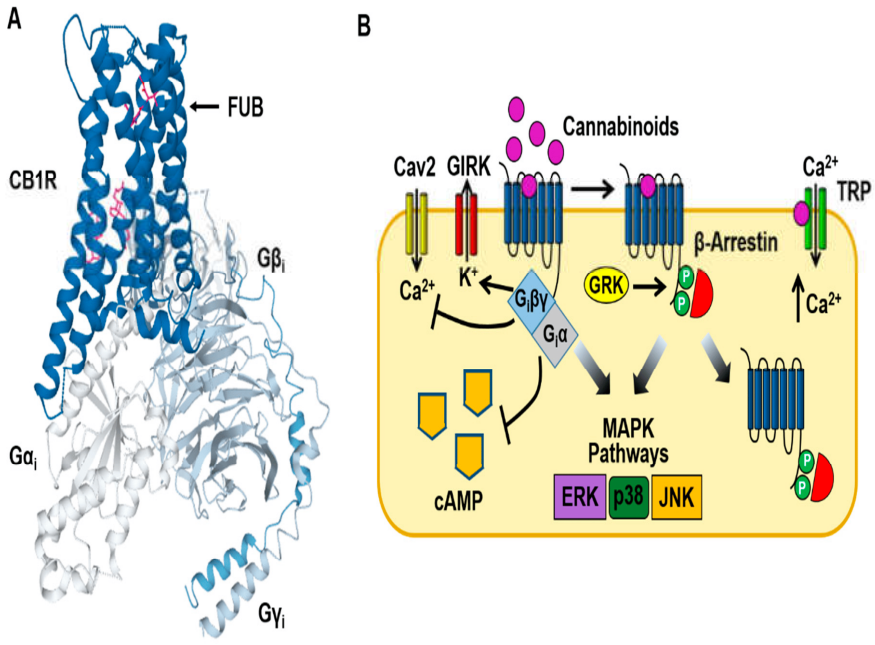


**Fig. 1.** Represent structure of receptor, B represent mechanism of cannabis in receptor.

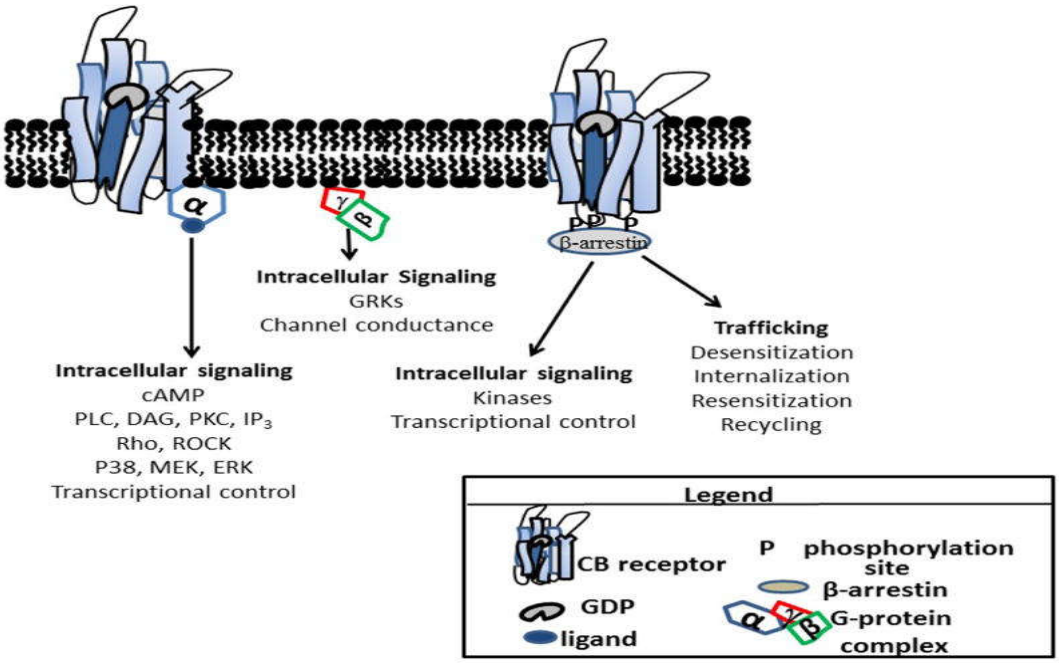


**Fig. 2.** It represents signalling mechanism of receptor binding site.

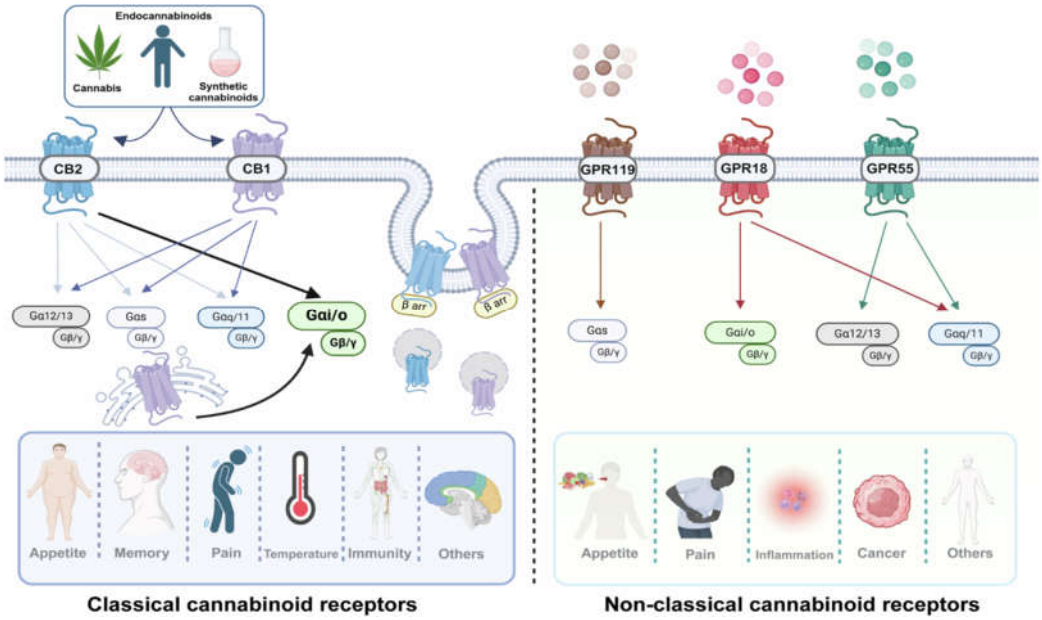


**Fig. 3.** Represent difference in classical and non-classical cannabinoids receptor.

Cannabinoids influence the mesolimbic dopamine system, which is central to reward processing. THC has been shown to increase dopamine release in the nucleus accumbent, mimicking the effects of classical drugs of abuse. CB1 receptor antagonists have been investigated as potential treatments for substance use disorders, though clinical trials have been limited by psychiatric side effects. The behavioural effects of cannabinoids on anxiety and mood are dose- and context-dependent. Low doses of THC may produce anxiolytic effects, while higher doses are often anxiogenic. Chronic exposure to cannabinoids may dysregulate the hypothalamic-pituitary-adrenal (HPA) axis and alter stress responses, contributing to mood instability. Traditionally associated with the immune system, CB2 receptors are now recognized for their role in modulating behaviour, particularly in response to inflammation and neuroimmune signalling. Recent studies suggest that CB2 activation can reduce drug-seeking behaviour and modulate consumption of substances like alcohol and food, offering a non-psychoactive target for

treating addiction. Cannabinoid receptor activation can impair cognitive functions such as memory, attention, and learning. CB1 receptor activation in the hippocampus has been associated with deficits in spatial memory and learning. These effects are particularly pronounced with chronic or high-dose THC exposure, raising concerns about the cognitive impact of prolonged cannabis use. The therapeutic potential of cannabinoids is vast, encompassing pain management, anti-inflammatory effects, and treatment of neurological disorders. However, the psychoactive properties of THC pose significant challenges in clinical applications. Selective targeting of CB2 receptors or non-psychoactive cannabinoids like CBD may offer therapeutic benefits without the associated cognitive and psychiatric side effects. Advancements in understanding the structural dynamics of cannabinoid receptors and their signalling pathways are paving the way for the development of novel therapeutic agents that can selectively modulate specific receptor conformations, enhancing efficacy and minimizing adverse effects. The effects of cannabinoids on mood and anxiety are complex and dose-dependent. Low doses of THC may produce anxiolytic effects, while higher doses are often anxiogenic. Chronic exposure to cannabinoids can lead to dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, contributing to mood instability and anxiety disorders. Cannabidiol (CBD), a non-psychoactive cannabinoid, has shown promise in reducing anxiety and improving mood without the adverse effects associated with THC. CBD's anxiolytic properties may be mediated through its interaction with serotonin receptors and its ability to modulate endocannabinoid signalling. CB2 receptors are primarily located in peripheral tissues and immune cells but are also present in certain brain regions. While traditionally associated with immune modulation, emerging evidence indicates that CB2 receptors play a significant role in modulating behavior. Activation of CB2 receptors has been shown to attenuate the rewarding effects of substances like cocaine and alcohol, suggesting a potential therapeutic target for addiction treatment without the psychoactive side effects associated with CB1 receptor activation.

## 5. Research methodology

The selection of appropriate experimental subjects is a crucial component of preclinical behavioral pharmacology research. In this study, male Wistar rats were used as the animal model due to their well-characterized behavioral responses and widespread use in neuropharmacological investigations. The selected rats were 8–12 weeks old at the time of experimentation, with an average body weight of 200–250 grams. This age range ensures that the animals are young adults with fully developed nervous systems but not yet subject to age-related behavioral variability.

### Housing and Environmental Conditions

All animals were obtained from an institutional breeding facility certified for laboratory animal care. Upon arrival, the animals were housed in groups of 3–4 per cage under the following standardized environmental conditions: Room temperature: Maintained at 22 ± 2°C, Humidity: 55 ± 10%, Light cycle: 12-hour light/dark cycle (lights on at 07:00 AM), Ventilation: Rooms equipped with HEPA filtration systems to maintain air quality, Food and Water: Standard rodent chow and filtered water provided ad libitum. Environmental enrichment (e.g., nesting materials and tunnels) was provided to minimize stress, promote natural behaviour, and improve the well-being of the animals.

**Acclimatization**

To reduce stress and allow adaptation to the laboratory environment, all animals underwent a 7-day acclimatization period before the start of experiments. During this period, they were handled daily to habituate them to human interaction and experimental procedures.

**Randomization and Group Assignment**

Following acclimatization, animals were randomly assigned to experimental groups using a random number generator to ensure that treatment groups were balanced for weight and age. Randomization helps minimize selection bias and enhances the internal validity of the study.

**Table – 1** Randomisation to animal group.

| Group | Treatment | Dose (mg/kg) | N |
|---|---|---|---|
| G1 | Vehicle Control | 0 | 8 |
| G2 | THC Low Dose | 1 | 8 |
| G3 | THC Medium Dose | 3 | 8 |
| G4 | THC High Dose | 10 | 8 |
| G5 | CBD | 5 | 8 |
| G6 | CB1 Antagonist + THC | AM251 + THC | 8 |

**Ethical Approval and Welfare Monitoring**

All experimental procedures involving animals were conducted in strict accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by the Institutional Animal Ethics Committee (IAEC). The protocol number and date of approval are recorded in the appendix. Animal welfare was monitored daily. Parameters such as grooming behaviour, posture, movement, feeding habits, and body weight were observed to assess health status. Any signs of distress, pain, or abnormal behaviour were documented, and animals were removed from the study if humane endpoints were met.

**Drug Preparation and Administration**

The accurate preparation and administration of pharmacological agents are critical in behavioural pharmacology studies to ensure reproducibility and reliability of results.

**Cannabinoid Compounds**

The following compounds were used in this study: $\Delta^9$-Tetrahydrocannabinol (THC): the primary psychoactive constituent of cannabis. Cannabidiol (CBD): a non-psychoactive cannabinoid with anxiolytic and neuroprotective properties. AM251: a selective CB1 receptor antagonist/inverse agonist used to investigate the role of CB1 receptor activity. JWH-133: a selective CB2 receptor agonist to study the behavioral role of CB2 receptors. All drugs were procured from certified suppliers and stored according to manufacturer guidelines (typically at -20°C) to maintain stability.

**Drug Formulation**

**Solvent**: A vehicle consisting of a mixture of ethanol:cremophor:saline (1:1:18) was used to dissolve the lipophilic cannabinoid compounds. THC and CBD were sonicated to ensure uniform dispersion before use. The final injection volume was standardized to 1 mL/kg body weight for all animals.

**Dosage and Schedule**

THC: Administered at doses of 1, 3, and 10 mg/kg based on previous behavioral studies indicating dose-dependent effects. CBD: Administered at 5 mg/kg, a commonly studied effective anxiolytic dose in rodents. CB1 Antagonist (AM251): Administered at 2 mg/kg, 30 minutes prior to THC to block CB1 receptor-mediated responses. CB2 Agonist (JWH-133): Administered at 1 mg/kg to evaluate CB2-specific behavioral modulation.

**Route and drug administration**

Route: Intraperitoneal (i.p.) injections were used for systemic delivery due to consistent absorption and ease of use. Timing: All injections were performed between 09:00–11:00 AM to reduce circadian influences on behaviour. Animals were handled gently and briefly restrained during injection to minimize stress.

**Behavioural Tests**

Behavioural assessments are a fundamental aspect of pharmacological studies involving cannabinoids, as these substances are known to impact various domains such as anxiety, locomotion, reward, and cognition. The following validated behavioural paradigms were selected to comprehensively evaluate the effects of cannabinoid administration in rodents.

**Parameters Measured**:

Total distance moved (index of general locomotor activity). Time spent in the centre zone (index of anxiety-like behaviour; more center time indicates anxiolytic effects). Number of rearing and grooming behaviour (indices of exploratory activity and stress). Data Analysis: Behaviour is video recorded and analysed using automated tracking software (e.g., EthoVision XT).

**Elevated Plus Maze (EPM)**

To assess anxiety-related behaviour by measuring an animal's natural aversion to open elevated spaces. The EPM is a widely used model for studying anxiety, particularly sensitive to the effects of anxiolytic or anxiogenic drugs. Cannabinoids have shown dose-dependent effects on this paradigm. A plus-shaped maze elevated 50 cm above the floor, consisting of: Two open arms (50 cm × 10 cm). Two closed arms of the same size with 40 cm high walls. A central platform (10 cm × 10 cm). The animal is placed in the center of the maze facing an open arm. The session lasts 5 minutes. Lighting is kept consistent (~100 lux in open arms).

**Parameters Measured**:

Time spent in open and closed arms. Number of entries into each arm. Percentage of open arm entries (open entries / total entries × 100). Total arm entries (to assess general activity level).

**Conditioned Place Preference (CPP)**

To evaluate the rewarding or aversive effects of cannabinoids, indicating their potential for abuse or therapeutic value. CPP is a classical conditioning paradigm used to assess the motivational properties of drugs. A preference for a drug-paired environment indicates positive reinforcement. A two-chamber apparatus with distinct tactile (floor texture) and visual (wall patterns) cues. A removable partition allows for either separate or free movement between chambers.

**Experimental Phases**:

Pre-conditioning (Day 1): Rats are allowed to freely explore both chambers for 15 minutes. Time spent in each chamber is recorded to assess initial preference. Conditioning (Days 2–5): Animals receive the cannabinoid (e.g., THC) before being confined to one chamber for 30 minutes. On alternate days, they receive vehicle and are confined to the opposite chamber. Post-conditioning (Day 6): Rats again have free access to both chambers, and the time spent in each is recorded.

**6. Results and Discussion**

Low-dose THC (1 mg/kg): Significantly increased locomotor activity compared to control ($p < 0.05$). High-dose THC (10 mg/kg): Decreased total distance traveled ($p < 0.01$), suggesting sedative or anxiogenic effects. CBD (5 mg/kg): Increased center time without affecting locomotion, indicative of anxiolytic properties. These results are consistent with the biphasic effects of THC, where low doses may stimulate exploratory behavior while higher doses suppress activity due to CNS depression. The increase in center time following CBD treatment aligns with its known anxiolytic and non-psychoactive profile, possibly mediated via 5-HT1A receptor modulation.

**Elevated Plus Maze (EPM)**

THC at 1 mg/kg: Slight, non-significant increase in open arm entries. THC at 10 mg/kg: Significantly decreased time in open arms ($p < 0.01$). CBD-treated rats: Spent significantly more time in open arms compared to control ($p < 0.05$). AM251 (CB1 antagonist) reversed the anxiolytic effects of low-dose THC. High-dose THC produced anxiogenic-like effects, reflected in reduced open arm exploration, possibly due to CB1 receptor overstimulation in the amygdala and prefrontal cortex. CBD's anxiolytic effects further support its therapeutic potential in anxiety-related disorders. The reversal of THC effects by AM251 confirms the CB1-mediated mechanism. THC (3 mg/kg) produced significant place preference ($p < 0.01$), indicating rewarding properties. High-dose THC (10 mg/kg) resulted in no preference or slight aversion. CBD did not induce place preference or aversion. JWH-133 (CB2 agonist) did not elicit significant CPP but reduced THC-induced preference. These findings support the rewarding potential of moderate-dose THC, consistent with dopaminergic activation in the mesolimbic reward pathway. The aversive trend at higher doses reflects dose-dependent shifts in cannabinoid receptor signaling. The neutral profile of CBD confirms its lack of abuse liability, and the CB2 agonist's attenuation of THC-CPP suggests potential in addiction treatment. Control group: Showed progressive learning over 4 days with reduced escape latency. THC (10 mg/kg) group: Demonstrated significantly impaired learning and memory (longer escape latency and poor probe trial performance, $p < 0.01$). CBD group: Performed similarly to control, with slightly improved memory retention. THC + AM251: Showed

improved performance compared to THC alone ($p < 0.05$). High-dose THC impaired spatial learning and memory, likely due to CB1 receptor activation in the hippocampus, consistent with existing literature. CBD did not negatively affect memory and may exert neuroprotective or cognition-enhancing effects. The partial reversal by AM251 highlights the role of CB1-mediated interference with long-term potentiation (LTP), a key mechanism of learning. Grooming and defensive postures were more frequent in the high-dose THC group. No mortality or severe adverse effects were recorded. Animals treated with CBD appeared more relaxed and socially interactive.

**Table 2 -** Summary of key findings.

| **Treatment** | **Locomotion** | **Anxiety** | **Reward** | **Memory** |
|---|---|---|---|---|
| Control | Normal | Baseline | None | Normal |
| THC Low | ↑ | ↓ Anxiety | Mild reward | Normal |
| THC High | ↓ | ↑ Anxiety | No preference / aversion | ↓↓ |
| CBD | ↔ | ↓ Anxiety | Neutral | ↔ / slight ↑ |
| AM251 + THC | ↔ / ↑ | ↓ Anxiety | ↓ Reward | ↑ |
| JWH-133 | ↔ | Neutral | ↓ THC reward | Not tested |

This study reinforces the complex, dose-dependent behavioral effects of cannabinoids: CB1 activation is central to both the rewarding and cognitive-impairing effects of THC. CBD demonstrates therapeutic potential by reducing anxiety and avoiding cognitive or addictive liabilities. CB2 modulation, while not affecting behaviour directly, may have protective roles in reducing drug-seeking tendencies. These findings contribute to the growing body of evidence advocating for CBD as a safer therapeutic alternative and highlight the importance of receptor-specific cannabinoid pharmacology in drug development.

**7. Conclusion**

The present study examined the behavioural pharmacology of cannabinoids, with a specific focus on $\Delta^9$-Tetrahydrocannabinol (THC) and Cannabidiol (CBD), using well-established rodent models. Through the use of behavioural assays—namely, the open field test, elevated plus maze, conditioned place preference, and Morri's water maze—the study evaluated how different cannabinoid compounds affect locomotion, anxiety, reward, and cognitive performance. The findings revealed that THC exerts dose-dependent effects on behaviour. Low doses increased locomotor activity and reduced anxiety-like behaviour, while high doses suppressed locomotion, induced anxiety, and impaired spatial memory. These results are in line with previous studies indicating a biphasic pharmacodynamic profile of THC (Huestis et al., 2001; Wiley et al., 2007). Conversely, CBD exhibited anxiolytic properties without affecting locomotor or reward-seeking behavior and did not impair cognitive performance. This supports its emerging role as a non-intoxicating therapeutic agent with a favorable safety profile (Campos et al., 2012). Pharmacological

manipulation with AM251 (a CB1 receptor antagonist) and JWH-133 (a CB2 agonist) provided further insight into receptor-specific actions. AM251 reversed the behavioral effects of THC, confirming the involvement of CB1 receptors in mediating THC's psychoactive and cognitive effects. Meanwhile, JWH-133 appeared to attenuate the rewarding effects of THC, suggesting a modulatory role for CB2 receptors in addiction-related pathways. These findings contribute to a growing body of literature emphasizing the complexity of the endocannabinoid system and its implications in behavioral pharmacology. They also support the potential of CBD as a safer, therapeutic alternative to THC for conditions involving anxiety or cognitive dysfunction.

**Future directions**

Future research should investigate: The long-term effects of cannabinoids on behavior and brain function. Sex-specific responses to cannabinoids, especially in developmental and aging populations. The interaction of the endocannabinoid system with other neurotransmitter systems such as dopamine and serotonin. The potential neuroprotective mechanisms of CBD at the molecular and cellular levels.

**References**


1. D`Souza, D. C., et al. (2015). “Synthetic cannabinoid uses and psychosis.” Biological psychiatry.
2. Auwarter, V., et al. (2016). “Synthetic cannabinoids: Analysis and effects.” Drug Testing and analysis.
3. Browne, R. A., et al. (2016). “Behavioural pharmacology of synthetic cathinones.” Neuropharmacology.
4. Carhart – Harris, R. L, et al., (2016). “Psychedelics and the human connectome.” Proceedings of the National Academy of Science.
5. Kerr, T., et al. (2017).” The role of harm reduction in addressing NPS. “International Journal of Drug policy.
6. Kumar, A, et al. (2019).” Reinforcing effects of synthetic cannabinoids. “Addiction Biology”.
7. Tirado – Munoz, J., et al. (2018). ‘social factor in NPS use. “substance & Misuse.
8. Vansickel, A.R., et al. (2016).”synthetic cannabinoids and public health. “Clinical toxicology.
9. Fabrizio Schifano, G, Duccio papanti, Laura Orsolini & John M. Corkery.(2016). `Novel psychoactive substance; the pharmacology of stimulants and hallucinogens’, Expert Review of clinical pharmacology, vol9 (7).
10. Shulgin AT, Manning T, Daley PF. The Shulgin Index, volume one: Psychedelic Phenethylamines and related compounds, Berkeley : Transform press , 2011
11. K. Lutfy, R.N. Pechnick and Nardaran,et al.(2023)”editorial : Pharmacology of new psychoactive substance.”
12. Schifano, F., et al. (2011). "The Clinical and Forensic Toxicology of New Psychoactive Substances." *Journal of Psychopharmacology*, 25(3), 410-417.

13. Gibbons, S. (2015). "Synthetic Cannabinoids: A New Class of Psychoactive Drugs." *British Journal of Pharmacology*, 172(14), 3670-3683.
14. Cohen, J. (2016). "New Psychoactive Substances: A Public Health Challenge." *American Journal of Public Health*, 106(8), 1397-1399.
15. Wadley G. How psychoactive drugs shape human culture: a multi- disciplinary perspective. Brain Res Bull (2016) 126(Pt 1):138–51. doi:10.1016/ j.brainresbull.2016.04.008
16. Müller CP, Schumann G. Drugs as an instrument: a new framework for non-addictive psychoactive drug use. Behav Brain Sci (2011) 34(6):293–310.
17. Müller CP, Schumann G. To use or not to use: expanding the view on non- addictive psychoactive drug consumption and its implications. Behav Brain Sci (2011) 34(6):328–47.
18. Müller CP. Non addictive drug use: the way forward. In: Wolff K, White J, Karch S, editors. The SAGE Handbook of Drugs & Alcohol Studies – Biological Approaches. (Vol. 2, Chap. 42), London: SAGE (2017). p. 411–34.
19. Ma J, Yin WY, Zhou H, Liao XB, Cook JM. General approach to the total synthesis of 9-methoxy-substituted indole alkaloids: synthesis of mitragynine, as well as 9-methoxygeissoschizol and 9-methoxy-N-b-methylgeissoschizol. J Org Chem (2009)
20. Matsumoto K, Yamamoto LT, Watanabe K, Yano S, Shan J, Pang PK, et al. Inhibitory effect of mitragynine, an analgesic alkaloid from Thai herbal medi cine, on neurogenic contraction of the vas deferens. Life Sci (2005) 78:187–94.
21. Forrester MB, Kleinschmidt K, Schwarz E, Young A. Synthetic cannabinoid and marijuana exposures reported to poison centers. Hum Exp Toxicol (2012) 31(10):1006–11.
22. Castaneto MS, Gorelick DA, Desrosiers NA, Hartman RL, Pirard S, Huestis MA. Synthetic cannabinoids: epidemiology pharmacodynamics, and clinical implications. Drug Alcohol Depend (2014)
23. Hudson S, Ramsey J. The emergence and analysis of synthetic cannabinoids. Drug Test Anal (2011) 3(7–8)
24. Muller-Vahl K, Grotenhermen F. Cannabis therapy. Dtsch Arztebl Int (2013) 110(9)
25. Darmani NA. Delta(9)-tetrahydrocannabinol and synthetic cannabinoids prevent emesis produced by the cannabinoid CB(1) receptor antagonist/ inverse agonist SR 141716A. Neuropsychopharmacology (2001) 24(2): 198–203
26. Wallach JV. Endogenous hallucinogens as ligands of the trace amine recep tors: a possible role in sensory perception. Med Hypotheses (2009) 72:91–4.
27. Micaela Tirri 1,†, Giorgia Corli 1,†, Raffaella Arfè 1, Beatrice Marchetti 1, Sabrine Bilel 1, Tatiana Bernardi 2, Behavioural and Pharmacokinetics Studies of N-Methyl-2-Aminoindane (NM2AI) in Mice: An Aminoindanes Briefly Used in the Illicit Drug Market; 18 January 2023;page no. 2-6.
28. Pálení˘ cek, T.; Lhotková, E.; Žídková, M.; Balíková, M.; Kucha˘r, M.; Himl, M.; Mikšátková, P.; ˘ Cegan, M.; Valeš, K.; Tylš, F.; et al. Emerging toxicity of 5,6-methylenedioxy-2-aminoindane

(MDAI): Pharmacokinetics, behaviour, thermoregulation and LD50 in rats. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 2016, 69, 49–59.

29. Luethi, D.; Kolaczynska, K.E.; Docci, L.; Krähenbühl, S.; Hoener, M.C.; Liechti, M.E. Pharmacological profile of mephedrone analogs and related psychoactive substances. Neuropharmacology 2018, 134
30. De-Giorgio, F.; Bilel, S.; Ossato, A.; Tirri, M.; Arfè, R.; Foti, F.; Serpelloni, G.; Frisoni, P.; Neri, M.; Marti, M. Acute and repeated administration of MDPV increases aggressive behavior in mice: Forensic implications. Int. J. Leg. Med. 2019, 133, 1797–1808.(PubMed)
31. Dafters, R.I. Hyperthermia following MDMA administration in rats: Effects of ambient temperature, water consumption, and chronic dosing. Physiol. Behav. 1995, 58, 877–882.
32. Marks, D.M.; Shah, M.J.; Patkar, A.A.; Masand, P.S.; Park, G.Y.; Pae, C.U. Serotonin-norepinephrine reuptake inhibitors for pain control: Premise and promise. Curr. Neuropharmacol. 2009, 7, 331–336.
33. Elliott, S.; Evans, J. A 3-year review of new psychoactive substances in casework. Forensic Sci. Int. 2014, 243, 55–60
34. Cannon, J.G.; Perez, J.A.; Pease, J.P.; Long, J.P.; Flynn, J.R.; Rusterholz, D.B.; Dryer, S.E. Comparison of biological effects of N-alkylated congeners of beta-phenethylamine derived from 2-aminotetralin, 2-aminoindan, and 6-aminobenzocycloheptene. J. Med. Chem. 1980, 23, 745–749.
35. Canazza, I.; Ossato, A.; Trapella, C.; Fantinati, A.; De Luca, M.A.; Margiani, G.; Vincenzi, F.; Rimondo, C.; Di Rosa, F.; Gregori, A.; et al. Effect of the novel synthetic cannabinoids AKB48 and 5F-AKB48 on “tetrad”, sensorimotor, neurological and neurochemical responses in mice. In Vitro and In Vivo pharmacological studies. Psychopharmacology 2016, 233, 3685–3709.
36. Baumann,M.H.; Ayestas, M.A., Jr.; Partilla, J.S.; Sink, J.R.; Shulgin, A.T.; Daley, P.F.; Brandt, S.D.; Rothman, R.B.; Ruoho, A.E.; Cozzi, N.V. The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. Neuropsychopharmacology 2012, 37, 1192–1203.
37. Hassan Z, Bosch OG, Singh D, Narayanan S, Kasinather BV, Seifritz E, Kornhuber J, Quednow BB and Müller CP; Novel Psychoactive Substances—Recent Progress on Neuropharmacological Mechanisms of Action for Selected Drugs. Front. Psychiatry;2017,
38. Dr. Bilel Sabrine, Prof. Marti Matteo; In vitro and in vivo pharmaco-toxicological characterization of Novel Psychoactive Substances (NPS): focus on Novel Synthetic Opioids (NSOs);2023,144.
39. Tirri, M., Frisoni, P., Bilel, S., Arfè, R., Trapella, C., Fantinati, A., Corli, G., Marchetti, B., De Giorgio, F., Camuto, C., Mazzarino, M., Gaudio, R. M., Serpelloni, G., Schifano, F., Botrè, F., & Marti, M. (2021). Worsening of the Toxic Effects of (±)Cis-4,4'-DMAR Following Its Co-Administration with (±)Trans-4,4'-DMAR: Neuro-Behavioural, Physiological, Immunohistochemical and Metabolic Studies in Mice. International journal of molecular sciences, 22(16), 8771.
40. Mazdai, L., Fabbri, M., Tirri, M., Corli, G., Arfè, R., Marchetti, B., Bilel, S., Bergamin, E., Gaudio, R. M., Rubini, M., De-Giorgio, F., & Marti, M. (2022). Epigenetic Studies for Evaluation of NPS Toxicity: Focus on Synthetic Cannabinoids and Cathinones. Biomedicines, 10(6), 1398.

41. Tirri, M., Corli, G., Arfè, R., Marchetti, B., Bilel, S., Bernardi, T., Boccuto, F., Odoardi, S., Mestria, S., Strano-Rossi, S., & Marti, M. (2023). Behavioral and Pharmacokinetics 309 Studies of N-Methyl-2-Aminoindane (NM2AI) in Mice: An Aminoindane Briefly Used in the Illicit Drug Market. International journal of molecular sciences, 24(3), 1882.
42. Ross E.A., Reisfield G.M., Watson M.C., Chronister C.W., Goldberger B.A. Psychoactive “bath salts” intoxication with methylenedioxypyrovalerone. Am. J. Med. 2012;125(9):854–858. doi: 10.1016/j.amjmed.2012.02.019. [DOI] [PubMed] [Google Scholar]
43. Regunath H., Ariyamuthu V.K., Dalal P., Misra M. Bath salt intoxication causing acute kidney injury requiring hemodialysis. Hemodial. Int. 2012;16(Suppl. 1):S47–S49. doi: 10.1111/j.1542-4758.2012.00750.x. [DOI] [PubMed] [Google Scholar]
44. Gibbons S., Zloh M. An analysis of the ‘legal high’ mephedrone. Bioorg. Med. Chem. Lett. 2010;20(14):4135–4139. doi: 10.1016/j.bmcl.2010.05.065. [DOI] [PubMed] [Google Scholar]
45. .Petit A., Karila L., Sananes M., Lejoyeux M. Mephedrone: a new synthetic drug. Presse Med. 2013;42(10):1310–1316. doi: 10.1016/j.lpm.2012.11.019. [DOI] [PubMed] [Google Scholar]
46. Karila L., Reynaud M. Mephedrone: A designer drug legally available on the Web. Presse Med. 2010;39(7-8):834–835. doi: 10.1016/j.lpm.2010.04.012. [DOI] [PubMed] [Google Scholar]
47. Wood D.M., Dargan P.I. Mephedrone (4-methylmethcathinone): what is new in our understanding of its use and toxicity. Prog. Neuropsychopharmacol. Biol. Psychiatry. 2012;39(2):227–233. doi: 10.1016/j.pnpbp.2012.04.020. [DOI] [PubMed] [Google Scholar].
48. Campos, A. C., Moreira, F. A., Gomes, F. V., Del Bel, E. A., & Guimarães, F. S. (2012). Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philosophical Transactions of the Royal Society B: Biological Sciences, 367*(1607), 3364–3378. https://doi.org/10.1098/rstb.2011.0389
49. Huestis, M. A., Gorelick, D. A., Heishman, S. J., Preston, K. L., Nelson, R. A., Moolchan, E. T., & Frank, R. A. (2001). Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. *Archives of General Psychiatry, 58*(4), 322–328. https://doi.org/10.1001/archpsyc.58.4.322
50. Wiley, J. L., Burston, J. J., Leggett, D. C., Alekseeva, O. O., He, H., & Mahadevan, A. (2007). CB1 cannabinoid receptor-GABA(A) receptor interactions and memory performance in female rats. *European Journal of Pharmacology, 573*(1–3), 31–38. https://doi.org/10.1016/j.ejphar.2007.05.054
51. .Gustavsson D., Escher C. Mephedrone--Internet drug which seems to have come and stay. Fatal cases in Sweden have drawn attention to previously unknown substance. Lakartidningen. 2009;106(43):2769–2771. [PubMed] [Google Scholar]
52. Dickson A.J., Vorce S.P., Levine B., Past M.R. Multiple-drug toxicity caused by the coadministration of 4-methylmethcathinone (mephedrone) and heroin. J. Anal. Toxicol. 2010;34(3):162–168. doi: 10.1093/jat/34.3.162. [DOI] [PubMed] [Google Scholar]
53. Maskell P.D., De Paoli G., Seneviratne C., Pounder D.J. Mephedrone (4-methylmethcathinone)-related deaths. J. Anal. Toxicol. 2011;35(3):188–191. doi: 10.1093/anatox/35.3.188.

54. Howlett, A. C., Barth, F., Bonner, T. I., Cabral, G., Casellas, P., Devane, W. A., & Mechoulam, R. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacological Reviews*, 54(2), 161-202.
55. Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, 365(6441), 61-65.
56. Turcotte, C., Blanchet, M. R., Laviolette, M., & Flamand, N. (2016). The CB2 receptor and its role as a regulator of inflammation. *Cellular and Molecular Life Sciences*, 73(23), 4449–4470.
57. Biala, G., & Budzynska, B. (2008). Cannabinoid CB1 receptor antagonism reverses the behavioral sensitization to nicotine and improves spatial memory in rats. *Behavioural Brain Research, 186*(1), 17–25.
58. Howlett, A. C., et al. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacological Reviews*, 54(2), 161–202.
59. Munro, S., et al. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, 365(6441), 61–65.
60. Onaivi, E. S., et al. (2006). Functional expression of CB2 cannabinoid receptors in the brain. *Proceedings of the National Academy of Sciences*, 103(41), 15067–15072.
61. Volkow, N. D., et al. (2014). Adverse health effects of marijuana use. *New England Journal of Medicine*, 370(23), 2219–2227.