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Cannabidiol and Neurodevelopmental Disorders in Children

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Neurodevelopmental and neuropsychiatric disorders (such as autism spectrum disorder) have broad health implications for children, with no definitive cure for the vast majority of them. However, recently medicinal cannabis has been successfully trialled as a treatment to manage many of the patients' symptoms and improve quality of life. The cannabinoid cannabidiol, in particular, has been reported to be safe and well-tolerated with a plethora of anticonvulsant, anxiolytic and anti-inflammatory properties. Lately, the current consensus is that the endocannabinoid system is a crucial factor in neural development and health; research has found evidence that there are a multitude of signalling pathways involving neurotransmitters and the endocannabinoid system by which cannabinoids could potentially exert their therapeutic effects. A better understanding of the cannabinoids' mechanisms of action should lead to improved treatments for neurodevelopmental disorders.

Keywords: anxiety, autism, cannabinoid, cannabidiol, endocannabinoid system, neuroinflammation, neuropsychiatry, paediatrics

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NEURODEVELOPMENTAL AND PSYCHIATRIC DISORDERS

Neurodevelopmental disorders in children have profound impacts on the functioning of children and families particularly where an additional mental health diagnosis is present. The prevalence of any neurodevelopmental disorder seems to vary depending on the study; however, it seems to be around 15% of children (3–17 years) in the United States of America (USA) based on parental concerns (1). This includes diagnoses such as Attention Deficit Hyperactivity Disorder (ADHD), Autism Spectrum Disorder (ASD), Intellectual Disabilities (ID) and syndromic disabilities. According to Boyle et al., around 4% of affected children had at least 2 diagnoses. Those who have a neurodevelopmental disorder are often two to four times more at risk of developing a mental health problem than a typically developing child (1). Neurodevelopmental disorders can include anxiety and mood disorders, Tourette's syndrome, psychosis, and bipolar disorders. Individuals with neuroatypical presentations may pose particular challenges to assessment and understanding of the psychiatric diagnosis. They may resort to behavioural escalations (such as tantrums and self-injury) as a manifestation of their extreme distress and inability to communicate their distress and, as such, can be very difficult for families and communities to support (2, 3).

The aetiology of neurodevelopmental disorders is multifactorial with polygenic risk as well as the impact of perinatal exposures to biological or environmental factors that may act as

epigenetic modifiers of neuronal networks and structures. The biological underpinning of many of these disorders is only, in part, minimally understood and thus therapies are usually based on responses in typically developing individuals, older paediatric populations and adults. Treatment options for comorbid mental health problems are limited on the whole to symptomatic therapies and often evidence is restricted in these populations as to the treatment's effectiveness and the mechanisms involved. For example, stimulants for ADHD and some newer therapies are frequently used where attention and impulsivity issues are present in other, non-ADHD disorders while anxiety medication may be trialled off-label on a child with ID diagnosed with significant anxiety. Interestingly, a common trait of ASD and ASD-related disorders (such as Fragile X syndrome and 22q11.2 deletion syndrome) is anxiety and seizures (with or without epilepsy) (4–7). The use of atypical antipsychotics continues to be one of the only evidence-based treatments in children with autism and escalated behaviour; however, the side effect profile of antipsychotics is very difficult to manage, which relegates them to be used only as a short-term last resort. Clinicians are regularly trialling medication to support children and families in significant distress, leading to most of these medications to be prescribed off-label for neuroatypical children; therefore, new medications with clear relationships to aetiology and biological underpinnings are required to support these individuals as they develop into adulthood.

CANNABINOIDS AS POTENTIALLY THERAPEUTIC FOR PAEDIATRIC PSYCHIATRIC DISORDERS

There has been interest for a long time in the impact of medicinal cannabis on neurological and psychiatric disorders (8). Phytocannabinoids (cannabinoids) have been found to be molecules that could be pharmaceutically beneficial for some ailments (9). However, the prescription of medical cannabis has been very conservative because of its stigma as a substance of abuse in many jurisdictions (10). Thanks to some well-publicised case studies, a recent increase in community acceptance of cannabis's medical benefits (11) has been shifting government policy in favour of cannabis decriminalisation/legalisation in jurisdictions such as Canada, Israel, Uruguay, a majority of USA states, and the Food and Drug Administration (12–15). In Australia, the Therapeutic Goods Administration (TGA) currently allows strict, limited prescription of medical cannabis by registered medical practitioners (16), and in 2019 the Australian Capital Territory legalised the individual possession and cultivation of small amounts of cannabis (17). Consequently, this surge in therapeutic cannabinoid usage is encouraging a rise in cannabis research, as the cannabis farming industry, biotechnology and pharmaceutical corporations compete to develop more medical cannabinoid products and better commercialise their usage.

Among the 126 cannabinoids in the cannabis plant and its many variants (18), only delta-9-tetrahydrocannabinol (Δ9-THC or THC) is strongly psychoactive and its effects on the developing brain have been a concern for many clinicians as it can induce short-term alterations in mood, behaviour, appetite and cognition (19). Pathological and behavioural aberrations have been detected in chronic cannabis users and can vary with individuals as well as over time (20, 21), making the effects of long-term cannabis treatment on individuals difficult to predict with current methodology. The neurodevelopment of children and adolescents can be disrupted by the cannabinoids' wide-ranging effects on the central nervous system (CNS) (22). The uncertainty of THC's long-term safety has directed society's contemporary focus on cannabidiol (CBD) as the most promising therapeutic cannabinoid due to its relative abundance in the plant, lack of psychoactive effects, positive safety profile (23) and purported benefits (24). There are some synergies between THC and CBD [i.e., THC can reinforce CBD's beneficial properties while CBD dampens THC's psychotropic effects (25, 26)], but THC's psychoactive properties and strong neural interactions can be detrimental after long-term frequent exposure, especially in the developing brain. Indeed, significant alterations in brain structure/function have been observed in humans, adult and adolescent rodents (27–31) frequently consuming cannabis compared to cannabis-free controls. But there is no definitive consensus as other experiments have either reported no significant difference in brain morphology (32) or have been contradictory; for example, one study found thinner brain cortices in adolescent/young adult cannabis users (33) while another study reported increased cortical thickness in adolescent cannabis users (34), compared to non-users of cannabis. Such uncertainty about the long-term effects of cannabinoids on the human brain reinforces the need for in-depth investigations of the cannabinoids' positive and negative effects. There is still very little understanding of how the intake of THC, CBD, and/or other cannabinoids may affect developing neurodivergent brains and research is urgently needed as the use of medicinal cannabis becomes legalised in various parts of the world.

The precise mechanisms behind CBD's beneficial effects are currently not well-understood. CBD does not significantly interact with the cannabinoid receptors that THC interacts strongly with, and its actions have been attributed to inhibition of anandamide degradation (35), serotoninergic, anti-inflammatory and/or its antioxidant properties (36–39).

Abbreviations: 22QS, 22q11. 2 deletion syndrome; 2-AG, 2-arachidonoylglycerol; 5-HTR, 5-hydroxytryptamine receptor; AA, Arachidonic acid; ADHD, Attention Deficit Hyperactivity Disorder; AEA, N-arachidonoyl-ethanolamine or anandamide; AEDs, Anti-epileptic drugs; ASD, Autism Spectrum Disorder; BBB, Blood-brain barrier; CBD, Cannabidiol; CB1R, Cannabinoid receptor 1; CB2R, Cannabinoid receptor 2; Cys-LT, Cysteinyl leukotriene; CNS, Central nervous system; COX, Cyclooxygenase; CYP, Cytochrome P450; EA, Ethanolamide; ECS, Endocannabinoid system; EET, Epoxyeicosatrienoic acid; ENT1, Equilibrative nucleoside transporter; FAAH, Fatty acid amide hydrolase; FDA, Food and Drug Administration; FXS, Fragile X syndrome; GABA, γ-Aminobutyric acid; GPR, G-protein coupled receptor; HETE, Hydroxyeicosatetraenoic acid; HPETE, 5-hydroperoxyeicosatetraenoic acid; ID, Intellectual disabilities; IL-1β, Interleukin-1β; LOX, Lipoxygenase; LT, Leukotriene; MAGL, Monoacylglycerol lipase; MAM, Methylazoxymethanol acetate; PG, Prostaglandin; TGA, Therapeutic Goods Administration; TNF-α, Tumour necrosis factor-α; Δ9-THC or THC, Delta-9 tetrahydrocannabinol; TRPV, Transient Receptor Potential Vanilloid; VDAC1, Voltage-dependent anion selective channel protein 1.

Therapeutic administration of CBD has been demonstrated to alleviate a range of neuropsychiatric symptoms in schizophrenia (35, 40, 41), depression (42) and anxiety (24, 43, 44) (**Table 1** summarises a selection of experiments/trials). Encouraged by these findings, CBD therapy has recently been clinically tested in case studies of autism. Aran (3) and Barchel et al. (46) reported improvements in behaviour, anxiety, and communication in oral CBD treatment trials with ASD children—about 60–70% of patients responding well to the treatment, with the side-effects of somnolence and appetite loss being reasonably tolerated. Phase 1b-2 trials of CBD therapy in ASD have demonstrated a positive response in irritability scales on the Aberrant Behaviour Checklist-Community (ABC-C) as well as some core features such as hyperactivity, anxiety. Other trials in phase 2 and phase 3 are underway for anxiety/ behavioural outcomes in ASD, 22q11.2 deletion syndrome (22QS), ID and Tourette's syndrome, with the results of phase 3 studies being awaited. In the case of Fragile X syndrome (FXS) treatment, positive results have also been obtained with successful case studies (5) and clinical trials (45) that involved the participation of children; the studies reported clinically significant improvements in emotional and behavioural symptoms of FXS, namely anxiety, social avoidance, and irritability. The CBD in Heussler's study was administered by transdermal application of a CBD gel patented by Zynerba Pharmaceuticals (4). Most side-effects were mild enough for this novel CBD treatment to be deemed tolerable by the FXS patients (45). Unlike ASD and FXS, there have been no reports published on the efficacy of CBD treatment on 22QS patients as of the time of writing. There is an ongoing clinical trial sponsored by Zynerba Pharmaceuticals, where the efficacy of their CBD gel is being tested on 22QS minors. Due to the commonalities shared by ASD, FXS, and 22QS, the rationale is that CBD would exert anxiolytic and behavioural improvements, resembling those observed in CBD therapy of ASD and FXS (4, 45).

With many cases of epilepsy persistently resistant to the most common treatment options (48), families of affected epileptic individuals have advocated for the use of medical cannabis as an alternative treatment. CBD demonstrably acts on brain regions and neural pathways in animal and human models of epilepsy via anticonvulsant and neuroprotective effects (38, 49–52). Therefore, cannabinoids (particularly CBD) have been trialled for the management of epilepsy. Paediatric clinical trials are underway in many parts of the world to evaluate pharmaceutical CBD and its impact on a number of areas including completed randomised clinical trials in Dravet and Lennox-Gastaut syndromes (refractory epilepsy syndromes). Two trials focused on the treatment of Lennox-Gastaut syndrome while one trial selected patients affected by Dravet syndrome. All trials had participants regularly administered with a patented oral formulation of 98% CBD (Epidiolex® by GW Pharmaceuticals). In these trials, the participants' pre-existing treatment regime (including medications and/or interventions for epilepsy, such as a ketogenic diet and vagus nerve stimulation) remained unchanged throughout. According to these trials' findings (47, 53, 54), CBD-based pharmaceutical formulations show promise as effective supplementary anticonvulsants, especially to treat refractory epilepsy (55, 56).

Cannabinoid researchers are still attempting to determine the precise effects of each cannabinoid on the human body, and their interactions with each other as well as other xenobiotics (25). Challenges in developing the evidence base for clinical prescribing have been related to products of variable quality with minimal understanding of how various cannabinoids work either individually, together (entourage effect) or with other drugs. One of the ways by which the cannabinoids have been demonstrated to exert their effects is by their direct and indirect interactions with a crucial component of the CNS, called the endocannabinoid system (ECS) (57–59). The ECS is intrinsically linked to neuromodulation, and therefore may be critical in alleviating some neuropsychiatric symptoms (44, 60).

A BRIEF INTRODUCTION TO THE ENDOCANNABINOID SYSTEM

The ECS is a major axis of the CNS, primarily responsible for modulating excitatory and inhibitory synaptic activity through the release of endogenous cannabinoids (endocannabinoids) that interacts with cannabinoid (and non-cannabinoid) receptors (61). Critical features of neural development/health and synaptic plasticity are regulated by the ECS (62). The lipid-based endocannabinoids are secreted extracellularly from the post- to the pre-synaptic site where they bind to cannabinoid receptors to initiate retrograde synaptic signalling (i.e., a negative feedback mechanism that regulates pre-synaptic activity) (63). The cannabinoid receptors, belonging to the G-protein coupled receptor (GPR) family, are found throughout the entire human body—the most well-characterised receptors being the Cannabinoid 1, Cannabinoid 2 and GPR55 receptors.

Cannabinoid 1 receptors (CB1Rs) are particularly abundant in the basal ganglial, cerebellar, cortical and hippocampal regions, with the majority of them present on axon terminals and pre-terminal axon segments (61, 64). CB2 receptors (CB2Rs) are normally expressed at much lower levels in the CNS compared to CB1Rs; this receptor is primarily present in microglia, vascular elements, immune cells and some specific neurons (61, 65). However, when the blood-brain barrier (BBB) is disrupted (by insults such as neuroinflammation), CB2R expression levels in the brain increase due to immune cells flooding the CNS (66). The majority of GPR55 receptors are aggregated in the CNS and peripheral nervous system (67, 68), where their activation on neurons can upregulate intracellular calcium release and inhibit potassium release, resulting in increased neuronal excitability (69, 70).

Activation of the cannabinoid receptors by endocannabinoids can trigger downstream signalling, such as ion channel openings, changes in intracellular calcium ion concentrations and regulation of inflammatory pathways (71). The two most well-studied endocannabinoids are N-arachidonoyl-ethanolamine (anandamide or AEA) and 2-arachidonoylglycerol (2-AG). AEA acts as a high-affinity, partial agonist of CB1R, and barely interacts with CB2R while 2-AG is a full agonist at both CBRs with low-to-moderate affinity, with both endocannabinoids being GPR55 agonists (68, 72, 73). At the end of their normal

TABLE 1 | Summarised findings of some referenced experiments/clinical trials in humans, which demonstrate the wide range of neurological disorders that CBD therapy could potentially be effective for.

References	Disorder	Experimental/clinical model	Drug dose and route	Major findings
Leweke et al. (35)	Schizophrenia	42 adult schizophrenic patients	800 mg/d, oral	Alleviation of psychotic symptoms
Heussler et al. (45)	Fragile X syndrome	20 FXS patients, aged 6–17 years	Daily 50 mg dose, twice daily 50 mg dose or twice daily 125 mg dose, transdermal	Significant reductions in anxiety and behavioural symptoms
Barchel et al. (46)	Autism Spectrum Disorder	53 children diagnosed with ASD	16 mg/kg/d (maximum of 600 mg), oral	Alleviation of some ASD comorbidity symptoms
Solowij et al. (42)	Depression	20 adult frequent cannabis users	200 mg/d, oral	Significant decrease in depressive and psychotic-like symptoms
Shannon et al. (43)	Anxiety and sleep	72 adults presenting with high anxiety or poor sleep	25 mg/d (maximum of 175 mg for 1 patient), oral	Long-term decrease in anxiety scores within the 1st month of treatment
Devinsky et al. (47)	Refractory epilepsy	120 children and young adults with Dravet syndrome and refractory seizures	5–20 mg/kg/d, oral	Reduction in convulsive-seizure frequency, but higher rates of adverse events than placebo

lifecycle, AEA is mostly degraded to arachidonic acid (AA) and ethanolamine by fatty acid amide hydrolase (FAAH) (71), while 2-AG is majorly converted to AA and glycerol by monoacylglycerol lipase (MAGL) (74). Interestingly, AEA is also a full agonist (with a different affinity than for CB1R) of a non-ECS receptor named the Transient Receptor Potential Vanilloid 1 (TRPV1) that regulates extracellular calcium ion secretion and neuronal excitability (75).

Another potential way for the ECS to affect the progression and severity of neuropsychiatric disorders is via the gut-microbiome-brain axis (76, 77). The gut-microbiome–brain axis is constituted of signalling (neural and humoral) pathways that connect the gastrointestinal system (GIS) and its microbiota to the CNS in reciprocal relationships for homeostatic and defensive maintenance of the whole body. ECS receptors, namely CB1R and TRPV1, peroxisome proliferator-activated receptor alpha (PPAR-α) and GPR119 are strongly expressed throughout the gut-brain axis (e.g., intestinal epithelial cells, myenteric and vagal fibres). These receptors affect myenteric neuron activity, vagal and sympathetic nerve function, and the release of gastrointestinal neuropeptides (such as N-acyl amides), which may subsequently have a significant impact on brain neural activity (77).

The gut microbiota produce metabolites that can interact with the ECS (78, 79). The microbes are usually categorised as either deleterious or beneficial (probiotic) to the host organism, depending on their overall effects (80). Commensal microorganism-derived molecules produce neurotransmitters (e.g., serotonin, GABA), as well as ECS-like mediators that are capable of interacting with host ECS receptors; for example, commendamide is analogous to the human signalling molecules N-acyl amides and interacts with ECS GPRs (81). Currently, the exact effects of these ligands are still mostly unknown, but their existence strongly hint at complex layers of interaction between the gut-brain axis and gut microbiota (78).

Components of the ECS can thus strongly modulate behaviour and mood via interactions with underlying neurotransmission and the gut-microbiome-brain axis.

THE ROLE OF THE ENDOCANNABINOID SYSTEM IN REGULATING ANXIETY

Anxiety is usually manifested in affected individuals as disproportionate startle response, avoidance behaviour, autonomic hyperactivity, increased muscular tension and reduced motion (66). Anxiety is primarily mediated by glutamatergic (excitatory, i.e., increase likelihood of action potentials), serotoninergic and GABAergic (inhibitory, i.e., decrease likelihood of action potentials) pathways. GABA is the main inhibitory neurotransmitter, widespread throughout the cortex and counters the excitatory activity of glutamatergic neurons (82). Excessive anxiety as experienced by patients with anxiety disorders is theorised to be caused by an imbalance between excitatory and inhibitory signalling. Consequently, such an imbalance may lead to cortical hyper-reactivity and behavioural hypersensitivity in ASD. Puts et al. (83) and Sapey-Triomphe et al. (84) found that cortical GABA levels appear to be reduced in children and adults with ASD, respectively, in comparison to those of neurotypical controls (83, 84). However, Kolodny et al. (85) recently reported no differences in cortical concentrations of GABA and glutamate between neurotypical and ASD young adults (85). This discrepancy in findings could be attributed to low participant numbers and small differences in experimental methodologies. The proper functioning of the ECS is also disrupted in FXS. The loss of Fragile X mental retardation protein (which regulates the translation and transport of messenger RNAs in brain neuron dendrites) in FXS seems to impair the glutamate receptor-5

(mGluR5)-dependent 2-AG signalling at excitatory synapses (86). Additionally, administration of AEA in a mice model of FXS (FMR1 knockout mice) reduced social anxiety (87), suggesting a detrimental downregulation of AEA in FXS.

Functional CB1Rs and CB2Rs expressed (88, 89) in GABAergic, dopaminergic, glutamatergic, and serotoninergic neurons (90–93), could be crucial in regulating behavioural and emotional states (88, 89), which are heavily disrupted in psychiatric/mood disorders. CB1Rs, in particular, are highly expressed on GABAergic interneurons (90, 94), on glutamatergic terminals (90, 92) and on dopamine D1 receptor positive neurons (95). Agonism of CB1Rs can inhibit the secretion of GABA and glutamate from presynaptic terminals (96–99), which indicate that endocannabinoid activation of CB1R can influence the type of synaptic signalling. AEA-mediated TRPV1 activation is linked to an anxiogenic response, as opposed to the anxiolytic response elicited by AEA-mediated CB1R activation. This suggests that there might be an imbalance between CB1R and TRPV1 expression that might play a part in instilling excessive anxiety (100). Inhibition of FAAH by selective inhibitor URB597 was reported to activate serotoninergic neurons in the midbrain of stressed rats, by the associated increase in AEA-mediated signalling at CB1R (101). Inhibition of FAAH and MAGL by selective inhibitors produced anxiolytic effects in CB1R-deficient mice, but not in CB2R-deficient mice, suggesting that CB2R could play a role in regulating anxiety (102). Additionally, CB2R might play a role in regulating anxiety as augmented activation of CB2R by accumulation of 2-AG (via inhibition of MAGL) was found to exert anxiolytic effects in a rat model of stress (103).

From the evidence gathered so far, therapeutic modulation of synaptic signalling and plasticity could indeed be feasible by regulation of the ECS. Moreover, a well-regulated ECS is critical in ensuring good neural health and function as distressed neural cells can lead to further neurological issues such as epilepsy (104).

HOW THE ECS COULD BE INVOLVED IN NEUROINFLAMMATION AND EPILEPSY

The ECS is an important signalling axis for inflammatory pathways throughout the body. Many children affected by ASD, FXS, and 22QS suffer from epileptic/non-epileptic seizures that stem from detrimental mutations responsible for their disorders (5, 7, 105–107). 10–30% of people with ASD have comorbid epilepsy and several synaptic plasticity pathways appear to be involved in both disorders (105). As such, affected children are at increased risk of serious seizure-related accidents and have their neurodevelopment further impaired by frequent seizures (108). In recent years, epilepsy has been surmised to be strongly correlated with neuroinflammation (104, 109). Additionally, abnormally high levels of neuroinflammation have been associated with ASD (110); Vargas et al. (110) and Jyonouchi et al. (111) found higher levels of proinflammatory cytokines (e.g., tumour growth factor–β1) in the brain tissue, cerebrospinal fluid and peripheral blood of ASD patients (including children) (110, 111).

Neuroinflammation is the term given to a set of defensive responses to insult and/or injury in the neural environment that is mainly mediated by glial cells. The resident immune cells of the CNS, the microglia, primarily function in protecting the neuronal population; they are called into action by inflammatory stimuli such as foreign bodies, products from injured/inflamed neurons, blood-brain barrier disruptions, and by chemokines/cytokines [e.g., Interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α)] (112–114). Neuroinflammation is a protective physiological process but can be harmful when it is excessive and unregulated (115). Multiple parts of the ECS are involved in inflammatory pathways. Moreover, microglia express many components of the ECS (such as CB1R, CB2R and GPR55), via which they communicate with neurons via expression of endocannabinoids (116, 117). There is evidence of microglial involvement in ASD from both brain tissue immunohistochemistry and positron-emission tomography (PET)-imaging studies which revealed increased neuroinflammation and population of activated microglia in brains of ASD patients (118, 119) compared to non-ASD individuals. Therefore, artificially modulating microglial endocannabinoid signalling and treating neuroinflammation could potentially alleviate some ASDsymptoms (117).

Agonism of CB1R and CB2R have shown anti-inflammatory effects in human and animal models (120–123). Antagonism/non-expression of GPR55 also resulted in a reduction in neuronal and microglial inflammation (116, 124, 125). However, agonism of GPR55 in animal and human neural stem cells was found to elicit a neuroprotective effect and rescued neurogenesis after inflammatory insult (126). Additionally, activation of microglial GPR55 by the endogenous ligand l-α-lysophosphatidylinositol limited neuronal damage in rats (127). As Hill et al. suggest, the actions of GPR55 probably strongly depend on the cell type and cause of inflammation (126). Cyclooxygenase enzymes (COX) synthesise signalling intermediaries known as prostanoids, often derived from AA. The constitutive isoform of COX, COX-1, found in numerous cell types, regulates physiological responses, while the inducible isoform, COX-2, is induced rapidly in several cell types (including neurons and glial cells) after biochemical stimuli, such as cytokines and pro-inflammatory molecules (128). COX-2 is involved in the conversion of a minor proportion of AEA and 2-AG to prostaglandin ethanolamides (PG-EAs) (74) and prostaglandin glycerol esters (PG-Gs) (129), respectively—both of which can contribute to inflammatory responses (128). Other prostaglandins derived from AA by COX-1 and COX-2, prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), have neurotoxic properties (125, 130, 131). Suppression of MAGL activity (which leads to a downregulation in AA synthesis) has shown neuroprotective effects in mice (132). COX-2 levels have been found to be greatly increased in the brains of patients with epilepsy, compared to non-epileptic patients (133) and in animals that experience prolonged seizures (134), suggesting a relationship between epilepsy and neuroinflammation.

The Cytochrome P450 (CYP) family is another group of enzymes that breaks down endocannabinoids. The ubiquitous CYP enzymes are expressed at different levels across the

body, with variations across species and amongst individuals. The CYP enzymes are known for their ability to metabolise xenobiotics, with the metabolites sometimes causing side-effects (135). Changes in CYP activity can influence downstream endocannabinoid signalling pathways by virtue of changes in substrate and metabolite concentrations. CYP3A4, expressed in the human brain (136, 137), derives anti-inflammatory epoxyeicosatrienoic acids (EETs) and pro-inflammatory hydroxyeicosatetraenoic acids (HETEs) from AA (138–141). AEA can be broken down by CYP enzymes (namely CYP3A4, CYP2C19, CYP2D6, and CYP2J2) into EET-ethanolamides (EET-EAs) and HETE-ethanolamides (HETE-EAs) (142–144). Just like their precursor molecules, the EET-EAs and HETE-EAs can bind to CB1Rs and CB2Rs, albeit with different affinities, e.g., 5,6-EET-EA binds much more strongly with CB2R than AEA (145) while 20-HETE-EA and 14,15-EET-EA have only a weak affinity for CB1R (146) in murine models. CYP2J2 breaks down 2-AG to create two products, 2-11,12-epoxyeicosatrienoic glycerol (EET-G), and 2-14,15-EET-G (147), which interact strongly with both CBRs (especially CB1R) (148). Many CYP metabolites therefore are potentially endogenous ligands for some of the ECS receptors and could subsequently be involved in inflammation regulation.

The lipoxygenase (LOX) enzyme pathway is another metabolic route for endocannabinoids and other related fatty acids (149). The LOX pathway starts with the change of AA into leukotriene A4 by the 5-LOX enzyme (expressed on cell types such as neurons). Leukotriene A4 (LTA4) is rapidly catalysed into LTB4 and cysteinyl leukotrienes (i.e., Cys-LTs, which comprises LTC4, LTD4 and LTE4) (149, 150). LTD4 has been linked to blood-brain barrier dysfunction (151), a contributing factor of neuroinflammation (152), as evidenced by exposure of microglial Cys-LT1 and Cys-LT2 receptors to LTD4 resulting in microglial secretion of pro-inflammatory IL-1β in mice (153). In brief, the ongoing research on eicosanoids (collective term for the endocannabinoids and the many metabolites of the ECS) indicates that the ECS is thoroughly implicated in regulation of neuronal activity and neuroinflammation. But until the signalling pathways involved are thoroughly investigated, particularly in the human brain, how neuroinflammation is exactly linked to ECS dysfunction and psychiatric impairments remains to be elucidated. Interestingly, inflammation in the GIS could substantially affect the gut-microbiome-brain axis and subsequent neuronal activity as ASD individuals have been reported to suffer from gastrointestinal issues (such as diarrhoea and constipation) (154–157) and dysbiotic microbiota compared to neurotypical individuals (158). Perturbations in gut microbial diversity has been found to influence neuroinflammation (159) as some gut microbes can secrete pro-inflammatory metabolites and cytokines (160) that cross the blood-brain barrier. CB1R, TRPV1 and PPAR-α can modulate the permeability of the gut-vascular barrier that prevents the entry of intestinal bacteria into the bloodstream; if the GVB's selective permeability is compromised, the bacteria themselves can enter the bloodstream and cross the BBB, causing an inflammatory response (161).

In summary, there is little doubt that the ECS is likely to be central in the aetiology and occurrence of neuropathology, whereby the modulation of the ECS at multiple points by extraneous agents such as cannabinoids could achieve beneficent outcomes.

CANNABINOIDS INTERACT WITH THE ECS AND NEUROTRANSMISSION

The cannabinoids' interactions with multiple receptors and enzymes can be safely assumed to hold the key to their wide-ranging therapeutical benefits, but can also obscure the exact mechanisms of their effects. THC is a partial agonist of CB1Rs and CB2Rs, and an agonist of GPR55. On the other hand, CBD's antagonistic/negative allosteric modulating actions on the CB1 and CB2 receptors (57, 162–164) might help explain how CBD can dampen THC's psychoactivity (165) (**Figure 1**).

While CBD might not interact strongly with CB1R and CB2R when administered at therapeutical levels (166), it has been reported to regulate calcium ion homeostasis in neurons (167) and increase inhibitory neurotransmission via interactions with GPR55 (164). CBD therapy has been correlated with an increase in AEA blood levels and a reduction in the psychotic symptoms of treated schizophrenic patients vs. placebo-control patients (35); the mechanism behind CBD's beneficial effect in this instance could be due to an increase in AEA levels found to be lower in the cerebrospinal fluid of epileptic patients (168) and in the blood of ASD children (169, 170). Of note, the mechanism by which CBD increases AEA levels seems to differ between species; Elmes et al. reported that, in humans, this effect may be due to CBD binding preferentially to the fatty acid binding proteins on which AEA depends to be transported into cells for FAAH catalysis rather than the CBD-induced FAAH inhibition observed in rodents (171). This interaction between CBD and AEA metabolism in humans vs. rodents (171, 172), highlights that the differences in xenobiotics metabolism between species can limit the utility of animal models in cannabinoid research.

In animal models of ASD, an increase in AEA concentration has been correlated with improvements in social interactions. AEA can interact with oxytocin, a neuropeptide that promotes parental and social bonding. Indeed, recent evidence has demonstrated that oxytocin stimulates AEA release in the nucleus accumbens, a key region for the reinforcing properties of natural rewards, with AEA-mediated signalling a requirement for the pro-social effects of this neuropeptide (173). A model of defective oxytocin-driven AEA signalling in ASD could therefore explain how CBD intake ameliorates social interactions in ASD patients (3, 46). Upregulated *Magl* gene (gene that encodes for the MAGL enzyme) expression has been observed in rat hypothalami treated with 10 mg/kg THC (174), supporting the hypothesis that cannabinoids can modulate cerebral endocannabinoid tone. Cannabinoids, like CBD, have been found to inhibit COX-2 activity and hence reduce the production of pro-inflammatory prostaglandins, which could be an additional pathway by which cannabinoids increase the levels of the endocannabinoids, triggering an indirect anti-inflammatory and

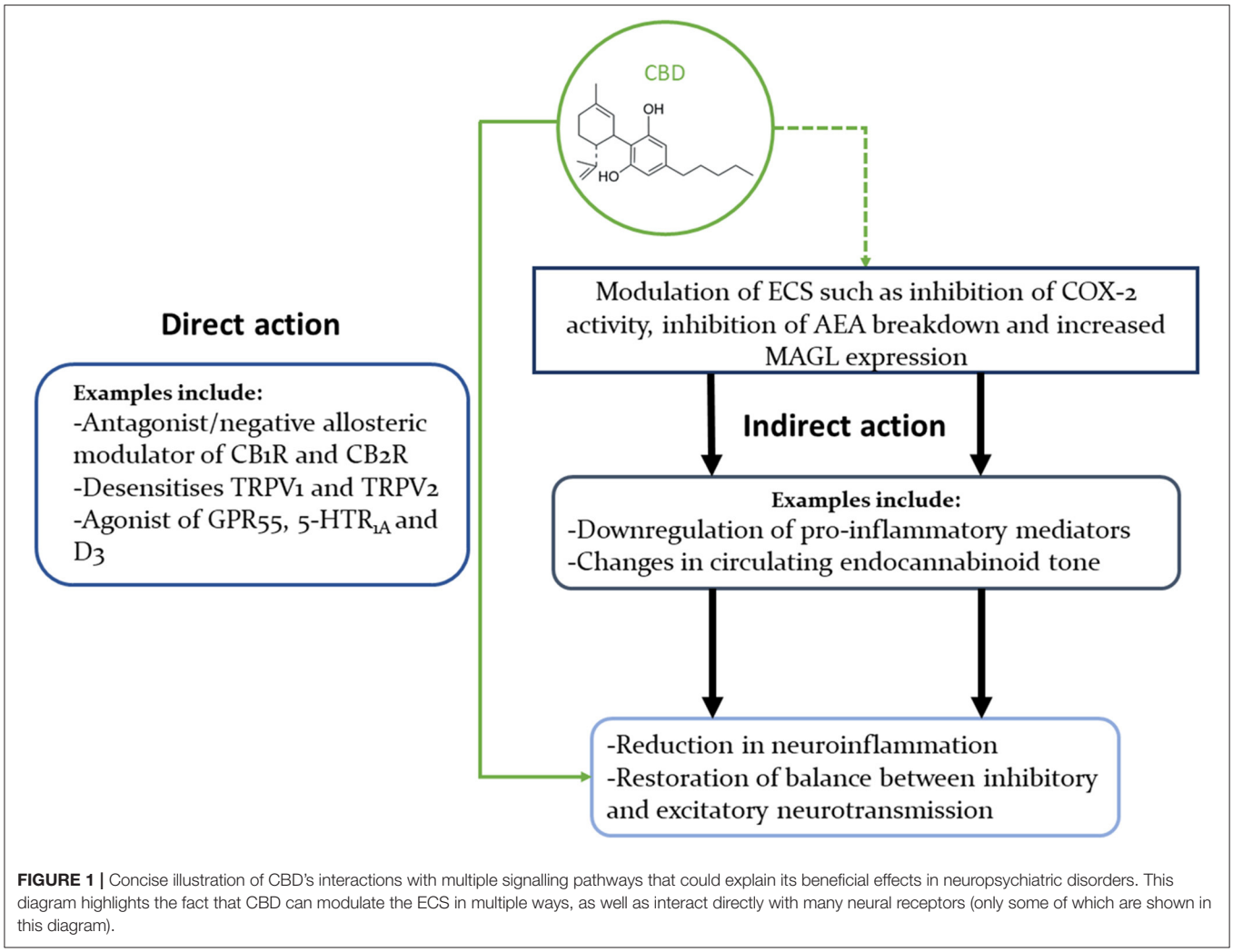

FIGURE 1 | Concise illustration of CBD's interactions with multiple signalling pathways that could explain its beneficial effects in neuropsychiatric disorders. This diagram highlights the fact that CBD can modulate the ECS in multiple ways, as well as interact directly with many neural receptors (only some of which are shown in this diagram).

anti-epileptic activity (175, 176). CBD's inhibition of cerebral CYP isoenzymes could, in turn, modulate the levels of EETs, EET-EAs and HETE-EAs. Therefore, even though CBD may not have a high affinity for CB1R, CB2R, and GPR55, the activation of these endocannabinoid receptors may be indirectly affected by CBD's upregulation/downregulation of endocannabinoids and eicosanoids (136); for example, Bornheim et al. found that CBD inhibited the CYP-driven formation of some AEA metabolites in mice (177) while Arnold et al. reported that THC and CBD inhibited the production of EET-EAs by cardiac CYP2J2 (178). Additionally, the activity and metabolite synthesis of 5-LOX was reduced in human tumour cells treated with CBD (179). Targeted inhibition of Cys-LT synthesis significantly attenuated seizures in treated mice (compared to untreated mice) (180, 181) and in epileptic patients (182), so CBD's inhibition of 5-LOX could have an anti-inflammatory effect.

Intriguingly, CBD has been shown to desensitise non-cannabinoid TRPV1s (75) and related TRPV2s, hence blocking the release of calcium ions outside cells and dampening hyperexcitability (contributor to aberrant neuronal activity) in neurons, suggesting another potential regulatory mechanism (172, 183). CBD has been reported to enhance microglial phagocytosis in rodent microglia partially via the activation of TRPV1 and probably TRPV2 receptor channel of the microglial cells (112); however, Hassan et al. cautioned that increasing microglial phagocytosis might not be a positive strategy for combating neuroinflammation, but their results might not be applicable to human physiology.

As we highlighted beforehand, the cannabinoids may indeed exert their effects differently between species. Another case of CBD's promiscuous interactions is its agonistic actions on the serotonin (5-hydroxytryptamine-1A) receptors (5-HTR_{1A}), which are deeply involved in activating anxiolytic responses and in neuronal electrochemical activity (36, 184, 185). In healthy and ASD human adults, CBD suppressed the activity of excitatory glutamatergic neurons in the prefrontal cortex via activation of 5-HTR_{1A} (186), which could contribute to restoring the balance between inhibitory and excitatory neurotransmission. Additionally, CBD inhibits the equilibrative nucleoside transporter (ENT1) responsible for the synaptic uptake of

adenosine, thereby increasing levels of extracellular adenosine. Consequently, an upregulation in extracellular adenosine can cascade into a decrease in neuronal hyperexcitability (187–189). CBD has anti-oxidative and anti-inflammatory properties that could counter neuroinflammation; modulation of TRPV1, CB2R, and GPR55 receptors can lead to downregulation of enzymes involved in the production of pro-inflammatory PGs, reactive oxygen species, and cytokines (190, 191). Another potential avenue for CBD's anti-inflammatory action could be its inhibition of voltage-dependent anion selective channel protein 1 (VDAC1) conductance, leading to a decrease in neuroinflammation (192). CBD was also found to enhance the inhibitory γ-Aminobutyric acid (GABA)'s activation of its associated $GABA_A$ receptors which regulate inhibitory neurotransmission (193) and are targeted by drugs such as clobazam; indeed, co-administration of CBD with clobazam significantly increased the inhibitory effects of GABA compared to either compound alone (194). Additionally, CBD's amplifying effects on GABA receptors could compensate for the reduced GABAergic transmission observed in FXS (195).

Lower levels of AEA (35) and higher expression/reduced methylation of *CNR1* (the gene coding for CB1R) (196, 197) in schizophrenic patients strongly suggest a pathological link with ECS dysfunction; CBD might compensate for this dysfunction by indirectly modulating endocannabinoid levels. Additionally, CBD is a partial agonist to dopamine D3 receptor, whose expression was demonstrated to be altered in the methylazoxymethanol acetate (MAM) murine neurodevelopmental model (198). Gestational MAM treatment of pregnant dams is a validated model that produces murine offspring with adult phenotype typical of schizophrenia, such as cognitive deficits, dopaminergic dysfunction, physical and behavioural abnormalities (196, 198, 199). Another murine model that mimics the development of the human schizophrenia phenotype is perinatal THC exposure of neonates as it results in similar neurodevelopmental impairments; the cognitive and social deficits were then demonstrated to be reversed by peripubertal CBD treatment (197). These experimental results reinforce the notion that early childhood treatment with CBD might be sufficient to minimise the impact of neurodevelopmental disorders into adulthood.

CBD's interactions with the GIS ECS might depend on the mode of administration; oral intake of CBD is subject to first-pass metabolism, which can result in most of the CBD being transformed by liver enzymes into its metabolites prior to reaching the gut (200). Conversely, more direct passage of CBD in circulating blood via dermal application or inhalation would hypothetically reduce CBD's availability to the GIS. Research on CBD's effects on the gut microbiome and gut ECS are few and limited to animal model studies (generally germ-free mice) (201), but CBD's anti-inflammatory properties could be potentially involved in counteracting gut cell inflammation, gut-vascular barrier leakage and subsequent neuroinflammation by dysbiotic gut microbes (202, 203).

CONCLUSION

Our review has hopefully shown that there is a strong body of evidence that early cannabinoid treatment may offer significant potential to safely alleviate many of the common symptoms affecting children with neurodevelopmental disorders. Continued research and evidence in establishing definite relationships between cannabinoid intake and alterations of the ECS are needed to determine clear risk-benefit profiles and to screen for potential individuals in whom benefit could be predicted. CBD is currently the most promising therapeutic cannabinoid for children due to its safety profile and broad-spectrum action. A fuller understanding of CBD's metabolism in the human body (especially how it might interact with the GIS and microbiota) and mechanisms of action could result in greater optimisation of cannabinoid delivery and better development of synthetic cannabinoid analogues.

AUTHOR CONTRIBUTIONS

KKC and HH contributed to the conceptualisation and writing of the article. MM the primary supervisor of KKC's research project, also reviewed, and contributed to the manuscript. All authors contributed to the article and approved the submitted version.

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The remaining 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.