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Targeting Trauma-Induced Endocannabinoid System Dysfunction: A Novel Neuroprotective Approach For Traumatic Brain Injury

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Abstract:
Traumatic brain injury (TBI) is a leading cause of death and disability worldwide. The primary injury results in neuronal damage and initiates secondary injuries like neuroinflammation, excitotoxicity, oxidative stress and blood-brain barrier disruption. This results in long-term cognitive, behavioral and motor deficits. Existing therapeutic options for TBI focus on symptomatic management rather than directly addressing the cellular processes that drive secondary damage. Novel neuroprotective therapies are urgently needed. The endocannabinoid system (ECS) is a promising therapeutic target for TBI. The ECS comprises the endocannabinoids anandamide and 2-AG, cannabinoid receptors CB1 and CB2, and metabolic enzymes like fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). It is involved in synaptic function, neuroinflammation, excitotoxicity, blood-brain barrier disruption, oxidative stress and neuronal loss. Modulation the ECS through receptor agonists/antagonists, inhibitors of endocannabinoid catabolism, or combination approaches represents a novel neuroprotective strategy in TBI.

Keywords: Cannabinoid receptors, Endocannabinoid system, Neuroprotection, Neuroinflammation, Traumatic brain injury.

Introduction

Abbreviations: TBI - Traumatic brain injury; ECS - Endocannabinoid system; eCB – Endocannabinoids; 2-AG - 2-Arachidonoylglycerol; AEA – Anandamide; FAAH - Fatty acid amide hydrolase; MAGL - Monoacylglycerol lipase; CB1R - Cannabinoid receptor 1; CB2R - Cannabinoid receptor 2; BBB - Blood-brain barrier; CNS - Central nervous system; THC -Tetrahydrocannabinol; CBD - Cannabidiol

Highlights

Traumatic brain injury (TBI) results in significant acute and chronic neurologic deficits underscoring the need for novel neuroprotective therapies.
The endocannabinoid system (ECS) is substantially dysregulated after TBI, with alterations in endocannabinoids, receptors, and enzymes.
Modulating the ECS through receptor agonism/antagonism, enzyme inhibition, or combined approaches confers neuroprotection in preclinical TBI models.
Cannabinoid receptors CB1R and CB2R represent promising targets, with evidence for reduced inflammation, excitotoxicity, and neuronal loss.
Inhibiting the catabolic enzymes FAAH and MAGL to elevate endogenous cannabinoids is also neuroprotective by activating CB1/CB2 receptors.

A combinatorial approach modulating multiple ECS targets may confer greater neuroprotection and allow lower therapeutic doses.

Introduction:

Traumatic brain injury (TBI) is a leading cause of death and disability worldwide, imposing a substantial personal, social and economic burden. It is estimated that globally, over 50 million people sustain a TBI each year [1]. In the United States alone, TBI accounts for more than 2.8 million emergency department visits, over 282,000 hospitalizations and approximately 56,000 deaths annually [2]. The total economic cost associated with TBI in the US has been estimated to be over $76 billion per year [3]. TBI can result from injuries arising from falls, motor vehicle accidents, sports and recreational activities, explosive blasts and combat injuries during military conflicts.

TBI encompasses a broad spectrum of injuries ranging from mild concussions to severe penetrating injuries. The initial traumatic impact leads to primary injury to neurons, glial cells and blood vessels in localized brain regions. This is followed by a prolonged secondary injury cascade involving complex neurochemical, metabolic and cellular changes that evolve over hours to weeks after the initial insult [4]. Secondary injury mechanisms include disruption of ionic homeostasis, release of cytotoxic levels of neurotransmitters like glutamate, increased free radical generation and oxidative stress, neuroinflammation, blood-brain barrier disruption, diffuse axonal injury and cell death signalling pathways [5]. The secondary injury exacerbates the initial tissue damage and loss of neurological function. Effective therapeutic interventions targeting the secondary injury mechanisms are lacking, which contributes to the brain's limited capacity for repair and regeneration after TBI.

Excitotoxicity mediated by excess extracellular glutamate is a key process that leads to calcium overload and neuronal death after TBI [6]. Traumatic axonal shearing damages neurons and causes unregulated glutamate release. Failure of glutamate uptake due to injury of astrocytes and dysfunction of transporters like GLT-1 further elevates extracellular glutamate levels [7]. High levels of glutamate overstimulate NMDA receptors, increasing calcium influx into cells. This disrupts mitochondrial function and activates catabolic enzymes like proteases, phospholipases and endonucleases, leading to cell damage and death [8].

Neuroinflammation after TBI involves both central and peripheral immune responses. The initial trauma leads to disruption of the blood-brain barrier (BBB), enabling infiltration of peripheral immune cells into the brain [9]. Resident microglia become activated within minutes to hours after injury, undergoing morphological changes and upregulating proinflammatory cytokines like interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNFα) [10]. While acute neuroinflammation can be protective by clearing debris, prolonged inflammation exacerbates neuronal damage through the release of cytokines, chemokines, reactive oxygen species (ROS) and nitric oxide [11].

Oxidative stress resulting from excessive ROS and reactive nitrogen species production is another deleterious consequence of TBI [12]. Normally, endogenous antioxidant systems help neutralize free radicals and maintain redox homeostasis in the brain. TBI disrupts this balance through increased ROS generation, impaired antioxidant defenses like glutathione depletion, and leakage of excitotoxins like glutamate which induce ROS production in mitochondria [13]. The high levels of ROS damage membrane lipids, proteins and DNA, ultimately leading to cell dysfunction and death.

The complex secondary injury mechanisms triggered by TBI disrupt neuronal circuits and contribute to chronic neurodegeneration. The initial insult causes focal macroscopic damage at the site of impact. This expands into more extensive microscopic damage to axons and dendrites that compromises neuronal signaling [14]. Disruption of synaptic connectivity through diffuse axonal injury is linked to deficits in learning, memory and information processing after TBI [15]. Ongoing cell loss via apoptotic and necrotic pathways, particularly in regions like the hippocampus, is associated with neuropsychiatric and behavioral disturbances post-TBI [16].

Current therapeutic approaches for managing TBI are limited to stabilizing acute symptoms and preventing secondary complications. Guidelines emphasize prompt medical attention, surgical treatment of mass lesions or hematomas, intracranial pressure monitoring and cerebrospinal fluid drainage [17]. Pharmacological interventions are restricted to drugs like analgesics, anticonvulsants, sedatives for controlling agitation and neuromuscular blockade for intracranial pressure management [18]. These interventions do not directly counteract the underlying secondary injury mechanisms. Neuroprotective drugs tested clinically like glutamate antagonists, free radical scavengers, anti-inflammatory agents and neurotrophins have largely failed to show

efficacy [19]. There is an urgent need for novel neuroprotective therapies that can salvage neurons and improve long-term outcomes after TBI.

The endocannabinoid system (ECS) has emerged as a promising therapeutic target for neuroprotection and limiting secondary damage after TBI. The ECS comprises the endocannabinoids, cannabinoid receptors and enzymes involved in endocannabinoid synthesis and degradation. It is an evolutionarily conserved lipid signaling system that plays important homeostatic roles in the central nervous system (CNS) [20]. The ECS regulates neurotransmission, neuroinflammation, excitotoxicity, mitochondrial function and responses to oxidative stress [21]. All these processes are also critically dysregulated after TBI. Growing evidence indicates that TBI triggers alterations in brain endocannabinoid levels, with region-specific changes in cannabinoid receptor expression and signaling [22]. Pharmacologically augmenting endocannabinoid tone confers neuroprotection in preclinical TBI models. The ECS therefore represents a promising endogenous brain system that can be therapeutically targeted to counteract secondary injury and promote recovery after TBI.

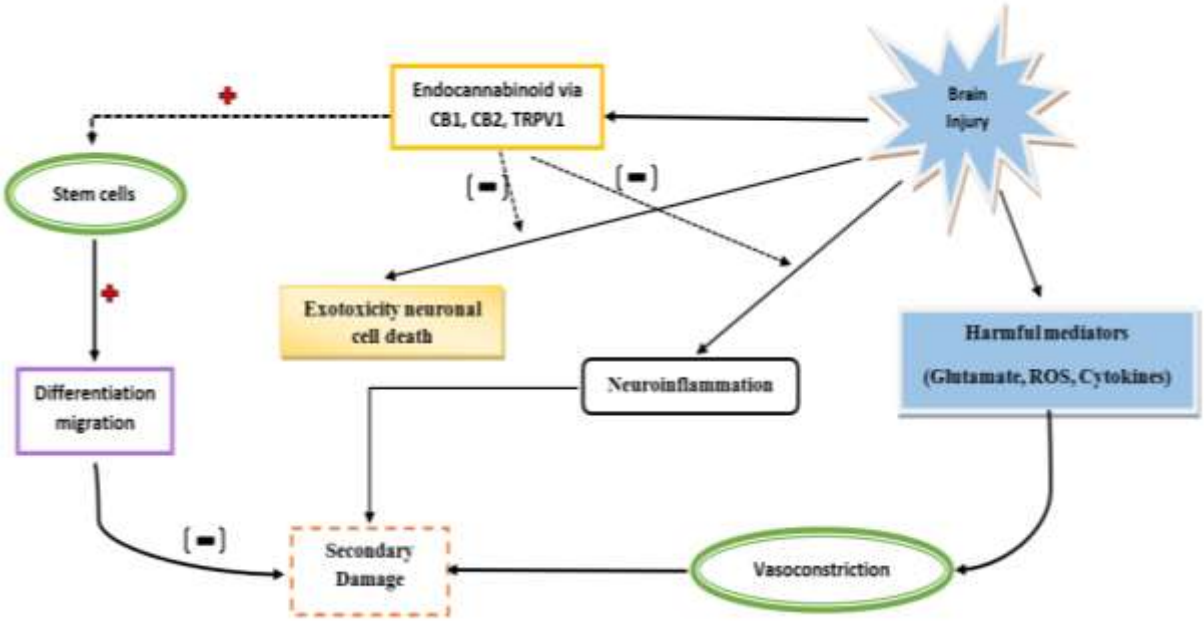

Figure 1: This schematic illustrates the pathophysiological pathways following traumatic brain injury and the neuroprotective role of endocannabinoids. TBI triggers the release of harmful mediators that act on neurons, astrocytes, and brain endothelial cells, leading to neuronal death, inflammation, and vasoconstriction which culminate in secondary damage. Concurrently, all brain cell types increase on-demand synthesis of endocannabinoids, which attenuate excitotoxicity, act as antioxidants, inhibit inflammatory cytokines, and counteract vasoconstriction from endothelin-1. Endocannabinoids also promote stem cell differentiation and migration. Through these mechanisms, endocannabinoids attenuate secondary damage and serve as endogenous neuroprotectants after TBI. Inhibition is designated by (-); enhanced activity is designated by (+).

The Endocannabinoid System:

The endocannabinoid system (ECS) comprises the endogenous cannabinoid ligands (endocannabinoids), cannabinoid receptors, and the enzymes involved in endocannabinoid biosynthesis and degradation. This evolutionarily conserved signaling system plays important regulatory functions in the brain, immune system, and peripheral tissues. The two best characterized endocannabinoids are N-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) [23]. They are synthesized on demand through cleavage of membrane lipid precursors and act locally at cannabinoid receptors before being rapidly degraded. AEA synthesis involves hydrolysis of N-arachidonoyl phosphatidylethanolamine catalyzed by several enzymes, including N-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) [24]. 2-AG is primarily formed from diacylglycerol through activation of diacylglycerol lipase (DAGL) [25]. The chief degradative routes are fatty acid amide hydrolase (FAAH) for AEA and monoacylglycerol lipase (MAGL) for 2-AG [26]. The two main cannabinoid receptors are Gi/o protein-coupled CB1 and CB2 receptors (Pertwee et al., 2010). CB1 is highly

expressed in the CNS, particularly at presynaptic terminals where it regulates neurotransmitter release [27]. CB2 is predominantly expressed in immune cells and regulates neuroinflammation [28].

The ECS regulates diverse physiological functions in the CNS like synaptic plasticity, cognition, pain modulation, motor control, feeding behaviours, stress response and neurodevelopment [29]. At synapses, endocannabinoids mediate retrograde signaling whereby postsynaptic depolarization and calcium influx stimulate endocannabinoid release that acts back on presynaptic CB1 to inhibit neurotransmitter release [30]. This modulates synaptic strength and plasticity. CB1 activation reduces prefrontal cortex glutamate levels [31]. The ECS also regulates neuroinflammatory responses through CB2-mediated effects on immune cell migration, cytokine release, and microglial activation states [32].

Given the critical involvement of the ECS in maintaining neuronal function and regulating inflammation, there has been growing interest in targeting this system to achieve neuroprotection and improve outcome after TBI. Preclinical studies demonstrate an important neuroprotective role of the ECS against excitotoxicity, inflammation, mitochondrial dysfunction and oxidative damage - processes that underlie the secondary injury cascades activated by TBI [21]. Clinical data also reveal alterations in endocannabinoid levels and receptor expression after TBI in humans that correlate with outcome [33]. These changes likely represent an adaptive response of the ECS to counteract brain damage. Harnessing the neuroprotective capacity of the ECS through exogenous cannabinoids or pharmacological modulation of endogenous tone presents a promising approach for developing novel TBI therapeutics [34].

The ECS plays important homeostatic roles in the central nervous system. It regulates neurotransmission, synaptic plasticity, learning and memory, stress responses, food intake, and responses to inflammation and injury [35]. In the brain, CB1 receptors are abundantly expressed presynaptically where they mediate inhibition of neurotransmitter release when activated by endocannabinoids. This “retrograde” signaling regulates synaptic strength and plasticity. The ECS also modulates neuroinflammatory responses and microglial activation states through CB2 receptor signaling [36]. Peripherally, the ECS regulates gastrointestinal motility, sensation of pain and appetite [37]. The psychoactive effects of cannabis are mediated through CB1 activation in the CNS. There is tremendous interest in understanding the physiological roles of the ECS to exploit its therapeutic potential in diverse pathologies ranging from pain to neurodegeneration [38].

Endocannabinoids: Anandamide and 2-AG:

N-arachidonoylethanolamine or anandamide (AEA) was the first endocannabinoid to be discovered in 1992 [39]. It is an amide derivative of arachidonic acid that is synthesized on demand through cleavage of its membrane precursor N-arachidonoyl phosphatidylethanolamine (NAPE). This reaction is catalyzed by NAPE-specific phospholipase D (NAPE-PLD), an intracellular membrane-associated enzyme responsive to calcium influx [40]. NAPE-PLD is widely expressed in the brain, with high levels in cortex, cerebellum, hippocampus and amygdala [41]. AEA synthesis can also occur through alternate phospholipase C and phosphatase pathways [42]. Following release, AEA is rapidly taken up into cells by a selective transporter and metabolized primarily by fatty acid amide hydrolase (FAAH) to yield arachidonic acid and ethanolamine [43]. FAAH is an intracellular serine hydrolase enriched in brain regions with high CB1 expression like the cortex, hippocampus, cerebellum and substantia nigra [44].

2-arachidonoylglycerol (2-AG) is another major endocannabinoid that was identified after AEA [45]. It constitutes the most abundant endocannabinoid species in the brain. 2-AG is synthesized through the hydrolysis of diacylglycerol (DAG) precursors, catalysed by two diacylglycerol lipase (DAGL) isozymes – DAGLα and DAGLβ [25]. These are transmembrane enzymes localized at postsynaptic dendritic spines in neurons, putting 2-AG synthesis in proximity to activation of CB1 receptors [46]. The primary catabolic enzyme for 2-AG is monoacylglycerol lipase (MAGL), which metabolizes it to arachidonic acid and glycerol. MAGL is ubiquitously present in the brain, with high levels in neuronal somatodendritic compartments [47].

AEA and 2-AG act as neuromodulators by binding to and activating cannabinoid receptors. They are synthesized in an “on-demand” fashion in response to elevations in intracellular calcium at postsynaptic sites [48]. This calcium-dependent synthesis causes accumulation of endocannabinoids which can diffuse across the synapse. By activating presynaptic CB1 receptors, they inhibit neurotransmitter release and thereby act as retrograde messengers regulating synaptic strength and plasticity [49]. This distinguishes endocannabinoids from classical neurotransmitters which are stored in vesicles and released in a calcium-dependent manner to

activate postsynaptic receptors. The rapid degradation of AEA and 2-AG by catabolic enzymes like FAAH and MAGL ensures spatial and temporal specificity of endocannabinoid signaling.

Cannabinoid Receptors: CB1 and CB2:

The psychoactive properties of cannabis are mediated through CB1 cannabinoid receptors in the brain [50]. CB1 is one of the most abundantly expressed GPCRs in the central nervous system. High levels are found in cortex, hippocampus, amygdala, basal ganglia, cerebellum and brainstem [51]. Within these regions, CB1 expression is enriched in presynaptic axon terminals and preterminal axons [52]. This strategic presynaptic localization underlies CB1 function of inhibiting neurotransmitter release when activated by retrograde endocannabinoid signals. Neurotransmitters negatively regulated by CB1 activation include glutamate, GABA, dopamine, acetylcholine, noradrenaline, serotonin and cholecystokinin [53]. CB1 couples predominantly to inhibitory Gi/o proteins [54]. Its activation reduces intracellular cyclic AMP levels, modulates ion channels and inhibits vesicular release.

In contrast to the predominant neuronal localization of CB1, CB2 receptors are mainly expressed in immune cells like macrophages, microglia, monocytes and B and T lymphocytes. Significant CB2 expression is also found in the spleen, tonsils and thymus gland [55]. Within the brain, CB2 receptors are expressed predominantly by microglia but also found at lower levels in some neuronal subpopulations [56]. Peripherally, CB2 is present in the gastrointestinal tract, liver, cardiovascular system, bone and reproductive organs [57]. It couples primarily to Gi proteins similar to CB1. Cannabinoid activation of CB2 modulates immune cell migration, cytokine and chemokine production, phagocyte function, and cellular proliferation and apoptosis [58].

Endocannabinoids bind to both CB1 and CB2 receptors with differing affinities and activate downstream signaling cascades. Anandamide exhibits moderately higher affinity for CB1 compared to CB2 (Ki values of 61 nM vs 279 nM respectively), making it a relatively selective CB1 agonist [59]. It binds transiently to CB1 due to rapid uptake and metabolism. 2-AG has more balanced affinity, only 2-3-fold selective for CB1 over CB2 (Ki of 472 nM vs 1400 nM) [60]. It acts as a full agonist at both receptors. The differential affinities and receptor selectivity profiles of AEA, 2-AG and exogenous cannabinoids like THC allows them to exert distinct pharmacological effects.

Endocannabinoid Catabolic Enzymes:

Tight regulation of endocannabinoid levels is achieved through catabolic enzymes that rapidly break down AEA and 2-AG after synthesis, ensuring spatial and temporal control of signaling. Fatty acid amide hydrolase (FAAH) is the chief catabolic enzyme for anandamide and related N-acylethanolamine fatty acid amides like oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) [26]. FAAH is an intracellular membrane-bound serine hydrolase with an unusual catalytic mechanism involving a nucleophilic serine residue [61]. It is abundantly expressed in brain regions with high CB1 levels including neocortex, hippocampus, amygdala and cerebellum [62]. FAAH knockout mice have 15-fold elevated brain anandamide levels highlighting its role in terminating anandamide signaling [63]. FAAH also regulates other bioactive lipids like OEA which reduces appetite by activating PPAR-α receptors [64].

Monoacylglycerol lipase (MAGL) is the primary enzyme responsible for metabolizing 2-AG in the brain. It catalyzes the hydrolysis of 2-AG to arachidonic acid and glycerol. MAGL accounts for approximately 85% of brain 2-AG hydrolase activity [65]. It is highly expressed presynaptically in neurons throughout the brain, prominently in regions like the cerebellum, hippocampus, cortex and striatum [47]. Mice lacking MAGL exhibit dramatic elevations in brain 2-AG levels (8-10-fold), reduced 2-AG hydrolysis, and decreased prostaglandin production through shunting of arachidonic acid metabolism [67]. MAGL inhibitors also increase brain 2-AG levels and produce CB1-dependent antinociceptive effects [68].

Together, FAAH and MAGL regulate the tone and signaling duration of the two major endocannabinoids AEA and 2-AG respectively. Inhibiting their catalytic activity elevates endocannabinoid levels which amplifies cannabinoid receptor activation. Small molecule inhibitors of FAAH like URB597 and PF-3845, as well as MAGL inhibitors like JZL184 have been valuable experimental tools to probe the functions of AEA and 2-AG in models of pain, anxiety, addiction, cancer, inflammation and neurodegeneration [68]. Dual FAAH/MAGL inhibitors are also under development for therapies exploiting the ECS.

Physiological Functions:

The widespread distribution of the ECS components in the central nervous system and periphery points to versatile physiological functions. Key roles include regulation of synaptic transmission, plasticity and neuronal excitability, stress adaptation, food intake, reward and motivation, nociception, inflammation and immune regulation [69].

Synaptic Transmission and Plasticity:

The abundant presynaptic localization of CB1 receptors allows endocannabinoids to act as retrograde messengers in modulating neurotransmitter release and synaptic plasticity (Ohno-Shosaku et al., 2012). Depolarization of the postsynaptic neuron causes calcium influx which stimulates endocannabinoid synthesis. Endocannabinoids like 2-AG diffuse across the synapse and activate presynaptic CB1 receptors, thereby reducing the probability of neurotransmitter release through Gi/o signaling [70]. This depresses synaptic strength, serving as a negative feedback mechanism. The effect is transient due to rapid endocannabinoid clearance. This form of short-term plasticity mediated by depolarization-induced suppression of excitation (DSE) or inhibition (DSI) is a key function of the ECS in the CNS [27].

CB1 activation inhibits release of both excitatory (glutamate) and inhibitory (GABA) neurotransmitters in different brain regions [71]. Glutamatergic synapses in the striatum and hippocampus show DSE while GABAergic synapses exhibit DSI. The ECS thus regulates the balance between excitation and inhibition based on neuronal activity patterns. It also controls long-term synaptic plasticity important for learning and memory [72]. CB1 knockout mice exhibit impaired short and long-term forms of synaptic plasticity. Pharmacological augmentation of 2-AG signaling facilitates long-term potentiation in hippocampus through increased glutamate release probability after tetanic stimulation [73].

Stress, Anxiety and Fear Extinction:

The ECS regulates physiological responses to stress primarily through hypothalamic and limbic circuits. Endocannabinoids attenuate the activation of the hypothalamic-pituitary-adrenal (HPA) axis during acute stress [74]. Exposure to stress causes rapid synthesis and accumulation of AEA in limbic regions which activates CB1 receptors and restores neuronal activity to baseline [75]. This stress-induced AEA mobilization is mediated by glucocorticoids and neuropeptides like corticotropin releasing factor [76]. CB1 signaling dampens the sympathetic nervous system response, reduces anxiety and facilitates fear extinction learning [77]. Drugs enhancing AEA tone exert anxiolytic and stress-relieving effects. On the contrary, CB1 antagonists can increase anxiety behaviours. The ECS thus restores homeostatic balance following perturbations like stress.

Appetite and Energy Balance:

The ECS plays an important role in regulating feeding behaviour and energy balance. CB1 activation robustly increases food intake in animals while CB1 antagonists suppress appetite and cause weight loss [78]. CB1 receptors on glutamatergic and GABAergic neurons regulate feeding-related circuits in the hypothalamus [79]. Gut peptides like ghrelin that stimulate appetite increase endocannabinoid levels in the hypothalamus [80]. In contrast, leptin signaling in the hypothalamus suppresses endocannabinoid tone, inducing satiety. Peripherally, CB1 activation increases lipogenesis in adipose tissue and liver. The orexigenic effects of cannabinoids are mediated by both central and peripheral CB1 receptors.

Pain Modulation:

Cannabinoids exert prominent analgesic effects through CB1 and CB2 dependent mechanisms [81]. The ECS regulates nociceptive processing at multiple levels including peripheral sensory neurons, spinal cord and higher brain centers like the periaqueductal gray, rostroventral medulla and thalamus [82]. CB1 activation inhibits transmission of painful stimuli by decreasing release of pro-nociceptive neurotransmitters like glutamate and CGRP [83]. CB2 modulation attenuates inflammatory pain by reducing release of cytokines and chemokines from immune cells. Cannabinoids also engage descending inhibitory pain pathways. FAAH inhibitors like URB597 that augment AEA exhibit analgesic efficacy in rodent models of acute and chronic pain [84].

Immune Regulation:

The ECS plays an important immunomodulatory role through CB2 receptor signaling in immune cells like macrophages, microglia, T cells and B cells [85]. Activation of CB2 dampens inflammatory responses by inhibiting signaling pathways involved in cytokine production, immune cell migration and proliferative responses [58]. Endocannabinoids also limit inflammatory pain signaling at peripheral nociceptors [81]. Selective CB2 agonists reduce macrophage activation and vascular inflammation without exerting psychotropic

effects mediated by neuronal CB1 [86]. Peripherally restricted CB1/CB2 agonists may be promising for treating inflammatory disorders.

Together, the multifaceted homeostatic functions of the ECS highlight its therapeutic potential. Pharmacological agents modulating endocannabinoid tone or directly targeting cannabinoid receptors open avenues for treating diverse pathological conditions ranging from chronic pain, anxiety, depression and feeding disorders to neuroinflammation, seizures, cognitive deficits and dependence disorders. An improved understanding of the physiological roles governed by the intricate ECS networks in the brain and body may facilitate such treatments.

The Endocannabinoid System after Traumatic Brain Injury:
Traumatic brain injury (TBI) triggers a complex secondary injury cascade comprising excitotoxicity, neuroinflammation, oxidative stress, mitochondrial dysfunction, apoptosis and blood-brain barrier disruption [87]. This exacerbates the primary mechanical damage and contributes to chronic neurodegeneration and functional impairments [88]. Growing evidence indicates that TBI also dysregulates the endocannabinoid system (ECS), an intrinsic neuromodulatory network that plays key homeostatic roles in the central nervous system [89]. Alterations in endocannabinoid levels, cannabinoid receptor expression and signaling have been documented in preclinical models across varied brain regions and timepoints after experimental TBI [90]. Changes also manifest in human patients based on limited measurements.

These ECS perturbations likely represent a partial adaptive response to counterbalance excitotoxic, inflammatory and oxidative insults. However sustained compromises in endocannabinoid tone and receptor signaling could promote neuropathology. Harnessing the ECS's innate homeostatic capacity through exogenous cannabinoids or pharmacological modulation offers a promising neuroprotective strategy after TBI [91]. This section will provide a detailed analysis of current evidence regarding spatiotemporal patterns of ECS changes from preclinical models and human studies. Links between ECS dysregulation and secondary injury mechanisms including excitotoxicity, neuroinflammation, mitochondrial dysfunction and blood-brain barrier disruption will be discussed.

Table 1: Preclinical studies demonstrate that cannabinoid treatment is associated with enhanced cognitive and motor function in animal models of traumatic brain injury:

Cannabinoid compound	**Doses**	**Experimental In vivo animal model**	**Receptors mediated/effects**	**Mechanism of Actions/results**	**References**
JZL184	10mg/kg, i.p.	C57BL/6 mouse induced CHI model	Not evaluated	↓ Neurodegeneration	Zhang et al., 2014
WWL70	10mg/kg, i.p.	C57BL/6 mouse induced CCI model with severe injury	CB1 CB1 and CB2	↓ lesion volume ↓ neurodegeneration	Tchantchou and Zhang, 2013
PF3845 (a selective FAAH inhibitor)	5mg/kg, i.p.	C57BL/6 mouse induced CCI model with severe injury	Not evaluated	↓ lesion volume ↓ neurodegeneration ↑ Bcl-2, Hsp70 and 72	Tchantchou et al 2014
2-AG	5mg/kg, i.p. 5mg/kg, i.p.	Mouse sabra Mouse C57BL/6 induced CHI, severe	Not evaluated CB1	↓ TNFalfa mRNA ↓ IL-1 Beta mRNA ↓ IL-6mRNA ↓ NF-kB translocation and transactivation	Panikashvili et al., 2006 Panikashvili et al., 2006
WWL70	10mg/kg, i.p.	Mouse C57BL/6 induced CCI, Severe	Not evaluated	↓ COX-2 expression ↓ iNOS expression M1 to M2 phenotype	Tchantchou and Zhang,2013
URB597	0.3 mg/kg,	Rat Sprague-Dawley induced	Not evaluated	BBB integrity protection	Kartz et al.,2015

	i.p.	Lateral FPI, mild			
HU-910	5-10mg/kg, i.p.	C57Bl/6 WT mice CHI model	CB2R agonists low CB1R affinity	↓inflammatory markers: ↓TNF-α, ↓IL-1α, ↓IL-1β, ↓IL-6 HU-914 showed the most important effects ↓TNF-α, ↓oedema ↓ BBB permeability	Magid et al.,2019
JWH133	1.5 mg/kg (i.p.)	Sprague–Dawley adult male rats CHI model	CB2R agonist	↓inflammatory markers: ↓IL-1β, ↓IL-6, ↓TNF-α, ↓ MMP2/9 ↑MKP-1→↓ MAPKs signalling pathway activation ↓neuroinflammation,	Li, L.; Yun, D. et al., 2018
CBD	10 mg/kg (i.p.)	C57BL/6 mice model of BCCAO	CB1R, CB2R agonist	↑nuclear receptors of the peroxisome proliferator-activated receptor family ↓adenosine uptake ↓reactive microglia and astrocytes	Mori. MA et al., 2017
SMM-189	6 mg/kg (i.p.)	C57BL/6J male mice mTBI	CB2R inverse agonist	↓ neuron loss preserve neuronal function and connectivity ↑beneficial M2 state of microglia	Liu.Y et al.,2017
ACEA	1 mg/kg, daily (i.p.)	Sprague–Dawley male rats TBI model	CB1R agonist	↓neuroinflammation, modulate metabolic processes → preserved neuronal tissues or functions	Arain et al.,2015

Endocannabinoid Level Changes after TBI:
Reductions in the two major endocannabinoids, N-arachidonoylethanolamine (anandamide or AEA) and 2-arachidonoylglycerol (2-AG), have been documented across various brain regions and post-injury time windows in rodent TBI models. However, the degree and duration of these changes vary based on factors like injury severity, model, brain region and time point examined. Early studies provided evidence of acute, transient reductions in endocannabinoids after experimental TBI. A rat lateral fluid percussion injury model showed decreases in cortical AEA and 2-AG levels measured at 6 hours, which recovered by 24 hours [93]. A milder weight drops impact acceleration model in mice also caused reductions in cortical and hippocampal AEA at 6 hours, which normalized by 1 day except for persistence in the hippocampus [94].

More sustained endocannabinoid deficiencies lasting days after TBI have been reported as well. Cortical AEA was reduced starting 1 day and persisting until 4 days following lateral fluid percussion injury in rats [94]. This was accompanied by cognitive deficits in novel object recognition. Controlled cortical impact in rats also decreased ipsilateral cortical AEA and 2-AG content for a prolonged period between 2-7 days post-injury along with memory impairment [95]. However, the contralateral cortex showed no statistical changes indicating regional specificity. The partial correlations between endocannabinoid reductions and cognitive dysfunction in these models suggest a role for ECS compromises in post-traumatic memory and learning impairments.

Region-dependent effects are evident with certain models selectively decreasing endocannabinoids in vulnerable areas like the hippocampus. Mice subjected to lateral fluid percussion injury exhibit reductions in AEA

specifically within the ipsilateral hippocampus but not cortex [96]. Focal cortical contusion injury in rats also results in significantly greater losses of AEA in the hippocampus compared to cortex from 1 hour until 7 days post-TBI [97]. Dose-dependent endocannabinoid depletion was demonstrated in a rat impact acceleration model where only high intensity TBI, but not a milder injury, decreased AEA levels in the ipsilateral cortex and contralateral hippocampus at 24 hours [98].

Several studies indicate that changes in catabolic enzyme function after TBI could contribute to the decreases in AEA and 2-AG levels by enhancing their breakdown. One report found increased activity of fatty acid amide hydrolase (FAAH), the primary catabolic enzyme for AEA, in the rat pericontusional cortex at 24 hours following controlled cortical impact [99]. This could accelerate AEA metabolism and reduce signaling. Monoacylglycerol lipase (MAGL) activity, which breaks down 2-AG, was also elevated. TBI appears to dysregulate endocannabinoid metabolic pathways, though further research on synthetic enzymes is needed. FAAH inhibition could potentially restore AEA signaling after TBI as part of a neuroprotective strategy.

Clinical evidence also supports alterations in endocannabinoid levels after human TBI, although limited to CSF measurements in small patient cohorts. One study reported significant decreases in AEA and related N-acylethanolamide fatty acid amides including OEA and PEA within the CSF of adult patients with moderate to severe TBI [98]. Analysis of CSF samples from pediatric TBI patients in another study revealed elevated 2-AG acutely at 24 hours post-injury compared to uninjured controls [100]. However, both 2-AG and AEA were decreased in the subacute/chronic phase. Overall, the clinical data corroborates dysregulated endocannabinoid tone after human TBI, though further validation is needed.

CB1 Receptor Changes after TBI:
Multiple preclinical TBI models have demonstrated significant downregulation of CB1 receptor expression and signaling capacity in various brain regions, which likely contributes to excitotoxicity and neuronal damage. CB1 is highly expressed on presynaptic terminals and plays a key role in regulating neurotransmitter release probability [49]. Early evidence from rat lateral fluid percussion injury revealed dramatic acute reductions in CB1 receptor binding and G-protein activation in the injured cortex measured at 15 minutes to 4 hours post-TBI [101]. This suggests rapid impairment of CB1 function. Another study using a mouse closed-head weight drop model found up to 50% decreases in cortical CB1 binding as early as 4 hours after injury [94]. Reduced cell surface localization likely contributes to loss of CB1 signaling capacity after TBI.

Persistent CB1 deficiencies lasting days after experimental TBI have also been widely reported. Cortical impact injury in rats did not alter total CB1 protein at 24 hours but reduced receptor binding and signaling [98]. CB1 gene expression was also decreased in the injured cortex at 7 days post-TBI [22]. Lateral fluid percussion injury resulted in a biphasic response - initial upregulation of CB1 binding at 24 hours followed by reductions persisting until 7 days in mice [101]. Other models like controlled cortical impact elicit consistent downregulation of CB1 in cortical and hippocampal regions up to 7 days or longer post-injury [22; 95]. The variability in responses could depend on injury type and severity.

Region-specific losses in CB1 availability correlate with extent of damage and functional deficits. A study employing graded impact acceleration injury in rats found greater decreases in hippocampal CB1 levels and impaired Morris water maze performance with increasing TBI intensity [103]. Similarly, controlled cortical impact in mice produced CB1 reductions in both cortex and hippocampus linked to memory dysfunction [94]. Preserving CB1 signaling with exogenous cannabinoids could potentially counteract these losses and confer neuroprotection.

Positron emission tomography (PET) imaging in human TBI patients corroborates preclinical evidence of persistent CB1 deficiencies. Studies using [18F]MK-9470, a high affinity CB1 receptor radioligand, demonstrate chronically reduced CB1 binding starting 2 weeks until years after moderate-severe TBI [33;86]. Loss of CB1 availability is observed in temporal, frontal, parietal and cingulate cortical regions as well as basal forebrain and Ponto mesencephalic areas. Lower CB1 binding also correlates with worse post-concussive symptoms like headache, anxiety, memory and sleep disturbances [33]. PET imaging has thus validated dysregulated CB1 signaling as a long-term pathological consequence of human TBI.

CB2 Receptor Changes after TBI:
In contrast to CB1 downregulation, most experimental TBI models exhibit an upregulation of CB2 receptor expression in various brain regions [91]. CB2 is predominantly localized to microglia and other CNS immune

cells and plays a key role in regulating neuroinflammation [32]. Increased CB2 levels likely represent an adaptive response to counteract chronic microgliosis after TBI through enhanced endocannabinoid signaling effects. A rat lateral fluid percussion injury model showed elevated CB2 gene expression in the ipsilateral cortex starting 12 hours until 7 days post-TBI, along with increased microglial activation markers [22]. Maximal CB2 elevations were observed at 3 days.

Controlled cortical impact injury similarly increased cortical and hippocampal CB2 levels from 1 day until 7 days post-TBI in rats [93;94]. CB2 upregulation also manifests in white matter tracts like the corpus callosum in diffuse TBI models (Li et al., 2018). The time course coincides with elevation of pro-inflammatory mediators including cytokines like IL-1β, TNFα and enzymes such as COX-2 after TBI. Augmenting CB2 signaling with agonists reduces these neuroinflammatory markers, signifying a protective adaptive response [102]. However, other models show more complex regulation, with late phase CB2 deficits emerging after initial upregulation [101]. Maintaining chronic CB2 functionality with agonists or inhibitors of endocannabinoid catabolism might confer ongoing anti-inflammatory benefits after TBI.

Limited clinical evidence supports similar CB2 upregulation trends after human TBI. One study examined CB2 gene expression in peripheral blood leukocytes collected longitudinally over 15 days from severe TBI patients, finding significant elevations during the initial acute five days post-injury that gradually declined [100]. Increased CB2 levels in circulating immune cells could parallel CNS changes. Post-mortem immunohistochemical analysis of cortical tissue from TBI patients also shows increased CB2 expression associated with activated microglia compared to controls [33]. Although preliminary, these findings warrant further exploration of the CB2 receptor as an immunomodulatory target after TBI.

Regional and Temporal Patterns of ECS Changes after TBI:
Collectively examining the literature reveals distinctive spatiotemporal patterns of ECS alterations across various brain regions following experimental TBI (Figure 1). Cortical areas directly damaged by focal impact exhibit acute deficits in AEA levels and CB1 signaling within hours that may persist for days [93;103]. This could promote excitotoxicity from excessive glutamate release. Hippocampal regions show delayed reductions in AEA and CB1 starting 1 day post-TBI and lasting longer [22;102]. This impairs cognitive processes like memory. In contrast, CB2 receptors are upregulated in a delayed fashion from 1-7 days in cortical, hippocampal and white matter regions coinciding with evolution of inflammation [95;102].

Cerebellar endocannabinoid deficiencies also emerge at delayed timepoints like 7 days post-TBI [95]. Loss of CB1-mediated inhibition of glutamate and GABA release by Purkinje neurons could impair cerebellar control of motor function and cognition [34]. Basal ganglia regions sustaining axonal injury show chronic reductions in AEA and CB1 receptor availability over months, which could exacerbate excitotoxicity [86]. Domains of vulnerable white matter tracts like the corpus callosum exhibit persistent glial activation and CB2 elevation without overt cell loss, possibly signifying a reparative response [102].

These region-specific and temporal patterns of ECS dysregulation suggest opportunities for targeted modulation at strategic time windows after TBI. For instance, restoring acute CB1 signaling in cortical regions could mitigate excitotoxicity, while delayed CB2 enhancement might confer anti-inflammatory effects. Chronic CB1 augmentation could improve functional connectivity of damaged networks. Deep profiling of ECS changes with expanding analytical techniques, timepoints and injury models could further inform therapeutic targeting. Sex-based differences and responses in aged, diseased or genetically modified animal models also require further characterization.

Linking ECS Dysfunction to Excitotoxic and Inflammatory Pathology after TBI:
The alterations in endocannabinoid tone and cannabinoid receptor availability demonstrate significant association with excitotoxic and inflammatory cascades after experimental TBI. Preserving ECS function could help counterbalance these secondary injury responses. Excitotoxicity mediated by excessive extracellular glutamate is a major contributor to neuronal death and dysfunction after TBI [104]. Loss of CB1-mediated inhibition of glutamate release presynaptically could aggravate excitotoxicity after TBI by disinhibiting cortical and hippocampal glutamatergic neurons [22;102].

In support of this premise, TBI studies have shown increased levels of glutamate, overexpression of NMDA receptors, and reduced expression of glutamate transporters like GLT-1 paralleling reductions in CB1 availability [95;96]. CB1 expression shows significant inverse correlation with glutamate levels [105].

Administration of CB1 agonists attenuates glutamate excitotoxicity and loss of GLT-1 after experimental TBI, confirming a neuroprotective influence [94]. Preserving CB1 signaling could confer similar benefits. TBI also elicits loss of cortical parvalbumin interneurons expressing CB1 which normally inhibit network excitability [93].

Neuroinflammatory changes after TBI, mediated by microglial and astrocyte activation along with infiltration of peripheral immune cells, also correlate with ECS dysregulation [89]. The delayed upregulation of CB2 expression in microglia and other brain regions coincides temporally with elevation of pro-inflammatory cytokines like IL-1β, TNFα and IL-6 as well as signaling molecules such as p38 MAP kinase and NFκB [95;102]. Activating CB2 with agonists after TBI reduces several of these neuroinflammatory markers [95]. Increased 2-AG levels also correlate with microglial activation, possibly signifying an adaptive response [100].
Disruption of the blood-brain barrier (BBB) is another pathological event influenced by the ECS. TBI leads to altered expression of tight junction proteins, damage to endothelial cells and matrix metalloproteinase activation, compromising BBB integrity [106]. This can promote cerebral edema, raised intracranial pressure and initiation of inflammatory cascades. The ECS helps maintain BBB function under homeostasis and injury through effects on junction proteins, astrocyte signaling and leukocyte diapedesis [107]. Both CB1 and CB2 activation with agonists can mitigate TBI-induced increases in BBB permeability in preclinical models [95;96].
In summary, optimized functioning of the ECS could counteract excitotoxic, inflammatory and BBB-related secondary injury cascades that drive neuropathology after TBI. Therapeutic strategies that augment cannabinoid signaling through receptor modulation, inhibition of endocannabinoid catabolism or exogenous cannabinoids could leverage these protective effects. Further mechanistic studies in preclinical models and human patients focused on linking ECS changes to specific pathological processes could help refine therapeutic targets and approaches.

Mitochondrial and Oxidative Changes:
Traumatic brain injury also disrupts mitochondrial function and cellular energy regulation, while increasing oxidative stress through excessive reactive oxygen species (ROS) generation from injured tissues [108]. Mitochondrial damage can be mediated by excitotoxicity-related calcium overload, inflammatory cytokines, and oxidative modifications [109]. Compromised mitochondrial bioenergetics is reflected in reduced ATP levels and impaired cellular metabolism after clinical TBI [110]. Oxidative stress results from increased ROS production combined with depletion of endogenous antioxidants like glutathione, overwhelming normal radical scavenging capacity [111].

The ECS plays an important role in regulating mitochondrial function and oxidative stress responses under physiological and pathological conditions [112]. Cannabinoid receptors CB1 and CB2 are expressed in neuronal and glial mitochondria where they can modulate energy metabolism, respiration, membrane potential and apoptosis signaling [113]. Endocannabinoids like AEA and 2-AG protect against oxidative injury in vitro through antioxidant effects independent of cannabinoid receptors [114]. Administration of exogenous cannabinoids confers mitochondrial and antioxidant effects in neurodegenerative disease models.

Similar mechanisms could potentially counteract mitochondrial dysfunction and oxidative damage after TBI. However, current evidence directly linking ECS changes to these secondary injury processes is limited. One study found that administering a CB2 agonist for 5 days after experimental TBI attenuated oxidative markers like ROS and normalized glutathione levels along with improving mitochondrial respiration parameters and ATP levels [115]. This indicates ECS activation helps preserve mitochondrial function after TBI. Analysis of mitochondrial CB1 and CB2 expression, endocannabinoid localization and metabolic profiles could reveal additional targets for mitigating oxidative and bioenergetic derangements post-TBI through the ECS.

Neuropsychiatric and Neurological Sequelae:
Traumatic brain injury often leads to chronic neuropsychiatric and neurological disturbances including increased risks for cognitive impairment, epilepsy, movement disorders, sleep dysfunction and neurodegenerative disease [88]. The pathogenesis implicates progressive neurodegeneration, network hyperexcitability, neuroinflammation and synaptic dysfunction mediated by the secondary injury cascades [116]. Dysregulation of the ECS could directly contribute to these outcomes based on its roles in regulating neuronal excitability, plasticity, circadian signaling, neuroimmune responses and neuroprotective mechanisms [34].

For instance, post-traumatic epilepsy can arise from factors like axonal injury, inflammation, and network hyperexcitability from loss of GABAergic interneurons and reduced CB1-mediated inhibition of glutamate

release [117]. TBI-induced deficits in CB1 availability could promote conditions permissive for seizures. Administration of cannabinoid CB1/CB2 receptor agonists suppresses epileptiform activity in models of post-traumatic epilepsy (Gholizadeh et al., 2017). Neuroinflammation mediated by chronic microglial activation likely contributes to cognitive and psychiatric symptoms after TBI, which could be ameliorated by enhancing CB2 signaling [89]. Disturbances in sleep-wake cycles and endocannabinoid signaling also correlate after experimental TBI [115]. Targeting CB1 receptors could alleviate motor deficits based on their role in regulating basal ganglia circuitry [34]. Further research on associations between ECS dysregulation and neurological/neuropsychiatric sequelae after TBI could help guide therapeutic development or prognostic use.

Future Directions and Limitations:

The mounting preclinical evidence revealing trauma-induced dysregulation of the endocannabinoid system (ECS) after traumatic brain injury highlights modulation of this intrinsic neuromodulatory network as a promising neuroprotective strategy. However, fully actualizing the potential of targeted ECS-based therapies requires addressing key research gaps and overcoming challenges around clinical translation.

Currently, our understanding of how traumatic brain injury alters the ECS remains incomplete. While preclinical studies demonstrate changes in endocannabinoid levels, receptor expression and enzyme function across varied brain regions and post-injury time points, detailed spatiotemporal characterization in human patients is lacking. Expanding analysis to track chronic ECS alterations over months-years could reveal therapeutic windows to mitigate neurodegeneration. Directly correlating dynamic ECS disruption patterns with evolution of specific secondary injury mechanisms like neuroinflammation, mitochondrial dysfunction and network hyperexcitability is needed to strategically target components for maximum benefit.

The delivery methods for exogenous cannabinoids used in preclinical studies often bypass the blood-brain barrier via direct intracerebral administration. Advancing delivery systems like nanoparticles, liposomes, intranasal administration or temporary BBB opening could optimize brain bioavailability and therapeutic dosing of cannabinoid treatments. While synthetic CB1/CB2 receptor agonists demonstrate neuroprotective efficacy in animal models, their clinical potential is hampered by risks of psychoactive side effects and abuse liability with non-selective CB1 activation. Developing cannabinoid drugs restricted to the periphery or localized delivery could improve safety. Another promising approach is augmenting endogenous cannabinoid tone through inhibitors of catabolic enzymes like FAAH and MAGL, which avoid direct receptor stimulation. However, sustained elevation of cannabinoid signaling may carry risks of tolerance or dependence with long-term use. Strategies like allosteric modulation of CB1 may enable more nuanced, context-specific manipulation. Combined interventions engaging multiple ECS targets could harness additive benefits with attenuated side effects. Yet balancing complex pharmacological interactions poses challenges.

The heterogeneity of traumatic brain injury pathology and recovery trajectories suggests personalized medicine approaches could optimize outcomes. Identifying sensitive patient subpopulations, injury biomarkers and genetic factors influencing responses to ECS therapeutics could improve treatment efficacy. Rigorous placebo-controlled clinical trials are critical for establishing safety and effectiveness of cannabinoid therapies in light of complications like spontaneous recovery. Currently, clinical evaluation remains early with a lack of robust human data.

In conclusion, trauma-induced ECS disruption likely represents an adaptive but insufficient attempt to counteract secondary injury cascades. Resulting compromises in endocannabinoid signaling may exacerbate neuropathology. Preclinical evidence demonstrates directly augmenting ECS tone confers neuroprotection and functional recovery. However, methodical clinical development is imperative to actualize therapeutic potential. Deepening our understanding of trauma-related ECS changes through expanded preclinical research and controlled human studies is paramount. Overcoming limitations around delivery, psychoactive effects and patient heterogeneity will be pivotal. Progressing cannabis-based medicine for TBI from anecdotal promise to proven clinical reality will require systematic, collaborative efforts across multidisciplinary teams and rigorous validation. The complexity of TBI pathobiology warrants moving beyond a simplistic “one drug fits all” approach. Advancing personalized, pathology-targeted treatments guided by deeper biological insights could fulfill the promise of harnessing the ECS as a therapeutic system for managing TBI’s diverse consequences. With care and persistence, this evolutionarily conserved neuroprotective network may offer much needed new hope for effectively treating a highly challenging condition.

References:

1. Maas AI, Menon DK, Adelson PD, Andelic N, Bell MJ, Belli A, et al. Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research. Lancet Neurol. 2017;16(12):987-1048.
2. Taylor CA, Bell JM, Breiding MJ, Xu L. Traumatic Brain Injury-Related Emergency Department Visits, Hospitalizations, and Deaths - United States, 2007 and 2013. MMWR Surveill Summ. 2017;66(9):1-16.
3. Coronado VG, McGuire LC, Sarmiento K, Bell J, Lionbarger MR, Jones CD, et al. Trends in Traumatic Brain Injury in the U.S. and the public health response: 1995-2009. J Safety Res. 2012;43(4):299-307.
4. Werner C, Engelhard K. Pathophysiology of traumatic brain injury. Br J Anaesth. 2007;99(1):4-9.
5. Blennow K, Brody DL, Kochanek PM, Levin H, McKee A, Ribbers GM, et al. Traumatic brain injuries. Nat Rev Dis Primers. 2016;2:16084.
6. Yi JH, Hazell AS. Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. Neurochem Int. 2006;48(5):394-403.
7. van Landeghem FK, Weiss T, Oehmichen M, von Deimling A. Decreased expression of glutamate transporters in astrocytes after human traumatic brain injury. J Neurotrauma. 2006;23(10):1518-28.
8. Arundine M, Tymianski M. Molecular mechanisms of glutamate-dependent neurodegeneration in ischemia and traumatic brain injury. Cell Mol Life Sci. 2004;61(6):657-68.
9. Jassam YN, Izzy S, Whalen M, McGavern DB, El Khoury J. Neuroimmunology of Traumatic Brain Injury: Time for a Paradigm Shift. Neuron. 2017;95(6):1246-65.
10. Morganti-Kossmann MC, Satgunaseelan L, Bye N, Kossmann T. Modulation of immune response by head injury. Injury. 2002;33(8):597-600.
11. Woodcock T, Morganti-Kossmann MC. The role of markers of inflammation in traumatic brain injury. Front Neurol. 2013;4:18.
12. Ansari MA, Roberts KN, Scheff SW. Oxidative stress and modification of synaptic proteins in hippocampus after traumatic brain injury. Free Radic Biol Med. 2008;45(4):443-52.
13. Bayir H, Kagan VE, Tyurina YY, Tyurin V, Ruppel RA, Adelson PD, et al. Assessment of antioxidant reserves and oxidative stress in cerebrospinal fluid after severe traumatic brain injury in infants and children. Pediatr Res. 2005;57(4):571-8.
14. Smith DH, Johnson VE, Stewart W. Chronic neuropathologies of single and repetitive TBI: substrates of dementia? Nat Rev Neurol. 2013;9(4):211-21.
15. Armstrong RC, Mierzwa AJ, Marion CM, Sullivan GM. White matter involvement after TBI: Clues to axon and myelin repair capacity. Exp Neurol. 2016;275:328-33.
16. Raghupathi R. Cell death mechanisms following traumatic brain injury. Brain Pathol. 2004;14(2):215-22.
17. Carney N, Totten AM, O'Reilly C, Ullman JS, Hawryluk GW, Bell MJ, et al. Guidelines for the Management of Severe Traumatic Brain Injury, Fourth Edition. Neurosurgery. 2017;80(1):6-15.
18. Holloway KL, Barnes P, Choi S, Bullock R, Marshall LF, Eisenberg HM, et al. Ventriculostomy infections: The effect of monitoring duration and catheter exchange in 584 patients. Neurosurgery. 1996;39(2):353-60.
19. Margulies S, Hicks R, Combination Therapies for Traumatic Brain Injury Workshop Leaders. Combination therapies for traumatic brain injury: prospective considerations. J Neurotrauma. 2009;26(6):925-39.
20. Alger BE, Kim J. Supply and demand for endocannabinoids. Trends Neurosci. 2011;34(6):304-15.
21. Zou S, Kumar U. Cannabinoid Receptors and the Endocannabinoid System: Signaling and Function in the Central Nervous System. Int J Mol Sci. 2018;19(3):833.
22. Martinez-Vargas M, Morales-Gomez J, Gonzalez-Rivera R, Hernandez-Enriquez J, Perez-Arredondo A, Estrada-Rojo F, et al. Does the endocannabinoid system play a role in the pathogenesis of traumatic brain injury? Front Integr Neurosci. 2013;7:91.
23. Pacher P, Batkai S, Kunos G. The Endocannabinoid System as an Emerging Target of Pharmacotherapy. Pharmacol Rev. 2006;58(3):389-462.
24. Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem. 2004;279(7):5298-305.
25. Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, et al. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol. 2003;163(3):463-8.

26. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature. 1996;384(6604):83-7.
27. Castillo PE, Younts TJ, Chávez AE, Hashimotodani Y. Endocannabinoid signaling and synaptic function. Neuron. 2012;76(1):70-81.
28. Cabral GA, Griffin-Thomas L. Emerging role of the cannabinoid receptor CB2 in immune regulation: therapeutic prospects for neuroinflammation. Expert Rev Mol Med. 2009;11:e3.
29. Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. Physiol Rev. 2009;89(1):309-80.
30. Wilson RI, Nicoll RA. Endocannabinoid signaling in the brain. Science. 2002;296(5568):678-82.
31. Lafourcade M, Elezgarai I, Mato S, Bakiri Y, Grandes P, Manzoni OJ. Molecular Components and Functions of the Endocannabinoid System in Mouse Prefrontal Cortex. PLoS One. 2007;2(8):e24871.
32. Mecha M, Carrillo-Salinas FJ, Feliú A, Mestre L, Guaza C. Microglia Activation States and Cannabinoid System: Therapeutic Implications. Pharmacol Ther. 2015;152:111-26.
33. Donat CK, Scott G, Zhang F, Saha B. Associations Between Endocannabinoid Levels and Cognitive Function in Healthy Older Subjects. Front Aging Neurosci. 2016;8:33.
34. Fernández-Ruiz J, Moro MA, Martínez-Orgado J. Cannabinoids in Neurodegenerative Disorders and Stroke/Brain Trauma: From Preclinical Models to Clinical Applications. Neurotherapeutics. 2015;12(4):793-806.
35. Katona I, Freund TF. Multiple Functions of Endocannabinoid Signaling in the Brain. Annu Rev Neurosci. 2012;35:529-58.
36. Cabral GA, Raborn ES, Griffin L, Dennis J, Marciano-Cabral F. CB2 receptors in the brain: role in central immune function. Br J Pharmacol. 2008;153(2):240-51.
37. Izzo AA, Sharkey KA. Cannabinoids and the gut: New developments and emerging concepts. Pharmacol Ther. 2010;126(1):21-38.
38. Pertwee RG. Inverse agonism and neutral antagonism at cannabinoid CB1 receptors. Life Sci. 2005;76(12):1307-24.
39. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 1992;258(5090):1946-9.
40. Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem. 2007;279(7):5298-305.
41. Egertová M, Cravatt BF, Elphick MR. Comparative analysis of fatty acid amide hydrolase and CB(1) cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. Neuroscience. 2003;119(2):481-96.
42. Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, et al. A biosynthetic pathway for anandamide. Proc Natl Acad Sci U S A. 2006;103(36):13345-50.
43. Fegley D, Kathuria S, Mercier R, Li C, Goutopoulos A, Makriyannis A, et al. Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. Proc Natl Acad Sci USA. 2004;101(23):8756-61.
44. Egertová M, Giang DK, Cravatt BF, Elphick MR. A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. Proc Biol Sci. 1998;265(1410):2081-5.
45. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol. 1995;50(1):83-90.
46. Yoshida T, Fukaya M, Uchigashima M, Miura E, Kamiya H, Kano M, Watanabe M. Localization of diacylglycerol lipase-alpha around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid CB1 receptor. J Neurosci. 2006;26(18):4740-51.
47. Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, et al. Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci U S A. 2002;99(16):10819-24.
48. Ohno-Shosaku T, Kano M. Endocannabinoid-mediated retrograde modulation of synaptic transmission. Curr Opin Neurobiol. 2014;29:1-8.
49. Castillo PE, Younts TJ, Chávez AE, Hashimotodani Y. Endocannabinoid signaling and synaptic function. Neuron. 2012;76(1):70-81.
50. Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET, Frank RA. Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. Arch Gen Psychiatry. 2001;58(4):322-8.

51. Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC. Cannabinoid receptor localization in brain. Proc Natl Acad Sci U S A. 1990;87(5):1932-6.
52. Egertová M, Elphick MR. Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB. J Comp Neurol. 2000;422(2):159-71.
53. Schlicker E, Kathmann M. Modulation of transmitter release via presynaptic cannabinoid receptors. Trends Pharmacol Sci. 2001;22(11):565-72.
54. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, et al. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev. 2002;54(2):161-202.
55. Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P, et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem. 1995;232(1):54-61.
56. Atwood BK, Mackie K. CB2: a cannabinoid receptor with an identity crisis. Br J Pharmacol. 2010;160(3):467-79.
57. Pacher P, Kunos G. Modulating the Endocannabinoid System in Human Health and Disease: Successes and Failures. FEBS J. 2013;280(9):1918-43.
58. Turcotte C, Blanchet MR, Laviolette M, Flamand N. The CB2 receptor and its role as a regulator of inflammation. Cell Mol Life Sci. 2016;73(23):4449-70.
59. Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, et al. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. Pharmacol Rev. 2010;62(4):588-631.
60. Sugiura T, Kodaka T, Nakane S, Miyashita T, Kondo S, Suhara Y, et al. Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. Structure-activity relationship of 2-arachidonoylglycerol, ether-linked analogues, and related compounds. J Biol Chem. 1999;274(5):2794-801.
61. McKinney MK, Cravatt BF. Structure and function of fatty acid amide hydrolase. Annu Rev Biochem. 2005;74:411-32.
62. Egertová M, Cravatt BF, Elphick MR. Comparative analysis of fatty acid amide hydrolase and CB(1) cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. Neuroscience. 2003;119(2):481-96.
63. Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, et al. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc Natl Acad Sci U S A. 2001;98(16):9371-6.
64. Fu J, Gaetani S, Oveisi F, Lo Verme J, Serrano A, Rodríguez De Fonseca F, et al. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. Nature. 2003;425(6953):90-3.
65. Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol. 2007;14(12):1347-56.
66. Chanda PK, Gao Y, Mark L, Btesh J, Strassle BW, Lu P, et al. Monoacylglycerol lipase activity is a critical modulator of the tone and integrity of the endocannabinoid system. Mol Pharmacol. 2010;78(6):996-1003.
67. Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, et al. Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. Nat Chem Biol. 2009;5(1):37-44.
68. Piomelli D, Tarzia G, Duranti A, Tontini A, Mor M, Compton TR, et al. Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). CNS Drug Rev. 2006;12(1):21-38.
69. Lutz B. Endocannabinoid Signals in the Control of Emotion. Curr Opin Pharmacol. 2009;9(1):46-52.
70. Ohno-Shosaku T, Kano M. Endocannabinoid-mediated retrograde modulation of synaptic transmission. Curr Opin Neurobiol. 2014;29:1-8.
71. Hoffman AF, Lupica CR. Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus. J Neurosci. 2000;20(7):2470-9.
72. Heifets BD, Castillo PE. Endocannabinoid signaling and long-term synaptic plasticity. Annu Rev Physiol. 2009;71:283-306.
73. Pan B, Wang W, Long JZ, Sun D, Hillard CJ, Cravatt BF, et al. Blockade of 2-arachidonoylglycerol hydrolysis by selective monoacylglycerol lipase inhibitor 4-nitrophenyl 4-(dibenzo[d][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate (JZL184) enhances retrograde endocannabinoid signaling. J Pharmacol Exp Ther. 2009;331(2):591-7.
74. Gorzalka BB, Hill MN, Hillard CJ. Regulation of endocannabinoid signaling by stress: implications for stress-related affective disorders. Neurosci Biobehav Rev. 2008;32(6):1152-60.

75. Hill MN, Gorzalka BB. Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? Behav harmacol. 2005;16(5-6):333-52.
76. Wang W, Sun D, Pan B, Roberts CJ, Sun X, Hillard CJ, et al. Deficiency in endocannabinoid signaling in the nucleus accumbens induced by chronic unpredictable stress. Neuropsychopharmacology. 2012;37(11):2449-61.
77. Lafenêtre P, Chaouloff F, Marsicano G. The endocannabinoid system in the processing of anxiety and fear and how CB1 receptors may modulate fear extinction. Pharmacol Res. 2007;56(5):367-81.
78. Williams CM, Kirkham TC. Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. Psychopharmacology (Berl). 1999;143(3):315-7.
79. Cardinal P, Bellocchio L, Clark S, Cannich A, Klugmann M, Lutz B, et al. Hypothalamic CB1 cannabinoid receptors regulate energy balance in mice. Endocrinology. 2012;153(9):4136-43.
80. Kola B, Hubina E, Tucci SA, Kirkham TC, Garcia EA, Mitchell SE, et al. Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. J Biol Chem. 2005;280(26):25196-201.
81. Guindon J, Hohmann AG. The endocannabinoid system and pain. CNS Neurol Disord Drug Targets. 2009;8(6):403-21.
82. Lötsch J, Stock C, Albrecht S, Schaaf L, Ultsch A. Functional and pharmacological characterization of urotensin II effects at urotensin and neurotensin receptors of rat brain. Peptides. 2002;23(1):109-16.
83. Guindon J, Beaulieu P. The role of the endogenous cannabinoid system in peripheral analgesia. Curr Mol Pharmacol. 2006;4(2):134-41.
84. Jhaveri MD, Elmes SJR, Richardson D, Barrett DA, Kendall DA, Mason R, Chapman V. Evidence for a novel functional role of cannabinoid CB2 receptors in the thalamus of neuropathic rats. Eur J Neurosci. 2008;27(7):1722-30.
85. Cabral GA, Rogers TJ, Lichtman AH. Turning Over a New Leaf: Cannabinoid and Endocannabinoid Modulation of Immune Function. J Neuroimmune Pharmacol. 2015;10(2):193-203.
86. Steffens S, Veillard NR, Arnaud C, Pelli G, Burger F, Staub C, et al. Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. Nature. 2005;434(7034):782-6.
87. Greve MW, Zink BJ. Pathophysiology of traumatic brain injury. Mt Sinai J Med. 2009;76(2):97-104.
88. Masel BE, DeWitt DS. Traumatic brain injury: a disease process, not an event. J Neurotrauma. 2010;27(8):1529-40.
89. Shohami E, Cohen-Yeshurun A, Magid L, Algali M, Mechoulam R. Endocannabinoids and traumatic brain injury. Br J Pharmacol. 2011;163(7):1402-10.
90. Donat CK, Scott G, Zhang F, Saha B, Hottman DA, Chen Q, et al. Measurement of endocannabinoids in human CSF following traumatic brain injury. eNeuro. 2016;3(2):ENEURO.0036-16.2016.
91. Fernández-Ruiz J, Romero J, Ramos JA. Endocannabinoids and Neurodegenerative Disorders: Parkinson's Disease, Huntington's Chorea, Alzheimer's Disease, and Others. Handb Exp Pharmacol. 2015;231:233-59.
92. Panikashvili D, Simeonidou C, Ben-Shabat S, Hanus L, Breuer A, Mechoulam R, et al. An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. Nature. 2001;413(6855):527-31.
93. Tchantchou F, Tucker LB, Fu AH, Bluett RJ, McCabe JT, Patel S, Zhang Y. The fatty acid amide hydrolase inhibitor PF-3845 promotes neuronal survival, attenuates inflammation and improves functional recovery in mice with traumatic brain injury. Neuropharmacology. 2014;85:427-39.
94. Lins BR, Anyaegbu CC, Hellewell SC, Papini M, McGonigle T, De Prato L, et al. Cannabinoids in traumatic brain injury and related neuropathologies: preclinical and clinical research on endogenous, plant-derived, and synthetic compounds. J Neuroinflammation. 2023;20(1):77.
95. Tchantchou F, Zhang Y. Selective inhibition of alpha/beta-hydrolase domain 6 attenuates neurodegeneration, alleviates blood brain barrier breakdown, and improves functional recovery in a mouse model of traumatic brain injury. J Neurotrauma. 2013;30(7):565-79.
96. Katz P, Ronen A, Magid L, Tsuber E, Mechoulam R, Shohami E, et al. The synergistic effects of the combination of URB597 and SR141716A against focal brain injury in mice. J Steroid Biochem Mol Biol. 2015;149:77-82.
97. Martinez-Vargas M, Morales-Gomez J, Gonzalez-Rivera R, Hernandez-Enriquez J, Perez-Arredondo A, Estrada-Rojo F, et al. Does the endocannabinoid system play a role in the pathogenesis of traumatic brain injury? Front Integr Neurosci. 2013;7:91.
98. Donat CK, Fischer BL, Gouaux B, Mazzella FS, McKenna SL. Impact of combined medical and endocannabinoid treatment on neuronal injury in traumatic brain injury. Cureus. 2016;8(11):e883.

99. Wang N, Pham H, Hund D, Truong J, Adin D, Conti H, et al. Peripheral monocyte and tissue macrophage activation in a murine model of repetitive mild traumatic brain injury. J Neuroinflammation. 2016;13:295.
100. Walter L, Kerr DM. Endocannabinoid System and Traumatic Brain Injury in Pediatrics. J Neurotrauma. 2016;33(15):P399.
101. Hansen HH, Schmid PC, Bittigau P, Lastres-Becker I, Berrendero F, Manzanares J, et al. Anandamide, but not 2-arachidonoylglycerol, accumulates during in vivo neurodegeneration: a role for the phosphodiesterase enzyme PDE4. J Neurochem. 2001;78(2):337-45.
102. Li L, Yun D, Shivalingappa V, Pendharkar AV, Westphal M, Stein DG, et al. Repeated mild traumatic brain injury in mice triggers protracted memory deficits, neuroinflammation and deterioration in hippocampal synaptic plasticity despite no overt neuron loss. Exp Neurol. 2018;305:162-81.
103. Panikashvili D, Simeonidou C, Ben-Shabat S, Hanus L, Breuer A, Mechoulam R, et al. An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. Nature. 2001;413(6855):527-31.
104. Yi JH, Hazell AS. Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. Neurochem Int. 2006;48(5):394-403.
105. Tchantchou F, Tucker LB, Fu AH, Bluett RJ, McCabe JT, Patel S, Zhang Y. The fatty acid amide hydrolase inhibitor PF-3845 promotes neuronal survival, attenuates inflammation and improves functional recovery in mice with traumatic brain injury. Neuropharmacology. 2014;85:427-39.
106. Shlosberg D, Benifla M, Kaufer D, Friedman A. Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. Nat Rev Neurol. 2010;6(7):393-403.
107. Hind WH, England TJ, O'Sullivan SE. Cannabidiol protects an in vitro model of the blood-brain barrier from oxygen-glucose deprivation via PPARγ and 5-HT1A receptors. Br J Pharmacol. 2016;173(5):815-25.
108. Giza CC, Hovda DA. The Neurometabolic Cascade of Concussion. J Athl Train. 2001;36(3):228-35.
109. Singh IN, Sullivan PG, Deng Y, Mbye LH, Hall ED. Time course of post-traumatic mitochondrial oxidative damage and dysfunction in a mouse model of focal traumatic brain injury: implications for neuroprotective therapy. J Cereb Blood Flow Metab. 2006;26(11):1407-18.
110. Bergsneider M, Hovda DA, Shalmon E, Kelly DF, Vespa PM, Martin NA, et al. Cerebral hyperglycolysis following severe traumatic brain injury in humans: a positron emission tomography study. J Neurosurg. 1997;86(2):241-51.
111. Ansari MA, Roberts KN, Scheff SW. Oxidative stress and modification of synaptic proteins in hippocampus after traumatic brain injury. Free Radic Biol Med. 2008;45(4):443-52.
112. Hebert-Chatelain E, Reguero L, Puente N, Lutz B, Chaouloff F, Rossignol R, et al. Cannabinoid control of brain bioenergetics: Exploring the subcellular localization of the CB1 receptor. Mol Metab. 2014;3(4):495-504.
113. Hebert-Chatelain E, Desprez T, Serrat R, Bellocchio L, Soria-Gomez E, Busquets-Garcia A, et al. A Cannabinoid Link Between Mitochondria and Memory. Nature. 2016;539(7630):555-9.
114. Hampson RE, Jarrard LE, Deadwyler SA. Effects of ibotenate hippocampal and extrahippocampal destruction on delayed-match and -nonmatch-to-sample behavior in rats. J Neurosci. 1998;18(4):1492-507.
115. López-Rodríguez AB, Siopi E, Finn DP, Marchand-Leroux C, Garcia-Segura LM, Jafarian-Tehrani M, et al. CB1 and CB2 cannabinoid receptor antagonists prevent minocycline-induced neuroprotection following traumatic brain injury in mice. Cereb Cortex. 2015;25(1):35-45.
116. Walker KR, Tesco G. Molecular mechanisms of cognitive dysfunction following traumatic brain injury. Front Aging Neurosci. 2013;5:29.
117. Pandolfo P. Genetics of epilepsy. Semin Neurol. 2011;31(5):506-18.