Review

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Posted Date: 5 May 2026

doi: 10.20944/preprints202605.0085.v1

Keywords: traumatic brain injury; endocannabinoid system; neuroinflammation; excitotoxicity; neuronal cell death

 doi:10.20944/preprints202605.0085.v1

Review

Molecular and Genetic Mechanisms of Traumatic Brain Injury: The Endocannabinoid System as a Modulatory Axis

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Abstract

Traumatic brain injury (TBI) ranks among the top causes of illness and death on a global scale, characterized by a range of complex and diverse pathophysiological processes that include mechanical disruption, excitotoxicity, oxidative stress, and neuroinflammation. At the molecular level, TBI triggers a series of primary and secondary injury mechanisms that affect neuronal survival, synaptic integrity, and long-term neurological outcomes. The endocannabinoid system (ECS), a lipid-based neuromodulatory framework, has been recognized as a vital regulator of neural homeostasis, synaptic plasticity, and immune responses. This brief review integrates molecular and mechanistic insights into TBI, positioning the ECS as a central adaptive regulator of neuroinflammation and neurodegeneration. Collectively, these insights position the ECS as a context-dependent modulatory system with therapeutic relevance in TBI.

Keywords: traumatic brain injury; endocannabinoid system; neuroinflammation; excitotoxicity; neuronal cell death

1. Introduction

Traumatic brain injury (TBI) constitutes one of the most prevalent and challenging neurological disorders worldwide, affecting millions of people every year and significantly contributing to long-term disability, mortality, and economic burden [1]. Epidemiological studies suggest that around 0.5 to 1 million patients are admitted to hospitals annually for TBI in the United Kingdom [2], where it is the primary cause of disability for those under the age of 40 and severely impacts 150 to 200 individuals per million each year [3]. In the United States, over 1.4 million people are affected by TBI every year, and the estimated economic impact surpasses $50 billion [4]. Likewise, in Canada, TBI is a significant public health issue, with hundreds of thousands of cases reported each year, incurring substantial healthcare and societal expenses related to emergency care, rehabilitation, and long-term disability [5]. These statistics likely underrepresent the actual burden since many mild injuries go unreported. Despite considerable progress in understanding the biological mechanisms underlying TBI, a notable gap persists between mechanistic knowledge and the development of effective clinical treatments, underscoring a pressing unmet need [6].

In addition to its immediate neurological impacts, TBI is increasingly acknowledged as a condition that can lead to long-term and often progressive consequences, such as cognitive impairments, behavioral issues, and an increased risk of neurodegenerative diseases [7]. Importantly, even injuries considered mild can lead to prolonged symptoms and incomplete healing, highlighting

 doi:10.20944/preprints202605.0085.v1

a gap between clinical classifications and the underlying biological complexities [8]. This inconsistency emphasizes the shortcomings of existing diagnostic and prognostic models, most of which lack sufficient validation and are thus underused in clinical settings.

A significant challenge in TBI research and treatment is the condition's pronounced variability [6]. The severity of injuries can vary from mild concussions to severe structural damage; however, clinical outcomes do not always align with initial evaluations. Growing evidence suggests that TBI is characterized not only by mechanical damage but also by intricate interactions between injury-induced molecular pathways and individual-specific factors such as age, pre-existing conditions, and genetic predispositions [9–11]. This variability complicates the understanding of mechanisms and the development of treatments, as patients with apparently similar injuries may experience markedly different patterns of recovery or deterioration [12].

At the molecular level, TBI is best viewed as a progressive process that begins with a primary mechanical insult and is perpetuated by a complex array of secondary injury mechanisms [13]. These mechanisms include excitotoxicity from excessive glutamate release, mitochondrial dysfunction and metabolic failure, oxidative stress, neuroinflammation, and disruption of the blood-brain barrier (BBB) [14]. Crucially, these processes are interrelated and connected through reinforcing feedback loops, creating a self-sustaining pathological environment that drives ongoing neuronal dysfunction and tissue degeneration [15,16]. Additionally, TBI affects more than the CNS, initiating systemic responses such as peripheral immune activation, metabolic dysregulation, and multi-organ interactions, thereby framing brain injury as a broad-spectrum systemic disorder [17,18].

Genetic differences further contribute to variability in TBI outcomes by affecting key pathways involved in inflammation, synaptic adaptability, and cellular repair [19]. Variants in genes that regulate immune responses, lipid metabolism, and neuronal resilience have been linked to differences in injury susceptibility, the severity of secondary damage, and long-term recovery [20,21]. These results support the notion that TBI outcomes stem not only from the injury's characteristics but also from inherent biological factors that influence the host's response.

Within this intricate framework, lipid-mediated signaling pathways have attracted growing interest as key regulators of neurobiological responses [22–24]. Notably, the ECS has emerged as a vital neuromodulatory system functioning at the crossroads of synaptic communication, immune regulation, and metabolic control [25,26]. This review aims to provide a thorough examination of the molecular and genetic mechanisms underlying TBI, with an emphasis on the interplay between lipid signaling pathways and neuroimmune interactions. Here, the ECS is proposed as a central regulatory system influencing the balance between injury progression and repair.

2. The ECS in the CNS and Brain Injury

The ECS encompasses endogenous lipid ligands, collectively known as endocannabinoids (eCBs), primarily anandamide (AEA) and 2-arachidonoylglycerol (2-AG), along with their synthesizing and degrading enzymes, and two primary cannabinoid receptors, CB_1R and CB_2R [27], yet the scope of the ECS continues to expand as research evolves [28]. Both CB_1R and CB_2R are G protein-coupled receptors (GPRs) that inhibit voltage-gated N-type and P/Q-type Ca2+ channels upon activation [29]. In the CNS, CB_1R is found on neurons at presynaptic terminals and on astrocytes, whereas CB_2R is located on microglia, the brain's resident immune cells that originate from macrophages/monocytes [30,31]. In addition to the two principal CB_1R and CB_2R, the ECS extends beyond them to include additional receptors, with ongoing research revealing their extent and significance. Among these additional receptors, the most noteworthy include transient receptor potential vanilloid-1 (TRPV1), various GPRs (GPR55, GPR13, GPR6, GPR12, GPR18), peroxisome proliferator-activated receptor-γ (PPARγ), serotonin receptor 5-HT1A, and adenosine receptor A2A [27,32]. Of these, TRPV1 and PPARγ are of particular interest in the context of TBI, as TRPV1 is known to play a role in pain management and PPARγ is involved in inflammation and neurodegeneration [33]. In contrast to traditional neurotransmitter systems, eCBs are produced "on demand" in response

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to cellular activity and stress, allowing rapid, localized adjustments in neuronal and immune functions [34,35].

Functionally, CB_1Rs are mainly found in neurons and modulate synaptic transmission by regulating neurotransmitter release, whereas CB_2Rs are predominantly expressed in immune cells and are significantly upregulated during inflammation and tissue injury. This inducible expression profile positions the CB_2R as a crucial player in neuroimmune interactions, particularly under pathological conditions such as TBI [27,36]. Emerging research shows that ECS signaling is activated in response to excitotoxicity, oxidative stress, and inflammatory triggers, potentially serving as an intrinsic protective mechanism to restore balance [37,38].

Despite these advancements, the exact role of the ECS in TBI remains partially unclear. Although preclinical investigations support its role in modulating neuroinflammation, synaptic impairment, and oxidative stress, translating these mechanisms into clinically significant outcomes remains limited [39–41]. Moreover, the interaction among ECS signaling, genetic variability, and systemic responses remains largely unexplored, with substantial implications for understanding differences in injury progression and recovery between individuals.

2.1. Cannabinoids and Related Compounds

In addition to the naturally occurring endocannabinoids AEA and 2-AG in the mammalian nervous system, exogenous compounds also modulate ECS signaling [42]. These substances, collectively known as cannabinoids, fall into three categories: endogenous cannabinoids (eCBs), those from the Cannabis plant (phytocannabinoids), and artificially created variants (synthetic cannabinoids) [43]. An overview of these cannabinoid classes and their functional roles in TBI pathophysiology, along with detailed experimental parameters, including dosing and routes of administration, is summarized in Table 1. Together, these findings demonstrate that both pharmacological and genetic enhancement of endocannabinoid signaling generally converge to reduce neurodegeneration and improve functional outcomes.

Table 1. Modulation of endocannabinoid signaling in experimental models of traumatic brain injury.

Approach	Compound-Strategy	Dose and Administration	Timing-Regimen	TBI Model	Observed Effects	Refs.
Endocannabinoid supplementation	2-AG	0.1–10 mg/kg, i.v.	Single administration shortly after injury (~15 min)	Closed-head weight-drop TBI	Reduced brain edema and lesion volume; decreased neuronal loss (notably in hippocampus); improved functional recovery	[44]
Pharmacological enhancement of 2-AG	MAGL inhibitor (MJN110)	0.5–2.5mg/kg, i.p.	Initiated 30 min post-injury and continued daily for 5 days	Repetitive closed-head injury (CCI-based paradigm)	Attenuated neuroinflammation; reduced cell death; restored glutamatergic and GABAergic receptor balance; improved cognitive and locomotor performance	[45]
Genetic enhancement of	Astrocyte-specific	-	Constitutive (genetic model)	Repetitive closed-head	Decreased neuroinflammatory responses; limited	[46]

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endocannabinoid signaling	MAGL deletion	injury (CCI paradigm)	injury-induced transcriptional alterations; reduced neurodegeneration; preserved cognitive function

2.2. Phytocannabinoids

Phytocannabinoids are naturally occurring compounds extracted from the Cannabis sativa plant that exhibit biological activity within the ECS [47]. The main phytocannabinoids include Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) [48]. THC is the most recognized phytocannabinoid and the primary psychoactive element of the cannabis plant [49]. THC's pharmacological effects mimic those of the eCB AEA, functioning as a partial agonist at CB_1R and CB_2R [50]. Additionally, like AEA, THC administration reduces neuronal firing at the presynaptic level [51]. Importantly, there are two synthetic THC products approved by the United States Food and Drug Administration for medical use in the USA: nabilone and dronabinol [52]. There is a possibility that THC could help alleviate nausea and vomiting in the context of acute TBI [53]. It has been studied directly in TBI pathology models and indirectly in certain clinical TBI populations, with promising results for therapeutic applications beyond nausea [54–56].

CBD, the other principal component of cannabis, is typically considered an antagonist of CB_1R and an inverse agonist of CB_2R, though other studies report it may act as a negative allosteric modulator of CB_1R [57]. CBD also enhances the effects of eCB AEA by inhibiting fatty acid amide hydrolase (FAAH), the enzyme that breaks down AEA [58]. This is significant for TBI, as FAAH inhibitors are known to restore BBB integrity and improve motor, cognitive, and mood-related outcomes in TBI models [59–61]. Additionally, CBD directly activates serotonin 5-HT1A receptors, which could impact mood and anxiety [62–64]. It acts as a weak agonist at TRPV1 receptors, potentially modulating pain and synaptic plasticity [65]. As a PPARγ agonist, it may also be connected to neurodegeneration and inflammation [66]. The actions of CBD at these receptors could offer benefits in TBI, particularly through PPARγ activation, which decreases neurodegeneration and inflammation [67].

2.3. ECS as an Intrinsic Neuroprotective Mechanism

The ECS has been identified as an essential neuromodulatory network with widespread presence and functional significance across various cell types within the central nervous system, including neurons, astrocytes, microglia, and components of the cerebrovasculature [68]. This extensive distribution underpins its role in a diverse array of physiological and pathological processes, particularly in neural injury.

Recent preclinical research across TBI models highlights the neuroprotective role of eCBs and the ECS in facilitating recovery from TBI. For example, the administration of synthetic 2-AG in a mouse model of closed-head TBI resulted in a significant reduction in edema and infarct size, decreased cell death in the hippocampus, and enhanced functional recovery [44]. These beneficial outcomes appear to be mediated by the CB_1R, suggesting a potential protective role of the ECS following injury. Further supporting evidence is drawn from a study that enhanced the effects of 2-AG by inhibiting its degrading enzyme, monoacylglycerol lipase (MAGL), in a controlled cortical impact (CCI) model. In this study, administration of the MAGL inhibitor MJN110 to CCI mice post-injury led to reduced inflammatory markers, decreased cell death, normalization of altered glutamate and gamma-aminobutyric acid (GABA) receptor levels, and improved cognitive and motor performance [45]. In a repeated mild TBI (mTBI) mouse model, deletion of astrocyte-specific MAGL reduced neuroinflammation, attenuated alterations in TBI-induced gene expression, and slowed neurodegenerative changes and cognitive decline. These favorable effects were also mediated by the CB_1R [46]. Additionally, the administration of the FAAH inhibitor URB597 exhibited neuroprotective

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properties in an oxygen-glucose deprivation injury model, demonstrating antioxidant, anti-inflammatory, and anti-apoptotic effects [69]. Lastly, in a kainic acid-induced excitotoxicity model, AEA levels in the hippocampus increased rapidly, conferring CB_1R-dependent neuroprotection [70]. Collectively, these data support the neuroprotective capabilities of the ECS in response to brain injury, suggesting that enhancing or supplementing eCBs may provide therapeutic benefits in the management of traumatic brain injuries.

2.4. Evaluation of Cannabinoid-Based Interventions in Preclinical Models of TBI

Several preclinical investigations have examined the primary phytocannabinoid constituents in TBI models, yielding promising results. In a mouse model of mTBI induced by weight-drop, the injury resulted in reduced sociability, heightened aggression, and tactile allodynia, observed 14 days post-injury. Notably, these symptoms improved with daily oral administration of 10% CBD oil for either a 14-day period or from days 50 to 60 following the injury. Additionally, the mTBI model showed elevated levels of D-aspartate, glutamate, and GABA in the medial prefrontal cortex, and CBD treatment effectively alleviated these alterations [71].

In a rat model employing weight-drop and craniotomy (modified Feeney's model), CBD (10 mg/kg) was administered 30 minutes prior to the injury and again six hours afterward. The results indicated that CBD treatment improved BBB integrity by decreasing aquaporin-4 expression and increasing claudin-5 and occludin expression. Direct observation of BBB leakage was conducted using Evans' Blue assay. Furthermore, CBD administration resulted in reduced GFAP expression and lower levels of the pro-inflammatory mediators Tumor necrosis factor-alpha (TNF-α) and Interleukin-1 beta (IL-1β) compared with the control group [72].

In another study involving rats with moderate TBI (using the CCI model), Friedman and colleagues applied various high CBD, low THC botanical formulations directly onto the open skull over the dura mater at the injury site, utilizing a gelfoam matrix immediately following the injury. Additional groups received both the cannabinoid gel foam matrix and systemic administration of CBD ten minutes post-injury, continuing for 14 non-consecutive days thereafter [73]. The combination of gelfoam and systemic administration proved superior to either method alone, resulting in reduced defecation scores (an indicator of anxiety), smaller lesion volumes, lesser loss of hippocampal neurons, diminished neural pathology, and exhibited anti-inflammatory properties, as evidenced by reduced GFAP immunoreactivity alongside concurrent improvements in motor and cognitive functions. In summary, these studies collectively indicate that CBD treatment has multifaceted effects, offering benefits in inflammation, cognition, neurobehavioral deficits, lesion size, and BBB disruption following TBI.

In contrast, data pertaining to THC treatment in TBI models is more complex, with sex and timing emerging as critical factors. In a repeated injury model utilizing weight-drop in rats, THC (1.25 mg/kg, i.p.) was administered in six intermittent pre-injury doses or twelve consecutive post-injury doses. Post-injury THC treatment was associated with an increase in anxiety-like behavior in the elevated plus maze, although it did not influence behavior in the open field task. Interestingly, depression-like behavior assessed in the forced swim task improved in male rats treated with THC post-injury, while female rats displayed a more pronounced depression-like state. Additionally, post-injury THC treatment successfully prevented telomere shortening following repeated mTBI [54].

Consistent with prior studies, the authors observed increased microglial activation following repeated mTBI [74,75]. However, they did not observe any impact of THC treatment on microglial activation in limbic system structures, such as the hippocampus and nucleus accumbens. Instead, THC was found to elevate IBA1 immunoreactivity in the prefrontal cortex, suggesting a lack of therapeutic benefit regarding neuroinflammation in that specific area [54].

Conversely, in a male-only mouse model of CCI for TBI, THC treatment (3 mg/kg, i.p., daily for three days) improved motor function; this effect is likely attributable to a higher dose and more frequent dosing than in the preceding rat study. This enhancement in motor performance coincided with elevated levels of brain-derived neurotrophic factor and glial-derived neurotrophic factor, both

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of which are associated with neuronal and glial repair across various brain regions [76]. Moreover, THC administration was associated with increased levels of 2-AG in the brain, which aligned with improved short-term working memory [56]. These findings provide grounds for optimism regarding the potential application of THC in the management of TBI.

3. Molecular Pathophysiology of Traumatic Brain Injury

The primary injury occurs at the point of impact and represents direct mechanical damage to neuronal, glial, and vascular elements [77]. This includes membrane disruptions, axonal shearing, and immediate disturbances in ionic balance [78,79]. Although the primary damage is mostly irreversible, it triggers a series of biochemical and molecular reactions that lead to secondary brain injury, which is the focus of acute clinical treatment [80].

The evolution of secondary injury is a complex interplay of excitotoxic, ischemic, inflammatory, and cytotoxic processes that unfold over time, exacerbating the initial injury [81]. One of the earliest and most significant mechanisms is the excessive release of excitatory neurotransmitters, especially glutamate and aspartate, following the initiating trigger [82]. Microdialysis research has shown that this surge in excitotoxins overactivates N-methyl-D-aspartate (NMDA) receptors, increasing membrane permeability and leading to a notable influx of calcium and sodium ions into neurons [83,84]. Elevated intracellular calcium levels activate signaling pathways involving calmodulin and calcineurin, ultimately leading to cytoskeletal disintegration, axonal degeneration, and neuronal death [85]. Concurrently, potassium efflux from damaged neurons and its uptake by astrocytes disrupt ionic equilibrium, leading to cellular swelling and further injury progression [86,87].

These excitotoxic processes are closely related to metabolic dysfunction and mitochondrial impairment, creating a self-reinforcing loop of neuronal stress [88]. Mitochondrial overload caused by calcium dysregulation hinders ATP production and increases reactive oxygen species generation, thereby linking excitotoxicity to oxidative damage and loss of cellular energy [89–94]. Importantly, these processes do not happen in isolation but are constantly interacting with inflammatory and vascular responses [95].

Neuroinflammation is a crucial aspect of this secondary injury cascade and arises quickly after TBI [96]. Activated microglia and astrocytes release numerous inflammatory mediators, including cytokines, chemokines, neurokinins, and growth factors, thereby contributing to local and systemic reactions [97–99]. Among these mediators, interleukins have a particularly significant role. Following TBI, increased levels of cytokines such as Interleukin-6 (IL-6) and Interleukin-10 (IL-10) have been associated with the degree of perilesional edema and mortality [100]. Notably, the role of these cytokines is multifaceted and context-dependent [101]. While pro-inflammatory mediators can exacerbate neuronal damage by enhancing excitotoxicity and oxidative stress, they also contribute to neuroprotective processes, such as promoting growth factors and repair mechanisms [102]. This dual nature illustrates that neuroinflammation during TBI is not exclusively harmful but represents a carefully regulated response with both damaging and protective elements.

It's essential to recognize that the inflammatory response extends beyond the central nervous system [103]. Systemic inflammation and multi-organ dysfunction are often observed after TBI, suggesting that brain injury triggers a broader physiological response, including peripheral immune activation [104,105]. This systemic aspect further intensifies the secondary injury cascade and complicates the overall understanding of TBI pathophysiology.

Cerebral ischemia is another significant factor contributing to secondary brain injury and is closely tied to both vascular disruption and metabolic failure [106]. The primary injury frequently leads to immediate disturbances in cerebral circulation, resulting in reduced blood flow and tissue hypoxia [107]. In fact, ischemic processes are thought to account for most fatalities following severe TBI [108]. This ischemic damage is made worse by conditions such as low blood pressure, hypoxia, increased intracranial pressure, cerebral edema, and microvascular injury [109].

The progression of cerebral blood flow after TBI can be generally categorized into three phases. An initial phase is marked by hypoperfusion and reduced cerebral blood flow immediately after the

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injury. This is succeeded by an intermediate phase, which usually occurs within the first few days, during which hyperemia and increased blood flow may be noted. In the later stages, a vasospastic phase can emerge, characterized by a significant drop in cerebral perfusion [110]. These phases show notable regional variability in blood flow, particularly in the perilesional region surrounding the ischemic core, where perfusion deficits are often resistant to treatments that increase cerebral perfusion pressure [111].

In summary, these observations emphasize that the pathophysiology of TBI is governed by an interconnected network of excitotoxic, metabolic, inflammatory, and vascular mechanisms. Rather than following a linear progression, these processes create a self-amplifying system where each element reinforces the others. Understanding this complex interplay is essential for developing effective therapeutic strategies to limit secondary injury and improve clinical outcomes.

3.1. Cell Death in the CNS

Neuronal loss resulting from TBI occurs almost immediately through necrotic cell death and persists for several months post-trauma via both necrotic and apoptotic pathways [112]. The primary injury creates a contused region, referred to as the primary lesion or infarct, which is encircled by the pericontusional penumbra, an area adjacent to the primary lesion that is susceptible to additional neurodegeneration. The development of the pericontusional penumbra is largely driven by secondary injury mechanisms, and it has long been considered a potential target for interventions to prevent or mitigate further damage [113]. Current research regarding cannabinoids in the context of traumatic CNS cell death has demonstrated efficacy in two primary areas: the reduction of neurodegeneration and the decrease of lesion volume [67].

Neurodegeneration has been notably reduced in murine models using CB_2R agonists and inhibitors of FAAH and MAGL [59,114,115]. Additionally, Tchantchou et al. (2014) demonstrated that inhibition of FAAH increased expression of the anti-apoptotic protein Bcl-2 [59].

Several enzymes participate in the hydrolysis of 2-AG, with MAGL accounting for approximately 85% of total hydrolysis, while alpha-beta hydrolase domain-containing protein 6 (ABHD6) and ABHD12 account for the remaining 15% [116]. Research conducted by Tchantchou and Zhang (2013) revealed that inhibition of ABHD6 also reduced lesion volume and neurodegeneration in a CCI mouse model. Notably, a CB_1R antagonist was shown to negate the protective effects regarding lesion volume, while antagonism of both CB_1R and CB_2R receptors blocked the neuroprotective effects [61].

In summary, this body of evidence suggests that inhibitors of eCB hydrolysis confer protection against TBI-induced cell death, implicating both CB_1R and CB_2R; however, the distinctions among eCBs remain to be elucidated. Few studies have investigated the interactions between AEA and 2-AG in laboratory models of TBI. One investigation using a model of focal cerebral ischemia demonstrated that the concurrent administration of AEA and 2-AG reduced infarct size in rats, albeit without additional benefits beyond those provided by each substance individually [117]. Given recent advances in dual FAAH/MAGL inhibitors, combined inhibition of these enzymes following TBI may yield further insights into the interplay between AEA and 2-AG in TBI-induced cell death [118,119].

3.2. Excitotoxicity and Its Implications in TBI

Excitotoxicity, a pathological process resulting from excessive activation of glutamate receptors, poses significant challenges following brain injuries [120]. Past therapeutic efforts aimed at countering the detrimental effects of excitotoxicity following TBI have primarily focused on NMDA receptor antagonists [121]. The underlying rationale was the assumption that reducing NMDA receptor activity would alleviate excitotoxic damage associated with TBI. While these medications showed promising results in animal studies, their translation to clinical settings proved disappointing [122]. Although they offered temporary relief by reducing intracranial pressure and enhancing cerebral perfusion pressure, sustained positive outcomes remained elusive [123,124].

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Recent research has shifted its focus towards understanding how alterations in the ECS influence glutamatergic function in the context of TBIs [67]. Among the eCBs, 2-AG has emerged as a focal point due to its complex role in maintaining the integrity of glutamate receptors [125].

A multitude of studies investigating the effects of cannabinoids in various animal models of TBI have examined modulation of glutamate receptor subtypes, namely metabotropic (mGluR1, mGluR5), AMPA (GluA1, GluA2), and NMDA (GluN1, GluN2A, GluN2B) receptors [126]. Notably, the administration of the MAGL inhibitor JZL184 in the post-injury phase successfully counteracted the observed reductions in the expression levels of GluN2A, GluN2B, and GluA1 receptors induced by TBI, while showing no significant impact on GluN1 or GluA2 receptor levels [115]. In a somewhat surprising finding, the CB_1R antagonist Rimonabant did not alter the injury-related decrease in mGluR1 expression; however, it did reverse the decline in mGluR5 receptor expression when assessed six weeks after the injury [127]. Both significant outcomes were observed thirty days post-injury, underscoring the persistent changes in glutamatergic signaling following acute cannabinoid treatment after trauma [115,127].

Despite these encouraging revelations, a notable lack of consistency exists across studies concerning receptor expression endpoints. For instance, the expression of GluA1 has shown conflicting results; it was found to decrease in a study investigating mice subjected to daily mild closed head injuries over a span of three consecutive days [115], while an increase in GluA1 expression was reported in rats that underwent a single lateral fluid percussion brain injury [128]. Intriguingly, in both experimental contexts, MAGL inhibition effectively alleviated the reductions [115] and increased [128] levels of GluA1 expression.

eCBs are well-documented for their capacity to inhibit glutamate release from presynaptic terminals, particularly 2-AG, which has garnered attention for its modulatory role in electrochemical neurotransmission [129–131]. The inhibition of MAGL has been shown to provide a protective effect against injury-induced increases in both the frequency and amplitude of excitatory postsynaptic currents (EPSC) within pyramidal neurons located at the site of injury [37]. This observation may suggest potential alterations in presynaptic neurotransmitter release mechanisms or modifications in postsynaptic responsiveness [132]. Furthermore, MAGL inhibition has conferred protection against deficits in long-term potentiation (LTP) at hippocampal CA3–CA1 synapses [115], indicating that restoring glutamate receptor function may play a crucial role in mitigating memory impairments associated with TBI. Ultimately, the excitotoxicity stemming from traumatic brain injury initiates a complex cascade of events that culminates in the release of harmful reactive oxygen species (ROS). Antioxidants are recognized for their ability to thwart oxidative damage caused by sudden spikes in ROS levels. Notably, eCBs have been linked to the neuroprotective promotion of antioxidant production; for instance, administering exogenous 2-AG following injury has been shown to elevate antioxidant levels [133].

In conclusion, the evidence points to MAGL as a promising target for counteracting the adverse effects of excitotoxicity following brain injury through multiple complementary molecular pathways. This highlights the potential of therapeutic strategies that harness the eCB system's protective properties to mitigate the consequences of excitotoxic brain damage.

3.3. Neuroinflammation and Its Implications in TBI

Neuroinflammation arises when hydrolytic enzymes degrade AEA and 2-AG, producing a common metabolic byproduct that releases free arachidonic acid (AA), a primary substrate for enzymes that synthesize pro-inflammatory eicosanoids [134]. Consequently, the oxidation of eCBs not only results in the deactivation of cannabinoid receptors but also contributes to the generation of bioactive lipids that participate in inflammatory responses during the initial injury phases [135]. Modifications to the eCB system have been shown to reduce inflammation in various experimental contexts, including inflammatory pain [136] and multiple sclerosis [137]. The correlation between cannabinoids and TBI is linked to two principal anti-inflammatory effects: attenuation of inflammatory cell activation and a reduction in pro-inflammatory cytokine production [67].

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Activated pro-inflammatory microglia have been implicated in exacerbating the neuroinflammatory response following TBI [138]. Thus, diminishing inflammatory cell activation resulting from TBI emerges as a promising therapeutic strategy. Inhibition of MAGL has been shown to confer protection against microglial activation induced by TBI [38,115], whereas inhibition of ABHD6 facilitates a transition in microglia/macrophages from the pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype [61]. A straightforward interpretation of these observations is that inhibiting 2-AG breakdown reduces AA levels, thereby mitigating the production of pro-inflammatory mediators [139]. Given the involvement of 2-AG metabolism in eicosanoid generation, it is not surprising that numerous studies have indicated eCBs possess pro-inflammatory properties, as demonstrated in models of nephropathy [140] and cardiomyopathy [141]. It is important to note that most of these pro-inflammatory effects are attributed to 2-AG rather than AEA, likely due to its higher concentrations [142]. Nonetheless, inhibition of FAAH has similarly shown protective effects against TBI-induced microglial activation [38], as has activation of CB_2R [114]. Therefore, it is critical to distinguish the potential contributions of 2-AG to pro-inflammatory processes from its role as a precursor to AA production and its anti-inflammatory effects mediated through cannabinoid receptors in the context of TBI.

Inhibition of the enzymes responsible for the degradation of eCBs has also resulted in decreased levels of pro-inflammatory mediators induced by TBI [37,61]. A significant reduction in the expression of enzymes that initiate eicosanoid production following brain injury, such as cyclooxygenase-2 (COX-2), which converts free AA into prostaglandins, and inducible nitric oxide synthase (iNOS), which produces the free radical nitric oxide in response to cytokine signaling, has been observed following ABHD6 inhibition [61] and FAAH inhibition [59]. Additionally, decreases in messenger RNA levels of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) have been documented after treatment with exogenous 2-AG [133]. These findings appear paradoxical, given the potential for the rapid oxidation of 2-AG and its subsequent role in eicosanoid production. However, exogenous 2-AG has been shown to reduce TBI-induced activation of nuclear factor kappa B (NF-κB) (associated with cytokine production) in wild-type mice but not in CB_1R knockout mice, indicating that the protective effects of exogenous 2-AG are mediated through CB_1Rs [143]. The mechanisms by which cannabinoids influence neuronal, inflammatory, and vascular components of TBI are outlined in Table 2. Importantly, cannabinoid-mediated effects are not pathway-specific but rather span multiple interconnected domains, supporting a systems-level mechanism of action.

Table 2. Cannabinoid-mediated modulation of TBI pathophysiological pathways (preclinical evidence).

Pathophysiological Domain	Intervention Strategy	Mechanistic Target(s)	Key Observed Effects	Proposed Functional Outcome	Refs.
Neuronal death / neurodegeneration	MAGL inhibition (JZL184), FAAH inhibition (PF3845), CB_2R agonists (O-1966)	eCB accumulation, CB_2R signaling	↓ lesion volume, ↓ neurodegeneration, ↑ Bcl-2, Hsp70	Enhanced neuronal survival	[59,114,115]
Excitotoxicity / synaptic dysfunction	2-AG administration, CB_1R modulation, enzyme inhibition	Presynaptic CB_1R signaling, glutamate receptor regulation	Restoration of glutamate receptor function, protection of LTP and EPSC	Stabilization of synaptic transmission	[69,128,133]

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Oxidative stress	eCB elevation (2-AG), indirect antioxidant effects	ROS modulation, lipid signaling	↑ antioxidant response, ↓ oxidative damage	Protection against mitochondrial dysfunction	[59,133]
Neuroinflammation	CB_2R agonists, FAAH/MAGL inhibition, 2-AG elevation	Microglial CB_2R activation, NF-κB signaling	↓ TNF-α, IL-1β, IL-6; ↓ COX-2 and iNOS; ↓ microglial activation	Attenuation of inflammatory response	[38,114,143]
BBB integrity	2-AG signaling, cannabinoid modulation	Endothelial signaling, inflammatory regulation	↓ BBB permeability, improved vascular integrity	Reduced edema and secondary injury	[61,143]
Synaptic plasticity and recovery	CB_1R-dependent modulation, enzyme inhibition	Retrograde signaling pathways	Preservation of LTP, GluA1 expression, EPSC stabilization	Improved cognitive recovery	[37,115]
Neurovascular/ systemic response	Cannabinoid-mediated vasomodulation	Vascular tone regulation	Improved perfusion, vasodilation	Support of cerebral blood flow	[69,133]

4. Discussion

The ECS, including endogenous ligands and potential entourage effects from other lipid mediators, can be further modulated through strategies such as antagonism and allosteric regulation, which may influence functional selectivity and consequently modulate TBI outcomes. Targeting this system with endogenous, plant-derived, or synthetic cannabinoids presents a promising avenue for developing therapeutic strategies for TBI. A critical step in understanding the ECS's role in TBI involves an in-depth analysis of ligands that target cannabinoid receptors and of the enzymes that regulate eCB levels. Notably, non-cannabinoid-receptor-binding phytocannabinoids also show therapeutic potential in TBI models [52–54,57,76]. Presently, the strongest evidence for neuroprotective properties is seen for compounds containing CBD, or those targeting CB_2R, while the effects of THC treatment are less consistent. In experimental models of TBI, modulation of the ECS consistently reduces key aspects of secondary damage, including excitotoxicity, neuroinflammation, oxidative stress, and disruption of the BBB [67]. These effects are observed across multiple intervention strategies, including eCB supplementation, enzyme inhibition, and receptor-specific modulation, resulting in improved histological and functional outcomes. Additionally, research must focus on how TBI affects cannabinoid receptors, eCB levels, and the enzymes that regulate eCB metabolism [44–46].

Another important area for future therapeutic exploration lies in non-CB_1R/CB_2R targets, such as TRPV1 receptors, and in bridging preclinical efficacy and clinical outcomes. Moreover, investigating alternative approaches to modulate CB_1R and CB_2R signaling may further refine therapeutic outcomes. In this context, plant-derived compounds remain an underexplored yet potentially valuable source of multi-target therapeutic agents.

To date, the only cannabinoid that has undergone specific study for TBI treatment in human populations is Dexanabinol, or HU211. While animal studies have demonstrated its promise [122], clinical trials have yielded mixed results: HU211 provided some acute benefits [123] but did not show long-term advantages, and one trial reported no immediate or lasting effect [124]. Studies examining cannabinoid exposure or cannabinoid-based interventions in TBI present a more varied and less conclusive perspective. Observational data further suggest possible links between cannabinoid use and lower mortality rates or enhanced short-term outcomes; however, these results are inconsistent and frequently complicated by differences in the severity of injuries, patient characteristics, and

 doi:10.20944/preprints202605.0085.v1

patterns of substance use [123,124]. This translational gap likely reflects the multifactorial nature of TBI, in which single-target interventions fail to adequately modulate the complex, temporally dynamic injury cascade, underscoring the need for multi-target or systems-level therapeutic strategies. Importantly, although HU211 is classified as a cannabinoid because it is an enantiomer of the potent synthetic cannabinoid agonist HU210, it does not activate cannabinoid receptors [144]. Instead, HU211 functions as a non-competitive antagonist at NMDA receptors, which raises important questions regarding cannabinoid classification [145]. A direct comparison between preclinical and clinical findings further highlights critical challenges in translating cannabinoid-based therapies for TBI [146–150]. Future studies integrating patient stratification, biomarker-guided interventions, and multi-target pharmacological approaches may improve clinical translation.

Importantly, these multi-target effects, acting across interconnected pathways rather than a single mechanism, may partly explain the discrepancy observed in clinical translation. Preclinical models typically involve controlled injury scenarios, consistent treatment timing, and uniform populations, which enable detailed mechanistic exploration. Additionally, many preclinical treatments target multiple pathological processes in parallel, whereas clinical trials have often focused on individual compounds administered within narrow therapeutic timeframes. The timing dynamics of TBI make this translation even more challenging, as the relative impacts of excitotoxicity, inflammation, and metabolic processes change over time, requiring treatments that are both time-sensitive and integrative across several pathways.

A frequently overlooked aspect of TBI research is the ability of systemically administered drugs to penetrate the CNS effectively. It is critical for therapeutic interventions to be evaluated based on their ability to cross the BBB. Additionally, TBI often disrupts the BBB, a condition that can persist for up to three days post-injury [151]. Given the often-biphasic effects of cannabinoid medications, it is essential to move beyond single-dose studies to comprehensive dose-response evaluations. This shift could enhance our understanding of the underlying mechanisms and therapeutic potential of cannabinoids in treating TBI. Overall, the ECS represents a biologically relevant, multi-target regulatory network that may provide a more effective framework for developing future therapeutic strategies for TBI.

5. Conclusions

TBI is a complex condition to manage due to its biological intricacies and patient variability. Although extensive preclinical studies indicate the therapeutic promise of targeting the ECS, translation into clinical settings has been limited, highlighting the need for methods that more accurately reflect the multifaceted nature of the injury. Future advancements will rely on combining mechanistic understanding with clinically applicable strategies, such as improved timing, dosing, and patient classification. In this regard, the ECS offers a useful framework for creating more effective and translatable therapeutic approaches.

Author Contributions: Conceptualization, H.K.; methodology, H.K.; software, H.K.; validation, H.K.; formal analysis, H.K.; investigation, H.K.; resources, H.K.; data curation, H.K.; writing—original draft preparation, H.K.; writing—review and editing, H.K.; visualization, H.K.; supervision, H.K.; project administration, H.K.; funding acquisition, H.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. A post-doctoral fellowship from IUCPQ supported H.K.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

 doi:10.20944/preprints202605.0085.v1

TBI	Traumatic brain injury
ECS	Endocannabinoid system
eCBs	Endocannabinoids
AEA	Anandamide (N-arachidonoylethanolamine)
2-AG	2-Arachidonoylglycerol
CB_1R	Cannabinoid receptor 1
CB_2R	annabinoid receptor 2
CBD	Cannabidiol
MAGL	Monoacylglycerol lipase
FAAH	Fatty acid amide hydrolase
GPR	G-protein-coupled receptors
BBB	Blood–brain barrier
CNS	Central nervous system
mTBI	mild TBI
CCI	Controlled Cortical Impact
HU-211	Dexanabinol
NMDA	N-methyl-D-aspartate
TNF-α	Tumor necrosis factor alpha
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
PPARγ	Peroxisome proliferator-activated receptor gamma
ROS	Reactive oxygen species
THC	Δ9-Tetrahydrocannabinol
TRPV1	Transient receptor potential vanilloid 1

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