Endocannabinoids: A Promising Impact for Traumatic Brain Injury

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The endogenous cannabinoid (endocannabinoid) system regulates a diverse array of physiological processes and unsurprisingly possesses considerable potential targets for the potential treatment of numerous disease states, including two receptors (i.e., CB1 and CB2 receptors) and enzymes regulating their endogenous ligands N-arachidonoyl ethanolamine (anandamide) and 2-arachidonyl glycerol (2-AG). Increases in brain levels of endocannabinoids to pathogenic events suggest this system plays a role in compensatory repair mechanisms. Traumatic brain injury (TBI) pathology remains mostly refractory to currently available drugs, perhaps due to its heterogeneous nature in etiology, clinical presentation, and severity. Here, we review pre-clinical studies assessing the therapeutic potential of cannabinoids and manipulations of the endocannabinoid system to ameliorate TBI pathology. Specifically, manipulations of endocannabinoid degradative enzymes (e.g., fatty acid amide hydrolase, monoacylglycerol lipase, and α/β-hydrolase domain-6), CB1 and CB2 receptors, and their endogenous ligands have shown promise in modulating cellular and molecular hallmarks of TBI pathology such as; cell death, excitotoxicity, neuroinflammation, cerebrovascular breakdown, and cell structure and remodeling. TBI-induced behavioral deficits, such as learning and memory, neurological motor impairments, post-traumatic convulsions or seizures, and anxiety also respond to manipulations of the endocannabinoid system. As such, the endocannabinoid system possesses potential drugable receptor and enzyme targets for the treatment of diverse TBI pathology. Yet, full characterization of TBI-induced changes in endocannabinoid ligands, enzymes, and receptor populations will be important to understand that role this system plays in TBI pathology. Promising classes of compounds, such as the plant-derived phytocannabinoids, synthetic cannabinoids, and endocannabinoids, as well as their non-cannabinoid receptor targets, such as TRPV1 receptors, represent important areas of basic research and potential therapeutic interest to treat TBI.

Keywords: traumatic brain injury, cannabinoid, endocannabinoid, neuroprotection, phytocannabinoid

Abbreviations: 2-AG, 2-arachidonyl glycerol; 2-LG, 2-linoleoyl-glycerol; 2-PG, 2-palmitoyl-glycerol; AA, arachidonic acid; ABHD6, α/β-hydrolase domain-6; ABHD12, α/β-hydrolase domain-12; AEA, anandamide; APP, amyloid precursor protein; BBB, blood brain barrier; CCI, controlled cortical impact model of TBI; CDTA, calcium-dependent transacylase enzyme; CHI, closed head injury model of TBI; COX-2, cyclooxygenase-2; cPLA2, cytosolic phospholipase A2; DAGL-α, diacylglycerol lipase-α; DAGL-β, diacylglycerol lipase-β; eCB, endocannabinoid; EPSC, excitatory post-synaptic current; FAAH, fatty acid amide hydrolase; FPI, fluid percussion injury model of TBI; LTP, long term potentiation; MAGL, monoacylglycerol lipase; NArPE, N-arachidonoyl phosphatidylethanolamine; NBS, neurological behavioral score; NMDA, N-methyl-D-aspartate; NPE, neurogenic pulmonary oedema; NSS, neurological severity score; PLA2, phospholipase A2 enzyme; PLC, phospholipase C enzyme; p-tau, hyperphosphorylated tau; ROS, reactive oxygen species; TBI, traumatic brain injury; TDP-43, TAR DNA-binding protein; THC, Δ2-tetrahydrocannabinol.
INTRODUCTION

Traumatic brain injury accounts for approximately 10 million deaths and/or hospitalizations annually in the world, and approximately 1.5 million annual emergency room visits and hospitalizations in the US (Langlois et al., 2006). Young men are consistently over-represented as being at greatest risk for TBI (Langlois et al., 2006). While half of all traumatic deaths in the USA are due to brain injury (Mayer and Badjatia, 2010), the majority of head injuries are considered mild and often never receive medical treatment (Corrigan et al., 2010). Survivors are consistently over-represented as being at greatest risk for approximately 1.5 million annual emergency room visits and deaths and/or hospitalizations annually in the world, and Traumatic brain injury accounts for approximately 10 million

THE ENDOCANNABINOID (eCB) SYSTEM

Originally, “Cannabinoid” was the collective name assigned to the set of naturally occurring aromatic hydrocarbon compounds in the Cannabis sativa plant (Mechoulam and Goani, 1967). Cannabinoid now more generally refers to a much more broad set of chemicals of diverse structure whose pharmacological actions or structure closely mimic that of plant-derived cannabinoids. Three predominant categories are currently in use; plant-derived phytocannabinoids (reviewed in Gertsch et al., 2010), synthetically produced cannabinoids used as research (Wiley et al., 2014) or recreational drugs (Mills et al., 2015), and the endogenous cannabinoids, N-arachidonylethanolamine (anandamide) (Devane et al., 1992) and 2-AG (Mechoulam et al., 1995; Sugiura et al., 1995).

These three broad categories of cannabinoids generally act through cannabinoid receptors, two types of which have so far been identified, CB1 (Devane et al., 1988) and CB2 (Munro et al., 1993). Both CB1 and CB2 receptors are coupled to signaling cascades predominantly through G11- coupled proteins. CB1 receptors mediate most of the psychomimetic effects of cannabis, its chief psychoactive constituent THC, and many other CNS active cannabinoids. These receptors are predominantly expressed on pre-synaptic axon terminals (Alger and Kim, 2011), are activated by endogenous cannabinoids that function as retrograde messengers, which are released from post-synaptic cells, and their activation ultimately dampens pre-synaptic neurotransmitter release (Mackie, 2006). Acting as a neuromodulatory network, the outcome of cannabinoid receptor signaling depends on cell type and location. CB1 receptors are highly expressed on neurons in the central nervous system (CNS) in areas such as cerebral cortex, hippocampus, caudate-putamen (Herkenham et al., 1991). In contrast, CB2 receptors are predominantly expressed on immune cells, microglia in the CNS, and macrophages, monocytes, CD4+ and CD8+ T cells, and B cells in the periphery (Cabral et al., 2008). Additionally, CB2 receptors are expressed on neurons, but to a much less extent than CB1 receptors (Atwood and MacKie, 2010). The abundant, yet heterogeneous, distribution of CB1 and CB2 receptors throughout the brain and periphery likely accounts for their ability to impact a wide variety of physiological and psychological processes (e.g., memory, anxiety, and pain perception, reviewed in Di Marzo, 2008) many of which are impacted following TBI.

Another unique property of the eCB system is the functional selectivity produced by its endogenous ligands. Traditional neurotransmitter systems elicit differential activation of signaling pathways through activation of receptor subtypes by one neurotransmitter (Siegel, 1999). However, it is the endogenous ligands of eCB receptors which produce such signaling specificity. Although several endogenous cannabinoids have been described (Porter et al., 2002; Chu et al., 2003; Heimann et al., 2007) the two most studied are anandamide (Devane et al., 1992) and 2-AG (Mechoulam et al., 1995; Sugiura et al., 1995). 2-AG levels are three orders of magnitude higher than those of anandamide in brain (Béquet et al., 2007). Additionally, their receptor affinity (Pertwee and Ross, 2002; Reggio, 2002) and efficacy differ, with
2-AG acting as a high efficacy agonist at CB1 and CB2 receptors, while anandamide behaves as a partial agonist (Hillard, 2000a). In addition, anandamide binds and activates TRPV1 receptors (Melck et al., 1999; Zygmunt et al., 1999; Smart et al., 2000), whereas 2-AG also binds GABA_A receptors (Sigel et al., 2011). As such, cannabinoid ligands differentially modulate similar physiological and pathological processes.

Distinct sets of enzymes, which regulate the biosynthesis and degradation of the eCBs and possess distinct anatomical distributions (see Figure 1), exert control over CB1 and CB2 receptor signaling. Inactivation of anandamide occurs predominantly through FAAH (Cravatt et al., 1996, 2001), localized to intracellular membranes of postsynaptic somata and dendrites (Gulyas et al., 2004), in areas such as the neocortex, cerebellar cortex, and hippocampus (Egertová et al., 1998). Inactivation of 2-AG proceeds primarily via MAGL (Dinh et al., 2002; Blankman et al., 2007), expressed on presynaptic axon terminals (Gulyas et al., 2004), and demonstrates highest expression in areas such as the thalamus, hippocampus, cortex, and cerebellum (Dinh et al., 2002). The availability of pharmacological inhibitors for eCB catabolic enzymes has allowed the selective amplification of anandamide and 2-AG levels following brain injury as a key strategy to enhance eCB signaling and to investigate their potential neuroprotective effects.

Finally, 2-AG functions not only as a major cannabinoid receptor signaling molecule, but also serves as a major precursor for AA, and therefore plays a role in inflammatory pathways (see Figure 2). Although AA is a degradative product of both 2-AG (Bell et al., 1979) and anandamide (Deutsch et al., 1997), MAGL represents a rate-limiting biosynthetic enzyme of highly bioactive lipid in brain, liver, and lung (Nomura et al., 2011). Historically, cPLA2 was considered to be the primary rate-limiting enzyme in AA production (reviewed in Buczynski et al., 2009). However, MAGL contributes ~80% and cPLA2 ~20% of LPS-stimulated eicosanoids in mouse brain. In contrast, cPLA2 is the dominant enzyme to control AA production in spleen (Nomura et al., 2011). Therefore, MAGL and cPLA2 appear to play differential roles in AA production, and concomitantly its eicosanoid metabolites in a tissue-specific manner (Nomura et al., 2011). As such, 2-AG functions not only as an endogenous CB1 and CB2 receptor ligand, but also an immunomodulator by virtue of its being a major precursor for AA, making it a versatile target for the treatment of TBI related pro-inflammatory pathologies. Understanding the biosynthesis mechanisms of eCBs may prove useful in modulating their entry into pro-inflammatory pathways. While 2-AG is known to be synthesized by DAGL-α and DAGL-β (Bisogno et al., 2003), the mechanisms mediating anandamide production are incompletely understood (Blankman and Cravatt, 2013).

**TRAUMATIC BRAIN INJURY PATHOLOGY**

Traumatic brain injuries are heterogeneous in their etiology, clinical presentation, severity, and pathology. The sequelae of molecular, biochemical, and physiological events that follow the application of an external mechanical force produce interacting acute and delayed pathologies, described as primary and secondary injuries. The initial insult produces an immediate mechanical disruption of brain tissue (Reilly, 2001). This primary injury consists of contusion, blood vessel disruption and brain oedema, localized necrotic cell death, as well as diffuse axonal injury producing degeneration of cerebral white matter (Adams et al., 1989; Gaetz, 2004).

Secondary injury mechanisms are initiated within minutes, in which necrotic and apoptotic cell death in contused areas and pericontusional penumbra continue over a period of days to months (Raghupathi, 2004). Neuronal disruption spills excitatory amino acids into the intersitial space, producing glutamate-mediated excitotoxicity (Bullock et al., 1998). Massive influx of Ca^2+ into cells (Floyd et al., 2010) produce mitochondrial dysfunction and the release of ROS which lead to further apoptosis (Zhao et al., 2005). Injury-induced activation of CNS resident glial cells, microglia, as well as recruitment of circulating inflammatory cells, e.g., macrophages, then produce secretion of inflammatory mediators, cytokines and chemokines (reviewed in Woodcock and Morganti-Kossmann, 2013). Increased intracranial pressure leads to reductions in cerebral blood flow (Shiina et al., 1998), while injury-induced breakdown of the cerebrovascular endothelium contributes to dysfunction of the BBB (Chodobski et al., 2012). Extracranial pathologies are also evident following TBI with pulmonary complications being the most common (Pelosi et al., 2005). NPE often develops early after brain injury, producing hypoxemia and further aggravating secondary brain injury (Brambrink and Dick, 1997; Oddo et al., 2010). These varied and interacting disease processes highlight the necessity to address the multiple targets associated with secondary injury cascades following TBI.

While there are many types of CNS injury models [e.g., spinal cord injury, lesion studies, focal and global ischemic injury etc. (Arai and Lo, 2009; Titomanlio et al., 2015)], this review will focus primarily on the work investigating manipulations of the eCB system in preclinical models of TBI.

**PRE-CLINICAL EVALUATION OF CANNABINOIDS TO TREAT TBI**

While basal anandamide and 2-AG levels differ within various structures in the CNS, levels increase on demand in response to a given stimuli [e.g., the induction of nausea (Sticht et al., 2016) or pain states (Costa et al., 2008)]. eCBs are lipid messengers not stored in synaptic vesicles (likely due to their hydrophobicity) but rather synthesized in an activity-dependent manner from membrane phospholipid precursors (Alger and Kim, 2011). Consequently, eCB signaling is enhanced by a stimulus-response synthesis and release mechanism.

Endocannabinoid levels increase in selected CNS tissue following neuronal damage, which may reflect a self-neuroprotective response. NMDA excitotoxicity produces elevations of anandamide in ipsilateral cortex of rats by 4-fold at 4 h and 14-fold at 24 h, but with no changes in 2-AG.
Schurman and Lichtman

Endocannabinoids and Traumatic Brain Injury

FIGURE 1 | Endocannabinoid system cell localization by CNS cell type. Endocannabinoid functional specialization among CNS cell types is determined by the cellular compartmentalization of biosynthetic and catabolic enzymes (biosynthesis by NAPE and DAGL-α, -β, catabolism by FAAH and MAGL). Cellular level changes in eCB biosynthetic and catabolic enzymes as a result of brain injury have yet to be investigated, though morphological and molecular reactivity by cell type is well documented.
levels (Hansen et al., 2002). Concussive head trauma in rats produces a similar pattern of findings in which modest increases of anandamide levels occur in ipsilateral cortex, and again with no change in 2-AG levels (Hansen et al., 2002). This pattern was replicated by Tchantchou et al. (2014), who found a 1.5-fold increase of anandamide levels at 3 days post-TBI in ipsilateral mouse brain, and with no change in 2-AG. In contrast, Panikashvili et al. (2001) reported that TBI in mice led to increases of 2-AG in ipsilateral brain from 1 to 24 h with elevations as high as 10-fold. Thus, further research is needed to discern whether species differences, the model used to elicit neurotrauma, and/or other procedural considerations contribute to the differential elevation of these eCBs (Mechoulam and Lichtman, 2003).

A lack of studies systematically investigating the consequences of TBI on changes in eCB levels in specific brain regions perhaps point to the difficulty in measuring changes in the volatile eCBs, prone to rapid degradation (Deutsch and Chin, 1993; Dinh et al., 2002). While pharmacological and genetic manipulations of the eCB system continue to be evaluated following TBI; full characterization of how eCB biosynthetic and degradative enzymes, receptors, and endogenous ligands, their precursors and catabolic products, change as a consequence of TBI remains to be fully illuminated.

Treatment of Cellular and Molecular Pathophysiology of TBI

In this section, we review preclinical studies of cannabinoids in the context of their potential to protect against cellular and molecular TBI pathology (see Table 1).
### TABLE 1 | Effect of cannabinoids on TBI-induced cellular and molecular pathophysiology.

<table>
<thead>
<tr>
<th>Compound/mutant</th>
<th>Dose</th>
<th>Species</th>
<th>TBI model/severity</th>
<th>Effect</th>
<th>Receptor mediated</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>CNS cell death</strong></td>
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<tr>
<td>O-1966</td>
<td>5 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, moderate</td>
<td>↓ Neurodegeneration</td>
<td>CB2</td>
<td>Amenta et al., 2012</td>
</tr>
<tr>
<td>PF3845</td>
<td>5 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, severe</td>
<td>↓ Lesion volume ↓ Neurodegeneration ↑ Bcl-2, Hsp70 and 72</td>
<td>Not evaluated</td>
<td>Tchantchou et al., 2014</td>
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<tr>
<td>JZL184</td>
<td>10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CHI, mild repetitive</td>
<td>↓ Neurodegeneration</td>
<td>Not evaluated</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
<td>WWL70</td>
<td>10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, severe</td>
<td>↓ Lesion volume ↓ Neurodegeneration</td>
<td>CB1 and CB2</td>
<td>Tchantchou and Zhang, 2013</td>
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<td><strong>Excitotoxicity</strong></td>
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<tr>
<td>Rimonabant</td>
<td>2 mg/kg, i.p.</td>
<td>Rat Sprague–Dawley</td>
<td>Lateral FPI, severe</td>
<td>mGluR5 receptor recovery at 6 weeks (no impact on mGluR1)</td>
<td>CB1</td>
<td>Wang et al., 2016</td>
</tr>
<tr>
<td>2-AG</td>
<td>5 mg/kg, i.p.</td>
<td>Mouse Sabra</td>
<td>CH1, severe</td>
<td>↑ Levels of weak antioxidants</td>
<td>Not evaluated</td>
<td>Panikashvili et al., 2006</td>
</tr>
<tr>
<td>JZL184</td>
<td>10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CH1, mild repetitive</td>
<td>Glutamate receptor recovery Injury-induced ↓ in LTP protection GluA1 expression protection Injury-induced ↑ in EPSC protection</td>
<td>Not evaluated</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
<td>WWL70</td>
<td>10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CH1, severe</td>
<td>Microglial activation protection</td>
<td>Not evaluated</td>
<td>Mayeux et al., 2016</td>
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<tr>
<td><strong>Neuroinflammation</strong></td>
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<tr>
<td>CB1-/-/-</td>
<td>N/A</td>
<td>Mouse C57BL/6</td>
<td>CH1, severe</td>
<td>No effect on NF-κB transactivation</td>
<td>N/A</td>
<td>Panikashvili et al., 2005</td>
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<tr>
<td>CB1-/-+2-AG</td>
<td>N/A</td>
<td>Mouse C57BL/6</td>
<td>CH1, severe</td>
<td>No effect on NF-κB transactivation</td>
<td>N/A</td>
<td>Panikashvili et al., 2005</td>
</tr>
<tr>
<td>O-1966</td>
<td>5 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, moderate</td>
<td>Microglial activation protection</td>
<td>CB2</td>
<td>Amenta et al., 2012</td>
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<tr>
<td>PF3845</td>
<td>5 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, severe</td>
<td>↓ COX-2 Expression ↓ iNos expression</td>
<td>Not evaluated</td>
<td>Tchantchou et al., 2014</td>
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<tr>
<td>URB597</td>
<td>0.3 mg/kg, i.p.</td>
<td>Rat Sprague–Dawley</td>
<td>Lateral FPI, mild</td>
<td>Microglial activation protection</td>
<td>Not evaluated</td>
<td>Katz et al., 2015</td>
</tr>
<tr>
<td>2-AG</td>
<td>5 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CH1, severe</td>
<td>↑ TNFα mRNA ↑ IL-1β mRNA ↑ IL-6 mRNA ↑ NF-κB translocation and transactivation</td>
<td>Not evaluated</td>
<td>Panikashvili et al., 2006</td>
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<tr>
<td>JZL184</td>
<td>10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CH1, mild repetitive</td>
<td>Microglial activation protection Microglial activation protection</td>
<td>Not evaluated</td>
<td>Katz et al., 2015</td>
</tr>
<tr>
<td>WWL70</td>
<td>10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, severe</td>
<td>Microglial activation protection Microglial activation protection</td>
<td>Not evaluated</td>
<td>Tchantchou and Zhang, 2013</td>
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<tr>
<td><strong>Cerebrovascular breakdown</strong></td>
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<tr>
<td>URB597</td>
<td>0.3 mg/kg, i.p.</td>
<td>Rat Sprague–Dawley</td>
<td>Lateral FPI, mild</td>
<td>BBB integrity protection</td>
<td>Not evaluated</td>
<td>Katz et al., 2015</td>
</tr>
<tr>
<td>2-AG</td>
<td>5 mg/kg, i.p.</td>
<td>Mouse Sabra</td>
<td>CH1, severe</td>
<td>BBB integrity protection</td>
<td>Not evaluated</td>
<td>Panikashvili et al., 2006</td>
</tr>
<tr>
<td>JZL184</td>
<td>16 mg/kg, i.p.</td>
<td>Rat Sprague–Dawley</td>
<td>Lateral FPI, mild</td>
<td>BBB integrity protection</td>
<td>Not evaluated</td>
<td>Katz et al., 2015</td>
</tr>
<tr>
<td>WWL70</td>
<td>10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, severe</td>
<td>BBB integrity protection</td>
<td>Not evaluated</td>
<td>Tchantchou and Zhang, 2013</td>
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<tr>
<td><strong>CNS cellular structure/remodeling</strong></td>
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<tr>
<td>Vehicle (saline-5% ETOH)</td>
<td>4 µL</td>
<td>Rat Wistar</td>
<td>CH1, moderate</td>
<td>Diurnal CB1 expression abolished ↑ Contralateral CB1 and CB2 expression</td>
<td>Not evaluated</td>
<td>Martinez-Vargas et al., 2013</td>
</tr>
</tbody>
</table>

(Continued)
volume and lowered neurodegeneration in a mouse CCI model. A CB1 receptor antagonist attenuated the protective effects on lesion volume, while CB1 and CB2 receptor antagonists prevented the protective effects on neurodegeneration (Tchantchou and Zhang, 2013).

Combined, this evidence suggests that inhibitors of eCB hydrolysis offer protection against TBI-induced cell death which involve CB1 and CB2 receptors, though the distinction between the eCBs remains to be clarified. Few studies have evaluated interactions between anandamide and 2-AG in laboratory models of TBI. One study using a model of cerebral focal ischemia found that exogenously administered anandamide and 2-AG in combination reduced infarct size in rats, but with no facilitatory effects beyond anandamide or 2-AG alone (Wang et al., 2009). Given the recent availability of dual FAAH/MAGL inhibitors (Long et al., 2009; Niphakis et al., 2012), simultaneous blockade of these enzymes following TBI may further reveal some insight into the relationship between anandamide and 2-AG on TBI-induced cell death.

**Excitotoxicity**

Previous efforts to attenuate the effects of excitotoxicity following brain injury focused on NMDA receptor antagonists, presumably with the understanding that the induction of depressed NMDA receptor function would counteract TBI-induced excitotoxicity. This class of drugs showed promise in laboratory animal models of TBI (Shohami et al., 1995), but failed to produce long-term beneficial outcomes in clinical trials, despite some acute benefits of improved intracranial pressure and cerebral perfusion pressure (Knoller et al., 2002; Maas et al., 2006). Research investigating manipulations of the eCB system on glutamatergic functioning following TBI have thus far focused primarily on 2-AG, and paradoxically, its effectiveness to protect the integrity of glutamate receptor function.

Several studies investigating the effects of cannabinoids in laboratory animal models of TBI have focused on expression changes of metabotropic (mGluR1, mGluR5), AMPA (GluA1, GluA2), and NMDA (GluN1, GluN2A, GluN2B) glutamatergic receptors. Specifically, post-injury administration of the MAGL inhibitor JZL184 reversed TBI-induced reductions of GluN2A, GluN2B, and GluA1 receptor expression, but with no impact on GluN1 or GluA2 receptors (Zhang et al., 2014). The CB1 receptor antagonist Rimonabant did not alter injury-induced lowered expression of mGluR1, but surprisingly reversed reduced mGluR5 receptor expression 6 weeks following TBI (Wang et al., 2016). Both findings were completed 30 days post injury (Zhang et al., 2014; Wang et al., 2016), suggesting long term changes in glutamatergic function following acute administration of cannabinoids post-injury. However, little overlap is found between receptor expression endpoints across papers. In an example of contradictory patterns of GluA1 expression after injury, GluA1 expression was reduced in a study that subjected mice to a daily mild CHI on three consecutive days (Zhang et al., 2014), and was increased in rats subjected to a single lateral fluid percussion brain injury (Mayeux et al., 2016). In these studies, MAGL inhibition ameliorated both the reduced (Zhang et al., 2014) and increased (Mayeux et al., 2016) GluA1 expression. As discussed above (see Pre-Clinical Evaluation of Cannabinoids to Treat TBI), systematic investigation of species (mice vs. rat), brain injury model, number of injuries, and other experimental variables are needed to understand the consequences of brain injury on glutamate receptor changes.

Endocannabinoids are known to depress glutamate release from pre-synaptic terminals, and in particular, 2-AG has been explored in its ability to influence the functioning of electrochemical neurotransmission. MAGL inhibition has been found to protect against injury-induced increases in frequency and amplitude of EPSC in pyramidal neurons at the site of injury...
et al., 2015), as too has activation of CB

anandamide, likely due to its considerable abundance over

pro-inflammatory effects are attributed to 2-AG and not

and experimental dermatitis (Oka et al., 2006). Most of such

of which include models of nephropathy (Mukhopadhyay

et al., 2010a), cardiomyopathy (Mukhopadhyay et al., 2010b),

and experimental dermatitis (Oka et al., 2006). The use of cannabinoids following

Microglia/macrophage shift from a pro-inflammatory M1 to

an anti-inflammatory M2 phenotype (Tchantchou and Zhang,

given the possibility of the rapid oxidation of 2-AG and its

inhibition (Tchantchou and Zhang, 2013) and FAAH inhibition

ments that CB1 receptors mediate the protective effects of

Cerebrovascular Breakdown
The blood vessels which carry oxygen rich blood to the brain

are lined by endothelial cells as well as astrocytes. These cells,

combined with specific transport proteins and enzymes, strictly

regulate movement between the general circulation and CNS

extracellular fluid, and are collectively known as the BBB. TBI

has been well documented in producing cerebral blood flow

pathology (Kelly et al., 1997) as well as interfering with BBB

integrity (Başkaya et al., 1997). Given that cannabinoids are

known to exert vascular effects, producing vasodilation as well

as hypotension (reviewed in Hillard, 2000b), their manipulation

may hold promise as protectants against cerebrovascular damage.
Below, we review studies examining the effects of cannabinoids

on TBI-induced disruption of BBB integrity.

Exogenous administration of 2-AG (Panikashvili et al., 2006),
as well as MAGL inhibition (Katz et al., 2015), and ABHD6

inhibition (Tchantchou and Zhang, 2013) administered post-

injury protect against BBB breakdown. However, Panikashvili

et al., 2006 found that the expression of proteolytic enzymes

implicated in BBB breakdown were unaffected by exogenous 2-

AG post-injury. These enzymes include matrix metalloproteinase-

9 (MMP9) involved in extracellular matrix degradation, and

tumor necrosis factor-α-converting enzyme (TACE), which

cleaves membrane-bound proteins. The mechanism by which 2-

AG acts as a protectant of BBB integrity following traumatic

insult is yet to be resolved.

One study found that post-surgery administration of a FAAH

inhibitor protected against BBB breakdown (Katz et al., 2015),
suggesting that anandamide and/or other substrates of this

enzyme play a protective role. While the mechanism underlying

the structural protection of the BBB was not explored following

TBI, anandamide has been found to decrease BBB permeability

in a model of ischaemic stroke by transient receptor potential
cation channel, subfamily V, member 1 (TRPV1) (Hind et al.,
2015). Given that activation of TRPV1 receptors disrupts BBB

Neuroinflammation
Hydrolytic enzymes of anandamide and 2-AG produce a shared

metabolic product in the formation of free AA, the major

substrate of the biosynthetic enzymes of pro-inflammatory
eicosanoids (Nomura et al., 2011). Therefore, eCB oxidation

not only produces inactivation at cannabinoid receptors, but

also leads to the production of bioactive lipids involved in

inflammatory responses during the early stages of injury.
Manipulations of the eCB system have proved effective in
downregulating inflammation in many experimental models,
such as inflammatory pain (Ahn et al., 2009), and multiple

sclerosis (Mestre et al., 2005). The use of cannabinoids following

TBI have thus far been linked to two predominant features of

inflammation; decreased inflammatory cell activation, and
decreases in pro-inflammatory cytokine production.

Pro-inflammatory activated microglia are known to

exacerbate TBI-induced neuroinflammation (Kigerl et al.,
2009). Thus, decreasing TBI- inductions of inflammatory cell

activation is an attractive treatment strategy. MAGL inhibition

protects against TBI-induced microglial activation (Zhang et al.,
2014; Katz et al., 2015), while ABHD6 inhibition promotes

microglia/macrophage shift from a pro-inflammatory M1 to

an anti-inflammatory M2 phenotype (Tchantchou and Zhang,
2013). A parsimonious explanation for these findings is that

prevention of 2-AG hydrolysis leads to reduced levels of AA

and concomitant reductions of pro-inflammatory mediators.

Given the contribution of 2-AG catabolism to eicosanoid

production, it is unsurprising that several studies have reported
eCBs as demonstrating pro-inflammatory roles, some examples

of which include models of nephropathy (Mukhopadhyay

et al., 2010a), cardiomyopathy (Mukhopadhyay et al., 2010b),

and experimental dermatitis (Oka et al., 2006). Most of such

pro-inflammatory effects are attributed to 2-AG and not

anandamide, likely due to its considerable abundance over

anandamide. However, FAAH inhibition, similarly has been

found to protect against TBI-induced microglial activation (Katz

et al., 2015), as too has activation of CB2 receptors (Amenta

et al., 2012). Thus, a need exists to disentangle the potential

contributions of 2-AG to pro-inflammatory processes from its

role as a substrate for AA production, versus anti-inflammatory
effects through cannabinoid receptors, following TBI.

Inhibition of eCB degradative enzymes has also produced
decreases in TBI-induced pro-inflammatory mediators. Reductions in the expression of inducible enzymes that

trigger eicosanoid production following brain injury, COX-2

enzyme (which converts free AA to prostaglandins) and iNos

(which produces the free radical nitric oxide in response
to cytokine signaling), are seen in response to ABHD6

inhibition (Tchantchou and Zhang, 2013) and FAAH inhibition

(Tchantchou et al., 2014). Reductions in TBI-induced pro-
inflammatory cytokine mRNA (IL-1β, TNFa, and IL-6) have

also been found following treatment with exogenous 2-AG

(Panikashvili et al., 2006). These findings seem counter-intuitive
given the possibility of the rapid oxidation of 2-AG and its

consequent contribution to eicosanoid production. However,
exogenous 2-AG has also been shown to ameliorate TBI-induced

transactivation of the nuclear factor NF-kB (linked to cytokine

production) in wild type mice, but not in CB1 knockout mice,
suggesting that CB1 receptors mediate the protective effects

of exogenous 2-AG (Panikashvili et al., 2005).
integrity (Hu et al., 2005), it is possible that anandamide, as a partial agonist at TRPV1 channels (Pertwee and Ross, 2002), may be acting as a functional antagonist against a high efficacy endogenous agonist to produce its structurally protective effects of the cerebral microvascular endothelium. The exploration of how anandamide may be exerting its protective effects of BBB integrity may yet yield further novel targets for the treatment of TBI.

In cerebral circulation, CB₁ receptor activation produces vasodilation. Indeed, the CB₁ receptor antagonist rimonabant inhibited hypotension induced by endotoxin shock and hemorrhagic shock, as well as increasing survival (Varga et al., 1998). Though cannabinoids are yet to be explored in the context of TBI-induced changes in cerebral blood flow, CB₁ receptor antagonism may prove to be a potential target for the treatment of TBI-induced hypotension.

Cell Structure/Remodeling

The key biological idea that structure dictates function also holds true for the neurophysiology of TBI. The shearing and tearing forces of TBI and subsequent secondary injury cascades produce changes in cell architecture, extracellular matrices, and the balance of fluid homeostasis, that impair neuronal function often both in a focal and/or diffuse manner throughout the brain (Gaetz, 2004). The use of cannabinoids has thus far been linked to protection against several of the CNS structural changes associated with TBI, with 2-AG being the most frequently studied eCB in this area.

While a traumatic insult can result in the rapid onset of cerebral oedema, exogenously administered 2-AG protects against TBI-induced oedema (Panikashvili et al., 2001, 2005). The observation that no such oedema protection was found following 2-AG administration in CB₁ receptor−/− mice (Panikashvili et al., 2005) suggests that this protection requires CB₁ receptor activation. Changes in protein physiology have also been found to occur following TBI. Specifically, the presence of protein aggregates such as amyloid-β plaques (Johnson et al., 2010), p-tau (Goldstein et al., 2012), and TDP-43 (Smith et al., 1999), have been found within hours following TBI. These proteins are thought to accumulate from damaged axons and as a result of a disturbed balance between genesis and catabolism (Johnson et al., 2010). MAGL inhibitors decrease amyloid-β protein and its precursor molecule APP, as well as p-tau and TDP-43 (Zhang et al., 2014). MAGL inhibition also decreases astrocyte activation (Mayeux et al., 2016), while exogenous 2-AG following TBI reduces hippocampal CA-3 neuron loss (Panikashvili et al., 2001). These consistent protective effects of 2-AG across varied TBI-related structural pathologies point to its important role in maintaining cell structure and promoting remodeling.

Protective roles played by anandamide in injury-induced structural changes are yet to be ascertained. Though FAAH inhibition decreases APP expression post-injury, as well as increases synaptophysin (Tchantchou et al., 2014), a synaptic vesicle protein whose elimination impairs object recognition and spatial learning in mice (Schmitt et al., 2009). Furthermore, eCBs may not be working alone to offer protection from TBI-induced structural impairments. For example, estradiol decreased the number of TBI-induced immunoreactive astrocytes, which was inhibited by CB₁ and CB₂ receptor antagonists, while also increasing cerebral cortex mRNA levels of CB₂ receptors (Lopez Rodriguez et al., 2011). These findings suggest that the regulatory activity of the eCB receptors in response to TBI may be mediated by endocrine as well as paracrine signaling mechanisms.

Traumatic brain injury is well described to increase CB₁ and CB₂ receptor expression, which includes disruption of diurnal rhythms of CB₁ receptor expression (Martínez-Vargas et al., 2013). Post-injury treatment with a CB₁ receptor antagonist reduces CB₁ receptor expression at 6 weeks following injury (Wang et al., 2016), whereas ABHD6 inhibition produces increased CB₁ and CB₂ receptor expression (Tchantchou and Zhang, 2013). As such, TBI-induced increases in cannabinoid receptor expression are perhaps facilitated by 2-AG.

Neurogenic Pulmonary Oedema

Pulmonary complications are reported in 20–25% of TBI patients (Holland et al., 2003), and its severity is related to brain injury magnitude (Alvarez et al., 2015). The exact CNS circuits involved in NPE have yet to be identified, though a sudden rise in intracranial pressure, rapid sympathetic surge, increased systemic vascular resistance and increase in hydrostatic pressure in the pulmonary vasculature, as well as release of pro-inflammatory mediators may all contribute to interstitial pulmonary oedema formation (Brambrink and Dick, 1997). NPE rapidly occurs within hours of TBI onset in clinical populations (Alvarez et al., 2015), and within minutes in animal models (Atkinson et al., 1998), producing CNS hypoxia (Oddo et al., 2010) which further contributes to secondary injury. NPE is a much needed area of interest in the study of TBI.

While at the present time there are no studies evaluating the contributions of, or protection by, the eCB system to NPE following TBI, this may prove an interesting area of future investigation. Specifically, the lung possesses a basal tone of 2-AG (Avraham et al., 2008; Nomura et al., 2008), and recently it has been shown that resident lung macrophages express major components of the eCB system, CB₁ and CB₂ receptors as well as anandamide and 2-AG (Staiano et al., 2015). Furthermore, MAGL inhibition has already been found to be protective against LPS-induced acute lung injury in mice, and attenuated with CB₁ and CB₂ receptor antagonists (Costela-de-Souza et al., 2013).

Treatment of Behavioral Deficits of TBI

The heterogeneous clinical presentation of TBI pathology in populations of survivors is reminiscent of its cellular and molecular pathophysiology described above. TBI patients report changes in mental health (depression, irritability, anxiety, and personality changes), sleep disturbance, post-traumatic headaches, persistent fatigue, epilepsy, learning and memory deficits (manifested also as impairments in attention and processing speed [Vakil, 2005]), and balance disorders (Stefán et al., 2016). Most frequently investigated measures in the pre-clinical TBI literature include neurological motor, and learning and memory impairments, leaving a
wide breadth of TBI clinical effects yet to be studied. Once again, components of the eCB system may become active to compensate for TBI symptomology given what is currently known of its regulatory effects within these areas, two examples being pain, and anxiety and depression (Corcoran et al., 2015).

In this section, we review what is currently known of cannabinoids in the context of their ability to alter post-traumatic animal behavior (see Table 2).

**Learning and Memory**

Learning and memory impairments are among the most frequently reported symptoms following TBI, and are slow to recover with deficiencies reported 10 years later (Zec et al., 2001). The eCB system has been shown to play a well-documented role in memory regulation (reviewed in Mechoulam and Parker, 2011), and as such its manipulation holds considerable promise to address such a profound consequence of TBI.

Inhibition of the eCB hydrolytic enzymes FAAH (Tchantchou et al., 2014), MAGL (Zhang et al., 2014), and ABHD6 (Tchantchou and Zhang, 2013) have been shown to protect against TBI-induced memory impairments, suggesting that anandamide and 2-AG elevation post-TBI may offer protection from TBI-induced learning and memory deficits. The protective effects of 2-AG appear to be task specific, with ABHD6 inhibition showing learning and memory protection in a Y-maze task, but not a Morris water maze task. To date, only a Y-maze task has been used to evaluate the memory

**TABLE 2 | Effect of cannabinoids on TBI-induced behavioral impairments.**

<table>
<thead>
<tr>
<th>Compound/mutant</th>
<th>Dose</th>
<th>Species</th>
<th>TBI model/severity</th>
<th>Effect</th>
<th>Receptor mediated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Learning and memory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF3845</td>
<td>5 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, severe</td>
<td>Y-maze deficit protection</td>
<td>CB₁</td>
<td>Tchantchou et al., 2014</td>
</tr>
<tr>
<td>JZL184</td>
<td>10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CHI, mild repetitive</td>
<td>MMW deficit reduction</td>
<td>Not evaluated</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
<td>WWL70</td>
<td>10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, severe</td>
<td>Y-maze deficit protection</td>
<td>No impact on MMW deficit</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Neurological motor deficits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB¹ --/–</td>
<td>N/A</td>
<td>Mouse C57BL/6</td>
<td>CHI, severe</td>
<td>Impaired NSS score</td>
<td>CB₁</td>
<td>Panikashvili et al., 2005</td>
</tr>
<tr>
<td>CB¹ --/– +2-AG</td>
<td>N/A</td>
<td>Mouse C57BL/6</td>
<td>CHI, severe</td>
<td>Impaired NSS score</td>
<td>CB₁</td>
<td>Panikashvili et al., 2005</td>
</tr>
<tr>
<td>O-1966</td>
<td>5 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, moderate</td>
<td>Rotarod deficit protection</td>
<td>Not evaluated</td>
<td>Amenta et al., 2012</td>
</tr>
<tr>
<td>Anandamide</td>
<td>1.25 μg/4 μL, ICV</td>
<td>Rat Wistar</td>
<td>CHI, moderate</td>
<td>Improved NSS score</td>
<td>Not evaluated</td>
<td>Martinez-Vargas et al., 2013</td>
</tr>
<tr>
<td>PF3845</td>
<td>5 and 10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, severe</td>
<td>Beam-walk deficit protection</td>
<td>Partial CB₁</td>
<td>Tchantchou et al., 2014</td>
</tr>
<tr>
<td>URB597</td>
<td>0.3 mg/kg, i.p.</td>
<td>Rat Sprague–Dawley</td>
<td>Lateral FPI, mild</td>
<td>No impact on NSS or NBS</td>
<td>Not evaluated</td>
<td>Katz et al., 2015</td>
</tr>
<tr>
<td>2-AG</td>
<td>5 mg/kg, i.v.</td>
<td>Mouse Sabra</td>
<td>CHI, severe</td>
<td>Improved NSS score</td>
<td>Not evaluated</td>
<td>Panikashvili et al., 2001</td>
</tr>
<tr>
<td>2-AG + 2-PG + 2-LG</td>
<td>1 mg/kg, i.v.</td>
<td>Mouse Sabra</td>
<td>CHI, severe</td>
<td>Improved NSS score</td>
<td>Not evaluated</td>
<td>Panikashvili et al., 2001</td>
</tr>
<tr>
<td>JZL184</td>
<td>10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CHI, mild repetitive</td>
<td>Improved NSS score</td>
<td>Partial CB₁</td>
<td>Not evaluated</td>
</tr>
<tr>
<td></td>
<td>16 mg/kg, i.p.</td>
<td></td>
<td>Lateral FPI, mild</td>
<td>Improved NSS and NBS score, out to 1 d</td>
<td>No CB₁, CB₂ reversal</td>
<td>Not evaluated</td>
</tr>
<tr>
<td></td>
<td>16 mg/kg, i.p.</td>
<td></td>
<td>Lateral FPI, mild</td>
<td>Improved NSS and NBS score, out to 14 d</td>
<td>Not evaluated</td>
<td>Mayeux et al., 2016</td>
</tr>
<tr>
<td>WWL70</td>
<td>10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, severe</td>
<td>Improved NSS score</td>
<td>Rotarod deficit protection</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Anxiety-like behavior</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF3845</td>
<td>5 and 10 mg/kg, i.p.</td>
<td>Mouse C57BL/6</td>
<td>CCI, severe</td>
<td>Zero-maze anxiety-like profile protection</td>
<td>No CB₁, CB₂ reversal</td>
<td>Tchantchou et al., 2014</td>
</tr>
<tr>
<td>Post-traumatic seizures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rimonabant</td>
<td>2 mg/kg, i.p.</td>
<td>Rat Sprague–Dawley</td>
<td>Lateral FPI, severe</td>
<td>Protective against seizure threshold deficits Lowered seizure mortality</td>
<td>CB₁</td>
<td>Wang et al., 2016</td>
</tr>
</tbody>
</table>

*Drug targets; CB₂ receptor agonist (O-1966), FAAH inhibitor (PF3845), MAGL inhibitors (JZL184 and URB597), ABHD6 inhibitor (WWL70), and CB₁ receptor antagonist (Rimonabant). TBI Model definitions; CCI (controlled cortical impact), CHI (closed head injury), and FPI (fluid percussion injury).*
protective effects of FAAH inhibition, and this task-specific effect did not occur with a MAGL inhibitor. Mice are a well-used pre-clinical model organism to study the memory effects of TBI; however, they are known to perform behavioral tasks more readily, and with less error, when the task does not rely on aversive motivation (Stranahan, 2011). This attribute of mice may, in some part, contribute to the task-related differences seen between the Y-maze task (which uses exploratory behaviors associated with novelty) and the aversively motivated escape behavior necessary in the Morris water maze. Regardless, in clinical populations the most common memory process vulnerable to TBI involves difficulties applying active or effortful strategy's in the learning or retrieval process (Vakil, 2005). Moving forward, the use of behavioral tasks able to selectively assess such frontal lobe-type memory impairments might improve the translational capacity of eCB TBI pre-clinical assessments (one such example being the Morris water maze Reversal Task, which evaluates cognitive flexibility).

Neurological Motor
Traumatic brain injury-induced neurological motor impairments currently represent the most frequently studied behavioral outcome measure in the TBI-cannabinoid literature. In clinical populations, neurological motor impairments seen as a result of TBI show spontaneous improvement over time, but one third of patients continue to experience neuromotor abnormalities 2 years after injury (Walker and Pickett, 2007). A variety of eCB system manipulations have thus far been found to be protective against the neurological motor deficits associated with murine models of TBI.

Both 2-AG and anandamide elevation provide protection against TBI-induced neurological motor deficits. MAGL inhibitors (Zhang et al., 2014; Katz et al., 2015; Mayeux et al., 2016), ABHD6 inhibitors (Tchantchou and Zhang, 2013), and exogenous 2-AG administration (Panikashvili et al., 2001), improve NSS in laboratory animal models of TBI. Moreover, ABHD6 inhibition also protects against TBI-induced rotarod deficits (Tchantchou and Zhang, 2013). Administration of exogenous 2-AG did not enhance NSS scores in CB1 receptor knockout mice subjected to TBI (Panikashvili et al., 2005), suggesting a CB1 receptor mechanism of action. FAAH inhibition has produced mixed findings in neurological motor tests, such as beam-walk deficit protection (Tchantchou et al., 2014) but no improvement on TBI-induced NSS deficits (Katz et al., 2015). In support of anandamide being protective against TBI-induced motor deficits, exogenous anandamide has also produced improved NSS performance (Martinez-Vargas et al., 2013). Full reversal, and partial reversal, of FAAH inhibitor mediated beam-walk deficit protection by respective CB2 and CB1 receptor antagonists (Tchantchou et al., 2014), suggest a role of both of these receptors in anandamide’s neuromotor deficit sparing effects. The involvement of the CB2 receptor is further supported by rotarod deficit protection from a CB2 receptor agonist (Amenta et al., 2012).

The role of entourage effects has also been evaluated in the area of TBI-induced neurological motor impairments. Corelease of endogenous fatty acid derivatives can potentiate 2-AG signaling, termed an entourage effect (Ben-Shabat et al., 1998; Lambet and Di Marzo, 1999; Lichtman et al., 2002). Administration of 2-AG with two related lipids that do not bind cannabinoid receptors, 2-LG and 2-PG, enhances recovery from TBI-induced NSS deficits (Panikashvili et al., 2001). Given FAAH is responsible for the degradation of various fatty acid amides in addition to anandamide (Boger et al., 2000), its various substrates may work in concert to ameliorate pathologies related to TBI. Thus any inferences drawn about anandamide through the use of FAAH inhibition needs to consider contributions of non-cannabinoid fatty acid amides.

Anxiety and Post-Traumatic Seizures
The signs of post-traumatic anxiety have been difficult to replicate in murine models of TBI (Tucker et al., 2016). Also, as there is a limited number of studies evaluating eCBs in this area, no definitive conclusions can be made. Thus far, only FAAH inhibition has been explored to address post-traumatic anxiety, and was found to protect against TBI-induced increases in anxiety-like behavior in mice (Tchantchou et al., 2014). This protection in the zero maze was unaffected by either CB1 or CB2 receptor antagonists, suggesting that these receptors are dispensable. Modeling post-traumatic epilepsy is time consuming and faces other challenges such as a low percentage of animals that develop epilepsy (Mazarati, 2006), however, recent models that produce consistent replication of spontaneous seizure activity following a TBI are available (Ping and Jin, 2016). Contrary to preclinical research demonstrating that the eCB system plays a protective roles against seizures (Wallace et al., 2001; Marsicano et al., 2003), a CB1 receptor antagonist has protected against injury-induced seizure threshold deficits as well as lowered seizure mortality (Wang et al., 2016), potentially through the disinhibition of GABAergic terminals.

This nascent body of data, suggests that eCB manipulations hold promise to treat injury-induced clinical symptoms outside of the more popular areas of learning and memory and neurological motor impairments.

PRIMARY PHYTOCANNABINOIDS AND TRAUMATIC BRAIN INJURY
Although currently well over one hundred phytocannabinoids have been elucidated from the Cannabis sativa plant (Elshohy et al., 2017), the most extensively studied of these are THC and cannabidiol (CBD). The investigation of phytocannabinoids on TBI pathology not only holds topical relevance, but also but also holds promise as potential treatment for TBI and other disorders.

Without exception, all of the experimental work reviewed and listed in Tables 1 and 2 have used post-injury drug administration times ranging from 15 min to several days,
clearly an attempt to simulate clinical intervention timing possibilities. However, clinical and pre-clinical findings provide evidence suggesting that the primary psychoactive constituent of *Cannabis sativa*, THC, is neuroprotective when administered prior to a traumatic insult. In a 3 year retrospective study of patients who had sustained a TBI, urine toxicology screen results showed decreased mortality in individuals with a positive THC screen (Nguyen et al., 2014). In two mouse models of CNS injury that yield cognitive deficits, pentylenetetrazole (an excitotoxic agent) and carbon monoxide induced hypoxic injury, prior administration of THC provided impairment protection (Assaf et al., 2011). Curiously, an extraordinarily low dose of THC (i.e., 0.002 mg.kg⁻¹) reduced injury-induced cognitive deficits in mice (Assaf et al., 2011). The authors explained this effect through the known biphasic effects of THC producing analgesia, acute hypothermia, and decreased locomotion at high doses (10 mg.kg⁻¹), and producing hyperalgesia, hyperthermia, and increased locomotion at a low dose (0.002 mg.kg⁻¹) (reviewed in Sarne et al., 2011). Such low dose effects of THC have been found to potentiate calcium entry into cells *in vitro* (Okada et al., 1992), increasing glutamate release, and thus may be mildly neurotoxic. Therefore, Assaf et al. (2011) hypothesized that low dose THC pre-treatment produced a pre-conditioning effect, where a mildly noxious stimulus becomes protective against a more severe subsequent insult, an effect known to occur in cardiology (Dirmagl et al., 2003) as well as cerebral ischaemia (Kitagawa et al., 1991). Moreover, the molecular signaling cascades behind cardiac and cerebral ischaemia preconditioning include activation of ERK and Akt (Dirmagl et al., 2003; Gidday, 2006), also shown to mediate the protective effects of ABHDB (Tchantchou and Zhang, 2013) and MAGL (Mayeux et al., 2016) inhibition following TBI.

Even though 80–90% of THC is excreted from individuals within 5 days of administration, the remaining slow release of lipophilic THC from lipid-storage compartments result in its long terminal half-life in plasma (Huestis, 2007). As such, individuals may experience very low plasma THC concentrations for prolonged periods after each administration. Although the clinical study of TBI-induced mortality reported no data to quantify levels of THC in the THC positive individuals, the low dose THC in CNS injured mice may mimic the pharmacokinetics of THC in humans. This presumed prolonged exposure of THC due to its pharmacokinetics, as well as other potentially neuroprotective cannabinoids, such as CBD (Perez et al., 2013), may be responsible for the survival effects found in cannabis-exposed TBI patients. A finding of increased clinical relevance, is that post-conditioning (when the mildly noxious stimulus is applied after the insult) with low dose THC also produced cognitive sparing effects in mice (Assaf et al., 2011). These findings, however, remain controversial, and are yet to be replicated in animal models of TBI.

The phytocannabinoid CBD, currently being investigated in clinical trials for its seizure reduction potential in Tuberous Sclerosis Complex (Gw Research Ltd, 2016), has known anti-inflammatory properties. Although CBD does not bind CB₁ and CB₂ receptors, it activates the g-protein coupled receptor GPR55 (Ryberg et al., 2007), inhibits nucleoside transporter 1 (Carrier et al., 2006), inhibits sodium channels (Hill et al., 2014), and produces increased extracellular adenosine concentrations that consequently downregulate inflammatory cells through the adenosine A₂A receptor (Ohta and Sitkovsky, 2001; Hasko and Pacher, 2008). While there are no studies at present which have investigated the anti-inflammatory effects of CBD following TBI, CBD has reduced FosB expression following cryogenic spinal cord injury (Kwiatkoski et al., 2012), and lowered iNos expression in a mouse model of tauopathy (Casarejos et al., 2013). As such CBD may be a promising future avenue of investigation in the study of neuroinflammation in response to brain injury.

**CONCLUDING REMARKS AND FUTURE DIRECTIONS**

The eCB system, through release of its endogenous ligands or by changes in cannabinoid receptor constitutive activity, possesses promise in the treatment of diverse TBI pathology. An important step forward in understanding the role that the eCB system plays in TBI pathology includes not only the full characterization of ligands targeting cannabinoid receptors and eCB regulating enzymes, but also changes in cannabinoid receptors, eCB levels, and eCB regulating enzymes as a consequence of TBI. Another future area of therapeutic interest is non-CB₁/CB₂ receptor targets, such as TRPV1 receptors, and their potential contribution to the protective effects following TBI. Furthermore, alternative activation of CB₁/CB₂ receptors, such as potential entourage effects from other fatty acid derivatives, antagonism, or allosteric modulation, might impact functional selectivity and thus TBI-related outcomes also warrants further investigation. So too do the plant-derived phytocannabinoids represent an understudied yet promising group of compounds given the neuroprotective results obtained from other types of CNS injury. In particular, CBD as well as other phytocannabinoids which do not bind cannabinoid receptors, represent promising molecules to treat TBI.

To date, the only reported cannabinoid to be specifically evaluated for the treatment of TBI in patient populations is Dexanabinol, also known as HU211. While HU211 showed promise in animal models of TBI (Shohami et al., 1995), it failed to produce long term patient outcomes in one clinical trial despite some acute benefits (Knoller et al., 2002), and in a second study showed no short or long term benefits (Maas et al., 2006). Although HU211 has been described as a cannabinoid by virtue that it is an enantiomer of the potent synthetic cannabinoid agonist HU210, it does not bind or activate cannabinoid receptors. Instead, HU211 acts as a non-competitive NMDA receptor antagonist (Feigenbaum et al., 1989). This therefore brings to light an important consideration of the classification of cannabinoids.

One consistently overlooked area across the study of TBI is the evaluation of the central penetration of systemically administered
drugs. Pharmacological treatments will need to be assessed for their ability to cross the BBB. Also, it should be noted that TBI rapidly disrupts the BBB and lasts for three days post-injury (Başkaya et al., 1997). Furthermore, given the often biphasic nature of cannabinoid drugs, it is critical to move away from single dose pharmacology to full dose-response assessments, which may yield an increased understanding of the mechanism and potential of cannabinoids to treat TBI. Overall, the abundant and growing pre-clinical research suggests that the eCB system possesses many promising targets for new and existing drugs that may ameliorate diverse TBI pathology.

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[References list is not shown in this response, but it is available in the document.]

AUTHOR CONTRIBUTIONS

LS performed the literature review and composed the article; AL contributed to the composition of the article.

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Endocannabinoids and Traumatic Brain Injury


Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.