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Healing autism spectrum disorder with cannabinoids: a neuroinflammatory story.

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Highlights

- Autism spectrum disorder has a multifactorial and complex etiology
- Changes in the endocannabinoid system are found in autistic patients
- Neuroinflammation is detected in autistic patients
- The endocannabinoid system has a key role in neuroinflammation
- Future therapies exploiting cannabinoid drugs

Abstract

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder with a multifactorial etiology. Latest researches are raising the hypothesis of a link between the onset of the main behavioral symptoms of ASD and the chronic neuroinflammatory condition of the autistic brain; increasing evidence of this connection is shedding light on new possible players in the pathogenesis of ASD. The endocannabinoid system (ECS) has a key role in neurodevelopment as well as in normal inflammatory responses and it is not surprising that many preclinical and clinical studies account for alterations of the endocannabinoid signaling in ASD.

These findings lay the foundation for a better understanding of the neurochemical mechanisms underlying ASD and for new therapeutic attempts aimed at exploiting the renowned anti-inflammatory properties of cannabinoids to treat pathologies encompassed in the autistic spectrum.

This review discusses the current preclinical and clinical evidence supporting a key role of the ECS in the neuroinflammatory state that characterizes ASD, providing hints to identify new biomarkers in ASD and promising therapies for the future.

Keywords

Autism spectrum disorder, neuroinflammation, microglia, endocannabinoid system, cannabinoids, cannabidiol.

Index

1. Introduction

The 5th Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) defines autism spectrum disorder (ASD) as a neurodevelopmental disorder characterized by persistent deficits in social communication and interaction and restricted-repetitive patterns of behavior, interests or activities. These symptoms typically occur at early childhood and produce clinically significant developmental impairment (American Psychiatric Association, 2013). Although it is among the most severe chronic childhood disorders, no effective treatment for ASD is yet available and its complex etiology is still being investigated. For instance, multiple factors are thought to be involved in the pathogenesis of the disease (i.e. both genetic and environmental factors and their interaction) thus explaining the heterogeneity of symptoms displayed by autistic patients (Jeste and Geschwind, 2014).

In recent years, clinical and preclinical studies have pointed to a strong inflammatory state associated with ASD (Prata et al., 2019; Rossignol and Frye, 2012; Siniscalco et al., 2018) which correlates with immune system dysfunctions (Estes and McAllister, 2015; Onore et al., 2012). For instance, increasing evidence about ASD as a non-classical immune-mediated disorder has been accumulating (Cristiano et al., 2018; Jiang et al., 2018; Siniscalco et al., 2018): by regulating innate and adaptive immunity, the immune system impacts neural development, cognitive functions and behavioral traits contributing to ASD pathogenesis (Dipasquale et al., 2017; Estes and McAllister, 2015; Filiano et al., 2015; Mead and Ashwood, 2015; Pardo and Eberhart, 2007). From fetal development to adulthood, the immune system reciprocally interacts with the central nervous system (CNS), influencing both peripheral and local CNS immune function (Meltzer and Van de Water, 2017). In ASD patients, an excessive neuroinflammation has been reported leading to brain underconnectivity (Rodriguez and Kern, 2011) as indicated, among others, by an augmentation of activated microglia and astrocytes (Koyama and Ikegaya, 2015; Patel et al., 2016; Vargas et al., 2005) and proinflammatory cytokines (Li et al., 2009; Tsilioni et al., 2019; Zimmerman et al., 2005) in autistic patients. Interestingly, the recent demonstration that

microglia, the resident immune cells of the CNS, contribute not only to inflammatory events but also to neural development (Delpech et al., 2015; Lenz and Nelson, 2018; Schafer and Stevens, 2015), has raised new hypotheses regarding a microglial role in the etiology of ASD.

Besides human evidence, research performed in animal models also suggests that neuroinflammation plays a fundamental role in the pathogenesis of ASD. Notably, animal studies highlighted that deficits in microglia activity during brain development negatively influence the formation of mature synapses, leading to an increase of immature synapses that could account for cognitive and ASD-like behavioral deficits (Paolicelli et al., 2011; Zhan et al., 2014). Therefore, in addition to genetic risk factors for inflammation, environmental factors leading to neuroinflammation, such as perturbation in microglial activity, are receiving scrutiny in the etiology of autism.

The endocannabinoid system (ECS) plays a key role in this scenario: it is a unique neuromodulatory system in mammalian physiology and it consists of cannabinoid receptors (CB1, discovered as a neuronal target of the psychoactive compound of the plant *Cannabis sativa*, Δ9-tetrahydrocannabinol (THC), and CB2), their endogenous lipid ligands i.e. the endocannabinoids (eCBs), including anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and the enzymes for ligand synthesis and degradation (Di Marzo et al., 2004; Piomelli, 2003). Notably, the ECS represents a link between the immune system and the CNS (Cabral et al., 2015). CB2 receptors are primarily located on immune system cells and serve as immune system modulators (Cabral and Griffin-Thomas, 2009), while CB1 receptors are located in the CNS (particularly in the hippocampus, cerebral cortex, basal ganglia, and cerebellum), peripheral nervous system (PNS) and peripheral organs (Zou and Kumar, 2018). Interestingly, cannabidiol (CBD), an abundant bioactive but non-psychotomimetic constituent of the plant *Cannabis sativa*, has emerged as a promising strategy to treat inflammation induced by microglial hyperactivation (Martin-Moreno et al., 2011; Mechoulam et al., 2007; Saito et al., 2012) and to attenuate oxidative and nitrosative stress in different animal models of neuroinflammatory diseases (Booz, 2011; Cassol et al., 2010;

Ligresti et al., 2016; Mukhopadhyay et al., 2011; Ruiz-Valdepenas et al., 2011), thus opening a novel window of opportunity to treat inflammatory and autoimmune disorders with cannabinoids.

Recently, highly purified CBD (approved in 2018 as Epidiolex® in the United States and more recently also by the European Medicines Agency) has demonstrated efficacy with an acceptable safety profile in patients with two epilepsy syndromes associated with ASD, i.e. Dravet Syndrome and Lennox-Gastaut Syndrome (Devinsky et al., 2019; Lattanzi et al., 2018). Moreover, cannabidivarin (CBDV), another non-psychoactive phytocannabinoid, is under clinical trial with a primary aim of studying its therapeutic effects in children with ASD. This evidence highlights the increasing interest in cannabinoids as a treatment for the core symptoms and co-morbidities of ASD. However, our understanding of the role of the ECS in ASD and the possible mechanisms involved, especially in relation to neuroinflammation, is still far from complete and the use of cannabinoids for children and adolescents suffering from ASD still remains controversial.

In this review, we will focus on the immunological hypotheses for ASD pathogenesis and provide a comprehensive study of the current scientific evidence about the interaction between neuroinflammation, cannabinoids and ASD.

2. Diagnosis of ASD

2.1 The DSM-5 Criteria of ASD diagnosis

ASD is a complex neurodevelopmental disorder that affects communication and behavior, particularly in the social domain. Symptoms generally appear in the first two years of life and are characterized by a wide variation in clinical presentation and severity. The DSM-5 presented an important innovation compared to the 4th Edition (DSM IV): a singular ASD diagnostic category replaced the previously distinct categories such as the autistic disorder, the Asperger syndrome, the pervasive developmental disorder-not otherwise specified (PDD-NOS), and the childhood disintegrative disorder. Revisiting diagnostic criteria for ASD has represented an important step forward in health advice and support especially because it is now clear that a timely and correct diagnosis together with early and individualized treatment, can help children with ASD to learn new skills, to make the most of their strengths and to improve their own and parent's quality of life.

Overall, the DSM-5 Autism Diagnostic Criteria include social/communicative deficits (criterion A) and restricted, repetitive patterns of behaviors, interests, or activities (criterion B). These symptoms are present since early childhood (criterion C) and limit or impair everyday life (criterion D) (American Psychiatric Association, 2013). Specifically, persistent deficits in social communication and social interaction vary across multiple contexts (criterion A): these deficits include social-emotional reciprocity, ranging from abnormal social approach and failure of normal back-and-forth conversation to reduced sharing of interests, emotions, or affect and failure to initiate or respond to social interactions. Moreover, people with ASD show deficits in nonverbal social communicative behaviors, including abnormalities in eye contact and body language or deficits in understanding and use of gestures related to nonverbal communication. Deficits in developing, maintaining and understanding relationships can manifest in the absence of interest in peers.

The restricted, repetitive patterns of behaviors and activities result in functional limitations in effective communication, social participation, social relationships, academic achievement, or occupational performance, individually or in combination (criterion B). The onset of ASD symptoms is in the early developmental period (criterion C), but deficits may not become fully manifest until social communication demands exceed limited capacities.

At this period, diagnosis is often a two-stage process that varies from a first general developmental screening during well-child checkups to an additional evaluation on the general cognitive level. For instance, thinking skills, language and age-adaptive abilities (i.e. those needed to complete daily activities independently, such as eating, dressing, and toileting) represent a first screening to be carefully evaluated by physicians. However, ASD is a complex disorder that frequently include persistent comorbid conditions; therefore, the comprehensive evaluation may not be simple and require additional medical outcomes including blood and hearing tests.

Despite the onset of ASD symptoms is usually in the early development, diagnosis may also occur later in life: thus, in older children and adolescents, ASD diagnosis is often made during the scholar period when subtle communication problems (i.e., understanding tone of voice, facial expression, body language, etc.) become evident together with troubles forming friendship with peers. Finally, diagnosing ASD in adults is often more difficult than diagnosing ASD in children. In adults, some ASD symptoms can overlap with symptoms of other mental health disorders, such as anxiety, psychosis or attention-deficit/hyperactivity disorder (ADHD), making it difficult to find the optimal therapeutic strategy (Lau-Zhu et al., 2019).

2.2 Inflammation in ASD: a biomarker analysis for a proper diagnosis

Two of the prominent clinical features of ASD are inflammation and neuroimmune system dysregulation (Onore et al., 2012; Siniscalco et al., 2013a; Xu et al., 2015) (detailed in chapter 4 - “Neuroinflammation in ASD”). Interestingly, enhanced inflammatory activity in ASD children has been demonstrated through pro-inflammatory biomarkers analysis (Brocker et al., 2010). For

instance, in the population-based case-control Childhood Autism Risks from Genetics and Environment (CHARGE) study, an over-production of pro-inflammatory cytokines was found in the plasma of 2–5 year-old autistic children compared with age-matched typically developing (TD) children and children with other developmental disorders (Ashwood et al., 2011). These results were supported by in vitro studies utilizing cultured and stimulated peripheral blood monocytes from ASD children which revealed an elevation of pro-inflammatory cytokines (Enstrom et al., 2010). Increased pro-inflammatory Th-2 cytokines were also found in peripheral blood mononuclear cells (PBMC) of 3–10 year-old children suffering from ASD compared to age-matched normally developing children (Molloy et al., 2006). However, it should be taken into account that part of the autistic children enrolled in these studies was prescribed psychotropic drugs (e.g., lithium, benzodiazepines, and the atypical antipsychotic clozapine) for controlling their ASD-related symptoms. Since these drugs can increase cytokine levels, this should be considered as a limitation of the causal-effect relationship.

Nevertheless, an emerging human diagnostic marker for ASD is represented by the detection of increased interleukin (IL)-6 concentrations in the umbilical cord plasma, as well as elevations in several other cytokines (Madsen-Bouterse et al., 2010). Indeed, most recently, elevated plasma levels of many pro-inflammatory cytokines and chemokines such as IL-1β, IL-6, IL-17, IL-12p40 and IL-12p70 and tumor necrosis factor-α (TNF-α) were found in 3–9 year-old children with a clinical diagnosis of ASD as compared to age-matched normally developing children (Inga Jacome et al., 2016; Xie et al., 2017). Interestingly, plasma cytokine profiles were correlated with severity of ASD symptoms (according to the Childhood Autism Rating Scale (CARS) test) and consequently, predictive of different ASD phenotypes: thus, higher levels of IL-12p40 were found in patients with mild disease severity and higher levels of TNF-α in patients with moderate severity (Inga Jacome et al., 2016; Xie et al., 2017), overall pointing to cytokines as important biomarkers in ASD. Moreover, TNF-α, IL-6 and IL-17 were significantly up-regulated in male but not female ASD patients compared to age- and sex-matched healthy controls, whereas IL-2

expression was down-regulated (Eftekharian et al., 2018). This sex-dependent evidence represents a very promising field of investigation especially for making a differential diagnosis, since boys are four times more likely to be diagnosed with autism than girls (Prata et al., 2017).

The inflammatory state associated with ASD is also reflected in the CNS as brain inflammation (Cristiano et al., 2018; Theoharides et al., 2013): human post-mortem samples, as well as animal models, suggest an over-production and subsequent increased levels of IL-6 in the autistic brain, where this cytokine may mediate neuro-anatomical abnormalities (Wei et al., 2013), especially by participating to both excitatory and inhibitory synaptic formation (Wei et al., 2012). Additionally, aberrant expression of many pro-inflammatory cytokines and chemokines, such as IL-1, IL-6, IL-8 and IL-12, as well as macrophage migration inhibitory factor (MIF) and platelet derived growth factor (PDGF) has been demonstrated in peripheral blood, cerebrospinal fluid (CSF) or brain tissues of ASD patients (Dipasquale et al., 2017; Pardo et al., 2017). Therefore, neuroinflammation, driven by increased production of pro-inflammatory cytokines, could be targeted as an important mechanism in the pathophysiology of ASD (Alabdali et al., 2014; El-Ansary and Al-Ayadhi, 2012; Prata et al., 2017) and the use of cytokine biomarkers could be useful to identify children at risk for ASD or ASD-associated comorbid conditions, thus helping to predict the developmental course of the disorder.

2.3 Implication of the maternal immune system during pregnancy in ASD

Several hypotheses have been proposed to explain ASD-related inflammation; among them, the dysregulation of the maternal immune system during pregnancy has raised attention as an important factor participating in the development of ASD (Croen et al., 2008). During fetal development, the activation of the maternal immune system may lead to changes in neural development representing an important risk factor for ASD (Jiang et al., 2016; Knuesel et al., 2014): thus, IgG antibodies derived from the mother enter the fetal compartment by freely crossing the placenta due to an incomplete fetal blood-brain barrier (BBB); these antibodies

recognize self-proteins and interfere with fetal development (Braunschweig and Van de Water, 2012; Brimberg et al., 2013; Croen et al., 2008). Interestingly, IgG reactivity against fetal brain proteins was found in plasma from some mothers whose children received a diagnosis of ASD later in life, but not in mothers of normally developing children (Braunschweig et al., 2008). Based on this evidence and on the fact that children with the presence of autoantibodies showed cognitive deficits and increased aberrant behaviors (e.g. irritability, social withdrawal, stereotypic behavior, hyperactivity/noncompliance and inappropriate speech) (Goines et al., 2011), it has been proposed that maternal autoantibodies could be used as useful markers for ASD diagnosis (Braunschweig et al., 2013), at least for specific ASD behavioral endophenotypes (Abou-Donia et al., 2019). However, additional studies targeting multiple sample populations are needed to determine the predictive ability of the maternal autoantibodies for determining ASD risk.

Since the early ’70s, it was discovered that viral infections during pregnancy (Chess, 1971; Deykin and MacMahon, 1979; Ghaziuddin et al., 1992; Yamashita et al., 2003; Zerbo et al., 2015) can create an inflammatory immune environment and trigger the production of maternal cytokines and chemokines, which can exert aberrant effects on fetus development by crossing the placenta and entering in the fetal compartment (Dipasquale et al., 2017; Meltzer and Van de Water, 2017).

Studies in laboratory animals corroborated this evidence demonstrating that environmental factors triggering maternal immune system activation (MIA) can induce phenotypes that resemble ASD or co-morbid conditions (Bauman et al., 2014; Careaga et al., 2017; Malkova et al., 2012; Weber-Stadlbauer et al., 2017).

Despite this evidence, understanding the mechanisms by which MIA and the subsequent neuroinflammatory response contribute to impaired brain development and to the onset of ASD still deserves further investigations.

2.4 The role of the Gut-Brain axis

The gut-brain axis is a complex path of communication that connects the sensory signals from the gastrointestinal tract to those of the CNS by involving the microbiota (Dinan and Cryan, 2017; Sherwin et al., 2019). This bidirectional communication between the brain and the gastrointestinal tract is based on a complex system, which includes the vagus nerve, but also the enteric and immune systems, or the direct effects of some microbiota metabolites. Research focusing on the interaction between microbiota and brain development has revealed that intestinal microbial colonization influences the brain development of mammals, since early life, by modulating essential behavioral and functional outcomes (Wang et al., 2018b). Therefore, it is possible that genetic and environmental factors could alter the composition of the normal intestinal microbiota, predisposing children to both acute and chronic inflammation up to the onset of neuropsychiatric disorders, including ASD (Ristori et al., 2019).

The hypothesis of an alteration of the microbiota composition in ASD is supported by clinical evidence reporting that half of the autistic patients present gastrointestinal problems with altered permeability of the intestinal barrier (Fowlie et al., 2018) and gut microbiota dysbiosis (Doenyas, 2018). Interestingly, it has been observed that ASD children are characterized by a strong food selectivity (they are called “picky eaters”) that consequently influences the nutritional quality of their diet and, as a consequence, their gut microbiota composition. For instance, intestinal barrier defects predispose ASD patients to be sensitized by environmental antigens (Navarro et al., 2015) and ASD-associated gastrointestinal symptoms could be a manifestation of an underlying inflammatory process (de Magistris et al., 2010; Navarro et al., 2016). In this context, it has been reported that the abnormal immune system in autistic subjects could be due to gluten/casein derived molecules that, once moved to a damaged intestinal barrier, are able to trigger pro-inflammatory processes including increased pro-inflammatory cytokines and monocytes (de Magistris et al., 2013). These pro-inflammatory mediators reach higher brain centers through the bloodstream and trigger brain neuroinflammation events (Fiorentino et al., 2016). Moreover, aggressive forms of *Candida spp*. have been identified in the fecal samples of ASD children

(Iovene et al., 2017) and different fecal flora has been observed in regressive ASD patients (Finegold et al., 2002) compared to their age-matched healthy controls.

The evidence of microbiota dysfunction and chronic gastrointestinal symptoms experienced by some ASD patients led to a recently proposed strategy aimed at restoring the intestinal bacterial flora of ASD patients. A study carried out on 18 children suffering from ASD and gastrointestinal problems showed significant improvements in both ASD- and gastrointestinal-related symptoms after a Microbiota Transfer Therapy (MTT), with prolonged beneficial effects up to 8 weeks after treatment (Kang et al., 2017). Surprisingly, a follow-up study on the same population showed beneficial effects of MTT even two years after treatment (Kang et al., 2019). Despite further studies are needed to support these findings, these results suggest the validity of MTT and lay the foundation for promising future therapies based on this approach.

Besides human evidence, animal studies are investigating the potential role of intestinal microbiota in ASD (Hughes et al., 2018; Nithianantharajah et al., 2017) trying to understand the possible mechanisms involved in microbial influence on brain and behavior. In germ-free (GF) mice, the architecture of the immune system is compromised and disorganized, and this makes them more susceptible to infections than mice kept in conventional facilities (Hughes et al., 2018). Interestingly, GF animals exhibit decreased sociability with unfamiliar partners (Clarke et al., 2013), which can be corrected by intestinal colonization with diverse commensal bacteria. To strengthen this evidence, it has been shown that the gut bacterium *Lactobacillus reuteri* (*L. reuteri*) reversed ASD-like symptoms in mice (Buffington et al., 2016), suggesting a new wave of interest in the relationship between gut microbiome and ASD.

3. Role of the endocannabinoid system in ASD: focus on CBD

The ECS represents a major neuromodulatory system involved in the regulation of emotional responses and cognitive states (Marco and Laviola, 2012) and there is increasing interest in cannabinoids, especially CBD, an abundant bioactive but non-psychotomimetic constituent of

Cannabis sativa, as a treatment for the core symptoms and co-morbidities of ASD (Aran et al., 2019; Barchel et al., 2018; Poleg et al., 2019; Pretzsch et al., 2019a; Salgado and Castellanos, 2018). From a general perspective, the ECS consists of cannabinoid receptors (CB1, discovered as a neuronal target of the psychoactive compound of the plant *Cannabis sativa*, Δ9-tetrahydrocannabinol (THC), and CB2, mainly expressed in the immune system, but recently found in the CNS as well), their endogenous lipid ligands (eCBs, including AEA and 2-arachidonoylglycerol; 2-AG), and the enzymes for ligand synthesis and degradation (Di Marzo et al., 2004; Piomelli, 2003). Evidence for the involvement of the ECS in ASD comes from both human and animal studies: reduced CB1 receptor expression was found in postmortem brains of autistic patients (Purcell et al., 2001), whereas up-regulation of CB2 but not CB1 gene expression was detected in PBMC of ASD individuals compared to healthy controls (Siniscalco et al., 2013b). More recently, lower circulating endocannabinoid levels (including AEA but not 2-AG) have been detected in children with ASD (Aran et al., 2019; Karhson et al., 2018) and heterozygous rare variants in diacylglycerol lipase α (DAGL-α), the major enzyme involved in 2-AG biosynthesis, have been found to be significantly associated with ASD (Smith et al., 2017). Interestingly, the non-psychotomimetic phytocannabinoid CBD (Epidiolex®) has been FDA- and EMA-approved for two epilepsy syndromes associated with ASD: Dravet Syndrome and Lennox-Gastaut Syndrome (Devinsky et al., 2017; Devinsky et al., 2016; Devinsky et al., 2018; Thiele et al., 2018). Another non-psychoactive phytocannabinoid, CBDV, is under clinical trial to assess its efficacy in people suffering from ASD (ClinicalTrials.gov Identifier: NCT03849456; NCT03202303). Related to this, it has been discovered that a single oral dose of CBDV modulates glutamatergic but not γ-aminobutyric acid (GABA) neurotransmission in adult male patients, although the biological response may differ between autistic individuals (Pretzsch et al., 2019b). This evidence suggests that cannabinoids may represent a promising pharmacological tool for ASD. Animal studies support these clinical findings showing that the ECS is involved in the modulation of many of the cellular functions, molecular pathways and behaviors that are altered

in ASD (Zamberletti et al., 2017). They also highlighted that CBD has beneficial effects on the seizures and social deficits observed in a mouse model of Dravet Syndrome (Kaplan et al., 2017) and improves the social and cognitive dysfunctions observed in a rat model of schizophrenia (Osborne et al., 2017). Moreover, preclinical evidence exists that CBDV improves behavioral and functional deficits in a mouse model of Rett syndrome (i.e., the early symptomatic Mecp2 mutant mice) (Vigli et al., 2018; Zamberletti et al., 2019a) and in an environmental rat model of ASD based on prenatal exposure to the antiepileptic valproic acid (VPA) (Zamberletti et al., 2019b). Despite these promising results, our understanding of the role of the ECS in ASD is still far from complete and the use of cannabinoids in general, and CBD in particular, for children and adolescents suffering from ASD still remains controversial (Bou Khalil, 2012; Poleg et al., 2019). CBD displays a plethora of therapeutic properties (Friedman et al., 2019; Premoli et al., 2019) and it is well tolerated by humans (Devinsky et al., 2014). Leweke and colleagues showed that CBD increases the serum levels of AEA in schizophrenic subjects (Leweke et al., 2012), by inhibiting the action of the enzyme fatty acid amide hydrolase (FAAH), responsible for AEA degradation. This evidence, together with retrospective studies showing positive effects in ASD children using CBD (Aran et al., 2019; Bar-Lev Schleider et al., 2019; Tartaglia et al., 2019), led researchers to theorize that CBD may have beneficial effects in ASD by inhibiting the action of FAAH and thereby normalizing the depletion of AEA tone observed in these patients (Aran et al., 2019; Karhson et al., 2018). In our opinion, this is a very appealing field of research which deserves further investigation. The assumption that an increase in endocannabinoid tone could ameliorate ASD symptoms has been explored by our and other groups. For instance, it has been demonstrated that URB597, a compound that increases AEA levels by inhibiting the enzyme FAAH, has therapeutic potential in rats prenatally exposed to VPA (i.e., environmentally-triggered ASD model) (Melancia et al., 2018; Servadio et al., 2016; Wu et al., 2020), by exerting its positive effects on socioemotional behavior (Bara et al., 2018; Manduca et al., 2016b; Trezza et al., 2012). Of note, enhancing AEA signaling through systemic administration of another

FAAH inhibitor PF3845 similarly attenuated the deficit in social behavior observed in VPA exposed male animals (Kerr et al., 2016), supporting a role of FAAH in the regulation of social behavioral deficits. Interestingly, these findings have been also extended to the genetic animal model of Fragile X syndrome (FXS), the most common form of inherited mental retardation and the leading genetic cause of ASD: thus, administration of URB597 improved aversive memory and anxiety-like behavior (Qin et al., 2015) and reversed the social impairment (Wei et al., 2016a) in mice with a knockout mutation for Fragile X mental retardation 1 (FMR1) gene, confirming that increasing AEA activity at CB1 receptors might offer an interesting therapeutic strategy for treating some of the clinical manifestations of FXS. It should be noted, however, that blockade of CB1 receptor has also been reported to ameliorate FXS-like symptoms in FMR1 knock-out mice (Busquets-Garcia et al., 2013; Gomis-Gonzalez et al., 2016), suggesting that other approaches targeting the ECS (i.e. modulation of CB1 receptors) may also show beneficial effects in FXS models. Besides AEA, evidence also supports a role of 2-AG in social behavior (Manduca et al., 2016a; Wei et al., 2015; Wei et al., 2016b), highlighting the different contributions of the two main eCBs in the modulation of emotionality (Manduca et al., 2015) and their involvement in neurobehavioral processes (e.g. social behavior and anxiety) highly compromised in ASD (Jung et al., 2012).

Interestingly, it has been found that gene expression for CB2 but not CB1 receptor was up-regulated in PBMC of ASD individuals compared to healthy controls (Siniscalco et al., 2013b), whereas gene expression for NAPE-hydrolyzing phospholipase D (NAPE-PLD), one of the enzymes responsible for the synthesis of AEA, was found significantly lower in individuals with ASD. One can argue that the decreased AEA tone observed in ASD (i.e. resulting from a decrease in AEA synthesis by NAPE-PLD enzyme) may have caused a compensatory increase in CB2 receptors and a decrease in plasma pro-inflammatory cytokines, thereby supporting the efficacy of CBD in the treatment for ASD. This represents a very promising field of investigation because cannabinoids showed great potential as anti-inflammatory agents and specific targeting of CB2

receptors holds promise for mediating immunosuppressive effects without exerting psychotropic side effects (Nagarkatti et al., 2009).

However, the exact mechanism of action of CBD in patients suffering from ASD and related comorbidities remains unclear, especially because of CBD polypharmacy. Due to the low affinity of CBD for both CB1 and CB2 receptors (Martinez-Pinilla et al., 2017; McPartland et al., 2015; Pertwee, 2008), few studies aimed at characterizing CB1- and CB2-independent modes of action for this phytocannabinoid in neuropsychiatric disorders. The non-endocannabinoid mediated mechanisms of action of CBD possibly involve the regulation of glutamatergic and GABAergic neurotransmission (Pretzsch et al., 2019a) or other receptors including the G protein-coupled receptor GPR55 (Kaplan et al., 2017), the 5-HT1a receptor, the α3 and α1 glycine receptors, and the transient receptor potential of ankyrin type 1 channel (Devinsky et al., 2014). To complicate this picture, it should be considered that CBD may also indirectly act through neuropeptides such as oxytocin (Wei et al., 2015) and vasopressin (Caldwell and Albers, 2016), which are involved in modulating social reward, that is compromised in individuals with ASD.

Another interesting hypothesis regarding the therapeutic potential of CBD in ASD is that CBD may alleviate many conditions co-occurring with autism, such as seizures (Khan et al., 2018), gastrointestinal problems (Couch et al., 2017), anxiety and depression (Lee et al., 2017; Sales et al., 2019; Silote et al., 2019), ADHD (Cooper et al., 2017), and sleep disturbances (Babson et al., 2017). Again, this evidence leads to the hypothesis that targeting the ECS may contribute to normalize different behavioral patterns compromised in ASD, such as social reward responsiveness, neuronal development, circadian rhythms, and anxiety-related symptoms (Chakrabarti et al., 2015). Despite this increasing scientific effort, our knowledge is still limited and the effects of CBD in individuals with ASD need to be further explored.

4. Neuroinflammation in ASD

During the last decade, the scientific community agreed on the involvement of neuroinflammatory processes in the pathogenesis of ASD and this is confirmed by the growing amount of literature available on this topic.

Neuroinflammation is a well-orchestrated process by various groups of glial cells, particularly microglial cells, which are exclusively present in the CNS and have a myeloid origin. They belong to the mononuclear phagocyte series of cells and constitute the first defense mechanism of the CNS. During development, there is a major migration of primitive myeloid precursor cells from the yolk sac into the CNS, resulting in microglial colonization of the fetal brain. This event starts in the late 1st trimester in humans and around embryonic day 9 in rodents (Chan et al., 2007; Matta et al., 2019).

At first, the migration seems to reach through the bloodstream some specific brain areas only, such as the hippocampus and corpus callosum, from where the colonization then continues, spreading in the whole brain. There is preclinical and clinical evidence of the continuous proliferation activity of microglia also during the first postnatal weeks (Bilbo et al., 2018).

Astrocytes, instead, are glial cells derived from the neuroectoderm and populate the brain parenchyma later than microglia during neurodevelopment (Matta et al., 2019). Together with microglia, they are fundamental in maintaining homeostasis within the CNS. Indeed, microglia and astroglia are of primary importance since they play a key role in synapse formation and function, as well as in synaptic pruning and they are connected with neurons through a complex signaling network from early stages of development (Petrelli et al., 2016).

These two glial cell types are strictly involved in neuroinflammation and they act in concert as a surveillance system in the CNS, ensuring efficient inflammatory processes against pathogens without disrupting homeostasis under physiological conditions, especially in the developing brain. For instance, microglia in the CNS usually remain in a non-reactive state, known as “resting”, which contributes to immune homeostasis, clearing debris, cytokines and growth factors secretion and communication with surrounding neurons (Chan et al., 2007; Ransohoff and Perry, 2009).

Since during this phase microglial cells are constantly checking the local environment through the extension of their branching processes (Napoli and Neumann, 2009), they are the first glial cell types to respond to pathological changes in the brain, carrying out diverse functions which can be either effective or harmful (Chan et al., 2007; Ransohoff and Perry, 2009).When signs of infection or tissue damage are detected, microglia can proliferate, migrate directly to the lesion sites (Adams and Gallo, 2018) and release inflammatory mediators like cytokines and prostaglandins, in order to recruit astrocytes during inflammation (Ransohoff and Perry, 2009).

Once the astrocytes are triggered, they undergo reactive gliosis, characterized by upregulation of the astrocyte intermediate filament protein or, most notably, glial fibrillary acidic protein (GFAP) (Pekny et al., 2014), which constitutes another important marker of neuroinflammation (Matta et al., 2019).

Both microglia and astrocytes modify their morphology to rapidly adapt to brain changes, influencing each other with a series of stimulatory signals, like cytokines, chemokines and ATP, to mount an immune response (Matta et al., 2019; Zhang et al., 2010).Together, reactive microglia and astrocytes form a glial scar at the site of injury to provide a structural barrier to prevent further damage and promote tissue repair (Adams and Gallo, 2018).

These mechanisms are critical for normal development and even a slight imbalance during early stages od development, because of the intense microglial proliferation and activity during the prenatal period, could lead to a loss of homeostasis, with subsequent impairment in synaptic functions and general chronic neuroinflammation, in which the aberrant and detrimental production of pro-inflammatory agents is considered to have a key role in the pathogenesis of ASD (Bilbo et al., 2018; Kalkman and Feuerbach, 2017; Petrelli et al., 2016).

Other key physiological functions carried out by microglia are neuronal plasticity and synaptic pruning (Salter and Stevens, 2017). An interesting hypothesis that supports the involvement of microglia abnormalities in the onset of ASD comes from some studies investigating their role in synaptic pruning; indeed, preclinical research showed how the dysregulation of specific immune

pathways that directly control microglial functions has a negative impact on synaptic connectivity (Lehrman et al., 2018). Related to this, it is interesting that structural synaptic changes also occur in a mouse model with autistic-like traits and with a knock-in mutation in the PTEN gene, whose dysregulation seems to have a role in pathologic microglial activation and, as a consequence, in aberrant synaptic pruning (Sarn et al., 2020). Although these results suggest that synaptic pruning dysregulation may contribute to abnormal synaptic functions in ASD, the direct cause-and-effect relationship between microglial-dependent synaptopathies and ASD remains to be elucidated.

Thus, if on one hand microglia exert primary tasks in the CNS and have the main function to remove cellular debris by phagocytosis under neuroinflammatory conditions, as well as macrophages do in the periphery, on the other hand, their unnecessary or prolonged activity in the absence of immune challenge leads to overcome their neuroprotective effects, resulting in neurotoxicity that occurs in multiple CNS diseases, including ASD (Bilbo et al., 2018; Matta et al., 2019).

Another harmful consequence of a prolonged neuroinflammatory condition is the release of pro-inflammatory molecules, as well as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can induce structural damage to tissues and vascular-endothelial dysfunctions (Mittal et al., 2014). These pro-inflammatory molecules act increasing permeability at the BBB through several different mechanisms, including altering expression and inducing reorganization of the cytoskeletal and tight junction proteins forming the BBB (Kealy et al., 2020). When this activity lasts typically beyond a period of weeks or months, it is considered a pathological hallmark of chronic neuroinflammation, leading to disease states.

Although ASD is not defined as a classical immune-mediated disorder, some of the complex neurological and behavioral abnormalities characterizing ASD can relate to dysfunctions found in the immune system and inflammation during development. There is an increasing number of data in literature suggesting specific immune profiles and markers for individuals with ASD, especially the pro-inflammatory markers, significantly different from healthy individuals or those without

ASD (Thom et al., 2019). Different studies reported an altered interplay between innate and adaptive immunity and CNS in ASD, leading to chronic low-grade neuroinflammation (Dipasquale et al., 2017).

4.1 Insights from clinical studies

Today, an increasing number of scientific papers are exploring the link between ASD and changes observed in the immune system. These seem to have a fundamental role in the pathogenesis of this disorder and their comprehension would thus not only contribute to a better understanding of the pathology itself, but also to the identification of new potential treatments (Lai et al., 2014).

A pivotal study by Vargas and colleagues investigated whether immune-mediated mechanisms are involved in the pathogenesis of ASD, through the analysis of the brain and CSF of autistic patients compared to healthy controls. Interestingly, they found out a marked neuroglia activation mainly correlated with innate immune response, involving both microglia and astrocytes, also displaying morphological and histological changes consistent with chronic and sustained neuroinflammation. These alterations are thought to be relevant to synaptic dysfunctions in autism, giving rise to the hypothesis of chronic neuroinflammation as a disturbing event during early brain development stages, causing subsequent behavioral and cognitive impairments. CSF analysis confirmed a prominent inflammatory cytokine profile specific to autistic patients (Vargas et al., 2005).

A more recent work, investigating the link between immunity and severity of ASD symptoms, supports previous findings considering ASD as a result of neurotoxicity and dysregulated immune response during neurodevelopment. Remarkably, it demonstrated the consistency of the hypothesis of chronic neuroinflammation by showing specific and atypical patterns of brain activity in ASD obtained by electroencephalogram (EEG) recordings (Inga Jacome et al., 2016).

Other studies further investigated the correlation between the BBB loss of function during neuroinflammation and the neurobehavioral changes observed in ASD. There is evidence, based

on molecular biomarkers such as S100B protein, GFAP and TNF-α, of the increased permeability and thus damage at the BBB. Indeed, a significantly higher level of these markers has been found in children with ASD (Esnafoglu et al., 2017; Guloksuz et al., 2017). A study by Fiorentino and colleagues provided proof of concrete injuries of the BBB through post-mortem analysis of brain tissues of autistic patients. They pointed out to a greater expression of proteins such as the translocator protein (TSPO), strongly implicated in immunomodulation and also considered a marker of neuroinflammation, and some components of tight junctions like claudins with the main function of sealing the BBB structure, indicating a possible compensatory mechanism to repair a compromised BBB (Fiorentino et al., 2016).

Another work, investigating new possible blood biomarkers for ASD, reported a higher concentration of autoantibodies against neuronal and glial proteins in the serum of children with ASD and their mothers, than in healthy controls. These data suggest a unique peripheral autoantibodies profile in ASD patients and thus provide new insights about the involvement of maternal immune system in the onset of autism, since MIA during pregnancy may produce molecules like IgG autoantibodies and cytokines able to cross the placenta and the developing fetal BBB, with subsequent consequences for early stages of neurodevelopment (Abou-Donia et al., 2019; Solek et al., 2018).

There is a number of studies providing evidence of an increased density and interactivity of reactive microglia (Morgan et al., 2012; Morgan et al., 2010) or microglial morphological changes typical of an altered activity (Tetreault et al., 2012) within the cerebral cortex of autistic patients, also according to results obtained from RNA sequencing, indicating a close relationship between ASD and genes related to glial activation (Voineagu et al., 2011). Likewise, other studies provide more details on microglial activation in the different brain regions in young adults with ASD, thanks to neuroimaging techniques as positron emission tomography (PET) (Suzuki et al., 2013; Thom et al., 2019).

These findings generally highlight the role of neuroinflammation in early developmental stages as one of the main mechanisms in the pathophysiology of ASD (Siniscalco et al., 2018).

4.1.1 Impact of neuroinflammation on brain and glia structural changes in ASD

Given the heterogeneous nature of ASD, it is hard to detect morphological changes and anatomical alterations universally valid. This field of investigation faces the challenge that the few brain samples available for direct investigation are usually restricted to post-mortem analysis and mainly in adult cohorts of patients.

Despite that, among the studies which tried to outline some specific features in the neuroanatomy of ASD, there is evidence of modifications in size and functionality of brain areas such as the hippocampus, amygdala, frontal cortex and cerebellum (Donovan and Basson, 2017; Schumann et al., 2004), four critical structures for emotional processing and cognition.

The use of magnetic resonance imaging (MRI) provided evidence of structural abnormalities of the hippocampus and amygdala of autistic patients. Despite there is no difference in terms of total cerebral volume, these two regions appear larger in patients with ASD than in TD controls, raising the hypothesis of a morpho-functional link (Schumann et al., 2004). The prefrontal cortex of autistic patients seems to undergo abnormal growth patterns, and alterations of cortical thickness and general disorganization among neurons in the cortical layers have been observed, potentially impacting on the connectivity with other brain regions (Donovan and Basson, 2017).

Concerning the cerebellum, some of the most consistent neuroanatomical alterations reported are reduced size, volume and number of Purkinje cells (Fatemi et al., 2012; Wegiel et al., 2014a; Wegiel et al., 2014b), which could contribute to some of the behavioral and cognitive deficits displayed by ASD patients. Interestingly, Whiting and colleagues described the case of a boy with a history of a complete prenatal cerebellar stroke, then diagnosed with the typical core symptoms of ASD, thus suggesting a link between cerebellum dysfunction and the onset of autism (Whiting et al., 2019). These findings could be also interpreted from another point of view, i.e., considering

the cerebellum damage as a consequence of the strong neuroinflammation evoked by the infarction during the prenatal period, the most sensitive for neurodevelopment. A review by Jayaraj et al. (2019) discusses the importance of the full understanding of neuroinflammatory mechanisms in order to potentiate therapeutic neuroprotective strategies for post-stroke inflammation and it is noteworthy that the chronicity of the same inflammatory processes during early developmental stages seems to be involved in ASD.

As reported in many studies, the altered spatial organization of glia and neuroinflammation-induced modifications in ASD could be at the origin of greater rearrangements in neurons and brain regions morphology (Morgan et al., 2012; Morgan et al., 2010; Tetreault et al., 2012; Vargas et al., 2005).

Glial activation is mainly determined by alternative states of microglia: in absence of external insults, microglia maintain the 'resting state' or M0 phase, characterized by a highly ramified shape able to constantly survey the local environment (Napoli and Neumann, 2009); when potential risk factors are detected, microglia undergo the 'classical activation state' or M1 phase, driven by factors such as lipopolysaccharide (LPS), interferon (IFN-) gamma and TNF-α, where the soma attains an amoeboid morphology and retracts its processes. The M1 state is the first line of defense in the CNS and it is involved in removing pathogens or infected cells, also increasing the antigen-presenting activity and upregulating genes related to enzymes for ROS or RNS production (Tanaka et al., 2020). After the classical activation, a resolution of the inflammatory state is required and it is represented by the 'alternative activation' or M2 phase of microglia; at this stage, microglia can undergo two different alternative states, triggered by different sets of cytokine expression and receptor profiles: the M2a state, promoted by cytokines like IL-4 and IL-13 (Tanaka et al., 2020), has an important role in resolving the inflammation and removing cell debris, while the M2c subtype, fostered by factors such as IL-10, transforming growth factor beta (TGF-β) and glucocorticoids (Franco and Fernandez-Suarez, 2015), constitutes the deactivating phenotype, aimed at restoring the original microenvironment (Mecha et al., 2016) [**Figure 1**].

Interestingly, it has been demonstrated that the two microglial phenotypes can simultaneously coexist within the local environment of the brain, thus suggesting a rate of inflammatory response dependent on the ratio between the different subtypes (Martinez and Gordon, 2014).

Microglia has a crucial regulatory role in neurodevelopment and neuroinflammation, as underlined by consequences of MIA in autism (Careaga et al., 2017).

Nowadays, evidence of microglia involvement in the onset of ASD is provided by many studies evaluating, for instance, microglial activation through PET-imaging (Suzuki et al., 2013) or large-scale transcriptomic analysis, confirming an up-regulated microglial signature in ASD (Gandal et al., 2018). This is likely to be responsible for the regulation of synaptic connectivity, also according to previous observation by Voineagu (Voineagu et al., 2011).

Overall, these findings suggest that spatial and morphological alterations of microglia and astrocytes in ASD, as well as their increased interactivity, lead to overtake the original neuroprotective threshold with subsequent impairment of healthy neurons (Matta et al., 2019) and, thus, contributing to the onset of the core symptoms of autism. Indeed, many of the autistic features as repetitive behaviors, impairments in verbal and nonverbal communication and social interactions, seem to have different severity levels also depending on changes in immune responses, confirming the intimate bond between neurodevelopment and immune processes (Careaga and Ashwood, 2012).

Certainly, further investigations about the role of neuroinflammation in ASD, and eventually its pharmacological modulation, are needed both from a genetic and immunological point of view.

In this context, animal models are of the utmost importance, as outlined in the following section.

4.2 Insights from preclinical studies

Despite the research performed in the last 10 years increased our understanding of the pathogenesis of ASD, several aspects of its etiology remain still controversial, with genetic, environmental (Karimi et al., 2017; Kim and Leventhal, 2015) and immunological factors

(Jyonouchi et al., 2005) being involved. In this scenario, animal models are essential to dissociate the role of each of these factors, and their eventual overlap, in the pathogenesis of ASD.

Given the great phenotypic variation within each core symptom domain, the best option to study neurobiological aspects of ASD is to focus on animal behaviors that mimic the core diagnostic features of ASD (Crawley, 2012; Servadio et al., 2015).

The contribution of new sequencing technologies to genetics allowed researchers to investigate ASD at a higher level, confirming that its etiology is generally multigenic and heterogeneous, while just few ASD-related diseases have monogenic causes, such as Rett syndrome, FXS, tuberous sclerosis and Schuurs – Hoeijmakers syndrome (Artuso et al., 2011; Stern et al., 2017; Woodbury-Smith and Scherer, 2018).

In addition to genetic factors, several prenatal and perinatal risk factors have been identified, including maternal treatment with drugs such as VPA and thalidomide, exposure to toxic agents as organophosphate, insecticides and heavy metals or intrauterine exposure to infections (Chaste and Leboyer, 2012).

In the last few years, there has been growing interest in neurodevelopmental disorders related to immune activation and several studies provided evidence of its involvement in the development of ASD, as suggested by the consequences reported in the population after many epidemics since the second half of the 20th century (Solek et al., 2018) and by the chronic CNS inflammation observed in ASD patients (Onore et al., 2012).

There are striking parallels between the clinical observations and the findings obtained in rodent models of ASD. For instance, FXS is the most common genetic cause of ASD and FXS patients frequently show an increased susceptibility to infections and gastrointestinal symptoms (Hagerman and Hagerman, 2002), together with atypical cytokine and chemokine profiles compared to healthy subjects (Ashwood et al., 2010), suggesting a possible role for immune dysfunctions in FXS. Accordingly, Fmr1-KO rodents not only display many of the typical autistic-like features at the behavioral (Asiminas et al., 2019; Connor et al., 2011; Melancia and

Trezza, 2018; Zamberletti et al., 2017), cellular (Berzhanskaya et al., 2017a; Berzhanskaya et al., 2017b; Dolen et al., 2007; Grossman et al., 2010; Ruby et al., 2015) and neurochemical (Busquets-Garcia et al., 2013; Maccarrone et al., 2010; Pietropaolo et al., 2020; Till et al., 2015; Zamberletti et al., 2017) level, but they also show immune dysfunction. Yuskaitis and colleagues reported a lower inhibitory serine-phosphorylation of glycogen synthase kinase 3 (GSK3) in Fmr1-KO mice, indicative of increased GSK3 activity, which is meaningful to alterations in the immune system since one of the functions of this protein is to trigger inflammation both in the periphery and in glia, a mechanism confirmed by the neuroprotective effect of GSK3 inhibitors, such as lithium (Beurel et al., 2015). They also demonstrated a neuroinflammatory condition in the CNS of Fmr1-KO mice through the astrogliosis marker GFAP, which significantly increased in the striatum, hippocampus and cerebral cortex. Interestingly, both the altered behaviors and inflammation displayed by Fmr1-KO mice ameliorated after lithium administration (Yuskaitis et al., 2010).

Another common animal model used in autism research is based on prenatal exposure to VPA in rodents (Tartaglione et al., 2019).

To date, VPA is considered one of the most teratogenic anti-epileptic drugs (Andrade, 2018) and several retrospective studies have shown that prenatal exposure to this drug is linked to an increased risk of ASD (Chomiak et al., 2013).

In addition to the altered behavioral, cellular and neurochemical features displayed by VPA-exposed rodents (Gogolla et al., 2009; Graciarena et al., 2018; Kataoka et al., 2013; Kerr et al., 2013; Mahmood et al., 2018; Nicolini and Fahnestock, 2018; Roullet et al., 2010; Servadio et al., 2018; Servadio et al., 2016; Wang et al., 2018a), there is evidence of immunological alterations (Nicolini and Fahnestock, 2018) and reactive microglia and astrocytes (Codagnone et al., 2015) induced by prenatal VPA exposure. Interestingly, the changes and activation of glia appear regions- and age-dependent, mainly occurring at young ages when all neuroglial cells are critical to correct brain development (Bronzuoli et al., 2018).

Collectively, these findings are in line with post-mortem analyses of human samples, highlighting a primary role for neuroinflammatory processes in the onset of ASD symptoms, even after the insult caused by an environmental agent such as VPA.

4.2.1 Maternal immune activation in rodents as preclinical model of ASD

The prenatal immune environment seems to have a key role in the onset of neurodevelopmental disorders (Estes and McAllister, 2015; Patterson, 2011) and its delicate equilibrium can be disrupted by perturbations in the maternal immune system (Abou-Donia et al., 2019).

The external insults involved range from infectious agents to maternal stress and include many other environmental and genetic factors that affect the inflammatory or immune pathways, potentially leading to alterations and dysfunctions in the developing brain (Bilbo et al., 2018). This is the reason why, according to a large-scale study performed in the United States, there is no evidence of a direct correlation between a given infectious agent and the onset of ASD, but there is probably increased odd of developing the pathology for maternal infections associated to fever and mainly acquired during the second trimester of pregnancy (Croen et al., 2019), suggesting that the impact of such infections is highly dependent on the severity of the maternal immune response and on the time of pregnancy.

These findings are consistent with the results of large cohort studies (Atladottir et al., 2012; Brucato et al., 2017; Hornig et al., 2018) and with the hypothesis of a direct relationship between the severity of the maternal immune response and the relative consequences for brain and behavioral development of children (Careaga et al., 2017).

To shed light on the mechanisms potentially involved in the development of ASD following MIA, different animal models have been developed. To date, the most widely investigated animal models of MIA in rodents are based on prenatal exposure to LPS, mimicking the consequences of a bacterial infection, or on prenatal exposure to polycytidylic acid (Poly(I:C)), mimicking instead a viral infection.

LPS is a bacterial endotoxin and a component of gram-negative bacteria wall. By binding to toll-like receptor (TLR) 4, it triggers antibacterial-like innate immune responses (Patterson, 2011), with the release of a pro-inflammatory cascade of cytokines such as IL-1β, IL-6 and TNF-α (Ronovsky et al., 2016). Conversely, poly(I:C) is a synthetic double-stranded viral RNA binding to TLR 3 and inducing an antiviral-like immune reaction with the production of antiviral interferons and inflammatory cytokines (Reisinger et al., 2015).

Timing plays an essential role in MIA induction. Organogenesis occurs during the first phase of pregnancy and more exactly in the late 1st trimester in humans, while the same event in rodents occurs around gestational days 8-10, when both neuronal proliferation and differentiation together with synaptic maturation are actively taking place (Chan et al., 2007).

In agreement with several studies, the administration of LPS, or poly(I:C) in rodents at this moment interferes with correct neurodevelopment, eliciting autistic-like symptoms in the offspring (Boksa, 2010; Kirsten et al., 2012; Solek et al., 2018).

Another key event in rodents brain development is marked by gestational day 12.5, when the closure of the neural tube occurs. Therefore, other studies were performed administering poly(I:C), LPS or inflammatory cytokines in pregnant rodents at this time point (Lammert and Lukens, 2019; Servadio et al., 2015; Smith et al., 2007; Xuan and Hampson, 2014).

There is evidence of several alterations in both mouse and rat models of prenatal exposure to LPS or poly(I:C), depending on the time and dose of injection (Solek et al., 2018), thus underlying different mechanisms involved in both CNS and immune system development.

Overall, among the studies modeling MIA in rodents with LPS, many reported increased anxiety in tasks such as the open field and elevated plus-maze (Depino, 2015; Hsueh et al., 2017; Lin et al., 2012), increased depressive-like behavior (Depino, 2015), communicative deficits with shorter ultrasonic vocalizations (USVs), sociability impairments, increased stereotypies (Fernandez de Cossio et al., 2017; Kirsten and Bernardi, 2017; Kirsten et al., 2015; Xuan and Hampson, 2014) and impaired learning and memory (Baharnoori et al., 2012; Golan et al., 2005).

Progressive decreased social interactions (Hao et al., 2010), reduced juvenile social play behavior (Taylor et al., 2012), impaired spatial discrimination (Kentner et al., 2016), as well as changes in locomotor activity (Straley et al., 2017), have also been found in MIA-exposed rodents.

These deficits in the behavioral domain are associated with altered microglial state and inflammatory biomarkers, which generally mirror the results obtained in human ASD studies (Solek et al., 2018).

A higher number of inducible nitric oxide synthase (iNOS) and IL-1β expressing cells (Cunningham et al., 2013) as well as increased levels of IL-1β (Kirsten et al., 2013) found in MIA-exposed rodents supports the augmented production of reactive species during neuroinflammation, having detrimental consequences on neurodevelopmental processes.

Interestingly, a study also detected lower mRNA levels of CX3CR1 in microglia (Fernandez de Cossio et al., 2017). CX3CR1 is a chemokine receptor with a role in synaptic pruning and anti-inflammatory responses (Paolicelli et al., 2011).

Furthermore, some studies reported increased levels of S100B, IL-6, TNF-α and CCL2 chemokine (de Souza et al., 2015; Dowling et al., 2012), and a greater expression of IL-1β and IL-6 mRNA in the cerebellum, hippocampus, prefrontal cortex and brainstem of the MIA offspring (Zhou, 2015).

Prenatal poly(I:C) injection also induces autism-like symptoms in rodents. At the behavioral level, rodent models of poly(I:C)-induced MIA show early social communication deficits with a reduction in USVs emission, increased stereotyped behaviors (Malkova et al., 2012), a general decrease in social interest (Malkova et al., 2012; Onore et al., 2014), impaired memory in the novel object recognition test (Luchicchi et al., 2016), increased anxiety and deficits in reversal learning (Meyer et al., 2006), as well as impaired prepulse inhibition (PPI) (Meyer et al., 2008).

Again, the behavioral findings positively correlate with immunohistochemical analyses, which revealed increased production of inflammatory agents such as IL-6, IL-17a, TNF-α, IFN-β, IL-1 β in rodents prenatally exposed to poly(I:C) (Choi et al., 2016).

In conclusion, the inflammatory dysregulations found in the offspring prenatally exposed to either LPS or poly(I:C) are in line with those observed in the brain of autistic patients (Jonakait, 2007; Patterson, 2009).Taken together, these findings highlight the link between the core symptoms of ASD and the LPS- or poly(I:C)-induced MIA, making these preclinical models widely used in autism research (Servadio et al., 2015).

5 The endocannabinoid system as anti-inflammatory mediator

As previously discussed, ASD is characterized by both ECS alterations and neuroinflammatory states, which seem to play a key role in the onset of the typical symptoms when affecting the CNS at the early stages of neurodevelopment.

On this basis, a new hypothesis is acquiring more consent in the last years, coming from the evidence that, as for macrophage-like cells, microglia have a complete and functional endocannabinoid signaling system (eCBSS), thus suggesting a regulatory role of eCBs for patterns of microglial maturation, differentiation and activation (Stella, 2009). This kind of regulation may also involve the control of neuroinflammation through an autocrine/paracrine signaling (Mecha et al., 2016).

For this reason, recently there has been growing interest in the interplay between the ECS and microglial alternative states, strictly connected to the pathways triggered by CB2 receptor activation (Araujo et al., 2019; Bisogno and Di Marzo, 2010; Brigida et al., 2017; Lunn et al., 2006).

The possible mechanism for the microglia eCBSS, proposed in some studies, is mainly based on the expression levels of the eCBs and cannabinoid receptors in microglia, depending on their activation state.

CB1 receptors are the most abundant G-protein coupled receptors in the CNS and they are generally expressed in neurons at the presynaptic terminals, where their activation modulates neurotransmission for cognition, motor functions, emotions, analgesia and memory; CB2

receptors, instead, are highly expressed in immune cells in the PNS and mainly in microglia at the CNS level, controlling immunomodulatory functions (Tanaka et al., 2020).

The activation of the canonical two cannabinoid receptors initiates different responses mainly depending on which receptor has been activated by a specific ligand at a given time (Atwood and Mackie, 2010; Zou and Kumar, 2018).

Although the full mechanisms through which the eCBSS drives the acquisition of alternative microglia phenotypes remains still unclear and needs further investigation, Mecha and colleagues provided strong evidence of the immunomodulatory and neuroprotective effects of the ECS on microglia.

To date, the proposed pathway for M2 alternative state acquisition sounds intriguing: first, microglia in the M0 state synthesize the eCBs under basal conditions; the stimulation by inflammatory agents induces the polarization to the M1 phenotype (primed microglia) with the subsequent immune response, where a general down-regulation of either the cannabinoid receptors and the biosynthetic and hydrolyzing enzymes has been observed; when switching to the M2a state, promoted by cytokines like IL-4 and IL-13, microglia display a significant up-regulation of both CB2 receptors and DAGL-α, the synthesizing enzyme for 2-AG, while showing a down-regulation of FAAH and monoacylglycerol lipase (MAGL), the eCBs degrading enzymes, resulting in a boosting of the eCBSS; last, when undergoing the M2c state (the deactivating phenotype), microglia show either a strong up-regulation of the CB1 and CB2 receptors and of the NAPE-PLD enzyme, that synthesizes AEA, a series of changes likely related to the restoration of the M0 phenotype (Mecha et al., 2015) [**Figure 2**].

Overall, although the proposed mechanism needs to be further examined, this hypothesis suggests the close correlation between the ECS and microglial functions, thus shedding some light on the concurrent dysfunctions of the ECS and the immune system in ASD.

It is interesting to consider this hypothesis on the light of the different properties of the two eCBs, with AEA acting as a high-affinity partial agonist of the CB1 receptor and being almost inactive

at the CB2 receptor, while 2-AG reveals to be a full agonist at both cannabinoid receptors (Zou and Kumar, 2018).

In the same way, it is important to keep in mind the effects of CB2 receptor activation, primary in the control of inflammatory conditions and in the restoration of homeostasis, two functions consistent with the up-regulation described in microglia alternative states aimed at restoring physiological conditions. Indeed, CB2 receptors can promote essential neuroprotective functions: their activation triggers some specific pathways such as the activation of phospholipase C with subsequent calcium release, the activation of phosphatidylinositol 3-kinase (PI3K), the induction of apoptosis and suppression of cell proliferation, the increase of anti-inflammatory cytokine production and the inhibition of pro-inflammatory agents production, or the modulation of microglial cell migration (Kelly et al., 2020).

As previously reported, many studies identified immune dysfunction in ASD and in particular there is evidence of chronic neuroinflammation, involving the altered spatial organization and morphological changes of microglia. These changes would impact on the normal functionality of the CNS immune system, with the loss of homeostasis and alterations in synaptic plasticity, leading to core ASD traits (Matta et al., 2019).

Neuroinflammation in ASD could be thus based on an imbalance of primed M1 microglia and M2 alternative states, with anti-inflammatory functions (Nakagawa and Chiba, 2016).

Furthermore, the ECS dysfunctions found at the clinical and preclinical levels strengthen the hypothesis on the link between the cannabinoid immunomodulation and the chronic neuroinflammatory condition in ASD.

This is the reason why non-canonical and canonical cannabinoid receptors, mainly CB2, constitute a promising therapeutic target for ASD, as for many other pathologies induced by inflammatory pathways. Indeed, the administration of cannabinoid compounds, either acting through stimulation of CB2 receptors or through pharmacological inhibition of eCB hydrolysis, improves the eCBSS, thus becoming a valid solution to exert a restorative anti-inflammatory

effect in the CNS (Nagarkatti et al., 2009) and potentially solve some behavioral and cognitive impairments typical of ASD.

6 Conclusions

Autism research has tremendously improved over the last 20 years, but yet we cannot provide definitive medical treatment. Recently, the use of *Cannabis sativa* and its non-psychotomimetic component CBD in children with autism has received increasing attention and a growing number of families with autistic children are considering these products to relieve severe behavioral symptoms, seizures and other challenging autism-related conditions.

Unfortunately, the limited scientific data currently available appear to have resulted in a growing body of anecdotal evidence reporting the benefits of cannabinoids and CBD for children with ASD. In this context, autism research is more important than ever, not only for individuals currently affected with ASD but as well as to support families in managing this disorder.

As emerged along this review, cannabinoids might represent a promising pharmacological tool in treating ASD neuroinflammation and in particular CBD, which is devoid of both the psychotropic side effects induced by direct cannabinoid agonists and the mood alterations induced by first-generation cannabinoid antagonists/inverse agonists.

Increasing our understanding of the neurobiology of ASD, including its neuroinflammatory component, is essential for the development of new effective and safe medications including cannabinoids in the treatment of this neurodevelopmental disorder.

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8 Appendix

Abbreviations:

2-AG	(2-arachidonoylglycerol)
ADHD	(attention-deficit/hyperactivity disorder)
AEA	(anandamide)
ASD	(autism spectrum disorder)
BBB	(blood-brain barrier)
CARS	(Childhood Autism Rating Scale)
CBD	(cannabidiol)
CBDV	(cannabidivarin)
CHARGE	(Childhood Autism Risks from Genetics and Environment)
CNS	(central nervous system)
CSF	(cerebrospinal fluid)
DAGL- α	(diacylglycerol lipase α)
DSM IV	(4th edition of the Diagnostic and Statistical Manual of Mental Disorders)
DSM-5	(5th edition of the Diagnostic and Statistical Manual of Mental Disorders)
eCBs	(endocannabinoids)
eCBSS	(endocannabinoid signaling system)
ECS	(endocannabinoid system)
EEG	(electroencephalogram)
EMA	(European Medicines Agency)
FAAH	(fatty acid amide hydrolase)
FDA	(Food and Drug Administration)
FMR1	(Fragile X mental retardation 1)
Fmr1-KO	(inactivation of the FMR1 gene)
FXS	(Fragile X syndrome)
GABA	(γ-aminobutyric acid)
GF	(germ-free)
GFAP	(glial fibrillary acidic protein)
GSK3	(glycogen synthase kinase 3)
IFN-	(interferon)
IL-	(interleukin)
iNOS	(inducible nitric oxide synthase)
L. reuteri	(Lactobacillus reuteri)
LPS	(lipopolysaccharide)
MAGL	(monoacylglycerol lipase)
MIA	(maternal immune system activation)
MIF	(macrophage migration inhibitory factor)
MRI	(magnetic resonance imaging)
MTT	(Microbiota Transfer Therapy)
NAPE-PLD	(NAPE-hydrolyzing phospholipase D)
NF-κB	(nuclear factor kappa-light-chain-enhancer of activated B cells)
PBMC	(peripheral blood mononuclear cells)
PDD-NOS	(pervasive developmental disorder-not otherwise specified)
PDGF	(platelet derived growth factor)
PET	(positron emission tomography)
PI3K	(phosphatidylinositol 3-kinase)
PNS	(peripheral nervous system)
Poly(I:C)	(polycytidylic acid)

PPI	(prepulse inhibition)
RNS	(reactive nitrogen species)
ROS	(reactive oxygen species)
TD	(typically developing)
TGF-β	(transforming growth factor beta)
THC	(Δ9-tetrahydrocannabinol)
TLR	(toll-like receptor)
TNF-α	(tumor necrosis factor-α)
TSPO	(translocator protein)
USVs	(ultrasonic vocalizations)
VPA	(valproic acid)

9 References

Abou-Donia, M.B., Suliman, H.B., Siniscalco, D., Antonucci, N., ElKafrawy, P., 2019. De novo Blood Biomarkers in Autism: Autoantibodies against Neuronal and Glial Proteins. Behav Sci (Basel) 9.

Adams, K.L., Gallo, V., 2018. The diversity and disparity of the glial scar. Nat Neurosci 21, 9-15.

Alabdali, A., Al-Ayadhi, L., El-Ansary, A., 2014. Association of social and cognitive impairment and biomarkers in autism spectrum disorders. J Neuroinflammation 11, 4.

American Psychiatric Association, a., 2013. Diagnostic and Statistical Manual of Mental Disorders, 5th ed, Washington, DC.

Andrade, C., 2018. Valproate in Pregnancy: Recent Research and Regulatory Responses. J Clin Psychiatry 79.

Aran, A., Cassuto, H., Lubotzky, A., Wattad, N., Hazan, E., 2019. Brief Report: Cannabidiol-Rich Cannabis in Children with Autism Spectrum Disorder and Severe Behavioral Problems-A Retrospective Feasibility Study. J Autism Dev Disord 49, 1284-1288.

Araujo, D.J., Tjoa, K., Saijo, K., 2019. The Endocannabinoid System as a Window Into Microglial Biology and Its Relationship to Autism. Front Cell Neurosci 13, 424.

Artuso, R., Papa, F.T., Grillo, E., Mucciolo, M., Yasui, D.H., Dunaway, K.W., Disciglio, V., Mencarelli, M.A., Pollazzon, M., Zappella, M., Hayek, G., Mari, F., Renieri, A., Lasalle, J.M., Ariani, F., 2011. Investigation of modifier genes within copy number variations in Rett syndrome. J Hum Genet 56, 508-515.

Ashwood, P., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Pessah, I., Van de Water, J., 2011. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 25, 40-45.

Ashwood, P., Nguyen, D.V., Hessl, D., Hagerman, R.J., Tassone, F., 2010. Plasma cytokine profiles in Fragile X subjects: is there a role for cytokines in the pathogenesis? Brain Behav Immun 24, 898-902.

Asiminas, A., Jackson, A.D., Louros, S.R., Till, S.M., Spano, T., Dando, O., Bear, M.F., Chattarji, S., Hardingham, G.E., Osterweil, E.K., Wyllie, D.J.A., Wood, E.R., Kind, P.C., 2019. Sustained correction of associative learning deficits after brief, early treatment in a rat model of Fragile X Syndrome. Sci Transl Med 11.

Atladottir, H.O., Henriksen, T.B., Schendel, D.E., Parner, E.T., 2012. Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. Pediatrics 130, e1447-1454.

Atwood, B.K., Mackie, K., 2010. CB2: a cannabinoid receptor with an identity crisis. Br J Pharmacol 160, 467-479.

Babson, K.A., Sottile, J., Morabito, D., 2017. Cannabis, Cannabinoids, and Sleep: a Review of the Literature. Curr Psychiatry Rep 19, 23.

Baharnoori, M., Bhardwaj, S.K., Srivastava, L.K., 2012. Neonatal behavioral changes in rats with gestational exposure to lipopolysaccharide: a prenatal infection model for developmental neuropsychiatric disorders. Schizophr Bull 38, 444-456.

Bar-Lev Schleider, L., Mechoulam, R., Saban, N., Meiri, G., Novack, V., 2019. Real life Experience of Medical Cannabis Treatment in Autism: Analysis of Safety and Efficacy. Sci Rep 9, 200.

Bara, A., Manduca, A., Bernabeu, A., Borsoi, M., Serviado, M., Lassalle, O., Murphy, M., Wager-Miller, J., Mackie, K., Pelissier-Alicot, A.L., Trezza, V., Manzoni, O.J., 2018. Sex-dependent effects of in utero cannabinoid exposure on cortical function. Elife 7.

Barchel, D., Stolar, O., De-Haan, T., Ziv-Baran, T., Saban, N., Fuchs, D.O., Koren, G., Berkovitch, M., 2018. Oral Cannabidiol Use in Children With Autism Spectrum Disorder to Treat Related Symptoms and Co-morbidities. Front Pharmacol 9, 1521.

Bauman, M.D., Iosif, A.M., Smith, S.E., Bregere, C., Amaral, D.G., Patterson, P.H., 2014. Activation of the maternal immune system during pregnancy alters behavioral development of rhesus monkey offspring. Biol Psychiatry 75, 332-341.

Berzhanskaya, J., Phillips, M.A., Gorin, A., Lai, C., Shen, J., Colonnese, M.T., 2017a. Disrupted Cortical State Regulation in a Rat Model of Fragile X Syndrome. Cereb Cortex 27, 1386-1400.

Berzhanskaya, J., Phillips, M.A., Shen, J., Colonnese, M.T., 2017b. Erratum: Sensory hypo-excitability in a rat model of fetal development in Fragile X Syndrome. Sci Rep 7, 44515.

Beurel, E., Grieco, S.F., Jope, R.S., 2015. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. Pharmacol Ther 148, 114-131.

Bilbo, S.D., Block, C.L., Bolton, J.L., Hanamsagar, R., Tran, P.K., 2018. Beyond infection - Maternal immune activation by environmental factors, microglial development, and relevance for autism spectrum disorders. Exp Neurol 299, 241-251.

Bisogno, T., Di Marzo, V., 2010. Cannabinoid receptors and endocannabinoids: role in neuroinflammatory and neurodegenerative disorders. CNS Neurol Disord Drug Targets 9, 564-573.

Boksa, P., 2010. Effects of prenatal infection on brain development and behavior: a review of findings from animal models. Brain Behav Immun 24, 881-897.

Booz, G.W., 2011. Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. Free Radic Biol Med 51, 1054-1061.

Bou Khalil, R., 2012. Would some cannabinoids ameliorate symptoms of autism? Eur Child Adolesc Psychiatry 21, 237-238.

Braunschweig, D., Ashwood, P., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Croen, L.A., Pessah, I.N., Van de Water, J., 2008. Autism: maternally derived antibodies specific for fetal brain proteins. Neurotoxicology 29, 226-231.

Braunschweig, D., Krakowiak, P., Duncanson, P., Boyce, R., Hansen, R.L., Ashwood, P., Hertz-Picciotto, I., Pessah, I.N., Van de Water, J., 2013. Autism-specific maternal autoantibodies recognize critical proteins in developing brain. Transl Psychiatry 3, e277.

Braunschweig, D., Van de Water, J., 2012. Maternal autoantibodies in autism. Arch Neurol 69, 693-699.

Brigida, A.L., Schultz, S., Cascone, M., Antonucci, N., Siniscalco, D., 2017. Endocannabinod Signal Dysregulation in Autism Spectrum Disorders: A Correlation Link between Inflammatory State and Neuro-Immune Alterations. Int J Mol Sci 18.

Brimberg, L., Sadiq, A., Gregersen, P.K., Diamond, B., 2013. Brain-reactive IgG correlates with autoimmunity in mothers of a child with an autism spectrum disorder. Mol Psychiatry 18, 1171-1177.

Brocker, C., Thompson, D., Matsumoto, A., Nebert, D.W., Vasiliou, V., 2010. Evolutionary divergence and functions of the human interleukin (IL) gene family. Hum Genomics 5, 30-55.

Bronzuoli, M.R., Facchinetti, R., Ingrassia, D., Sarvadio, M., Schiavi, S., Steardo, L., Verkhratsky, A., Trezza, V., Scuderi, C., 2018. Neuroglia in the autistic brain: evidence from a preclinical model. Mol Autism 9, 66.

Brucato, M., Ladd-Acosta, C., Li, M., Caruso, D., Hong, X., Kaczaniuk, J., Stuart, E.A., Fallin, M.D., Wang, X., 2017. Prenatal exposure to fever is associated with autism spectrum disorder in the boston birth cohort. Autism Res 10, 1878-1890.

Buffington, S.A., Di Prisco, G.V., Auchtung, T.A., Ajami, N.J., Petrosino, J.F., Costa-Mattioli, M., 2016. Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. Cell 165, 1762-1775.

Busquets-Garcia, A., Gomis-Gonzalez, M., Guegan, T., Agustin-Pavon, C., Pastor, A., Mato, S., Perez-Samartin, A., Matute, C., de la Torre, R., Dierssen, M., Maldonado, R., Ozaita, A., 2013. Targeting the endocannabinoid system in the treatment of fragile X syndrome. Nat Med 19, 603-607.

Cabral, G.A., Ferreira, G.A., Jamerson, M.J., 2015. Endocannabinoids and the Immune System in Health and Disease. Handb Exp Pharmacol 231, 185-211.

Cabral, G.A., Griffin-Thomas, L., 2009. Emerging role of the cannabinoid receptor CB2 in immune regulation: therapeutic prospects for neuroinflammation. Expert Rev Mol Med 11, e3.

Caldwell, H.K., Albers, H.E., 2016. Oxytocin, Vasopressin, and the Motivational Forces that Drive Social Behaviors. Curr Top Behav Neurosci 27, 51-103.

Careaga, M., Ashwood, P., 2012. Autism spectrum disorders: from immunity to behavior. Methods Mol Biol 934, 219-240.

Careaga, M., Murai, T., Bauman, M.D., 2017. Maternal Immune Activation and Autism Spectrum Disorder: From Rodents to Nonhuman and Human Primates. Biol Psychiatry 81, 391-401.

Cassol, O.J., Jr., Comim, C.M., Silva, B.R., Hermani, F.V., Constantino, L.S., Felisberto, F., Petronilho, F., Hallak, J.E., De Martinis, B.S., Zuardi, A.W., Crippa, J.A., Quevedo, J., Dal-Pizzol, F., 2010. Treatment with cannabidiol reverses oxidative stress parameters, cognitive impairment and mortality in rats submitted to sepsis by cecal ligation and puncture. Brain Res 1348, 128-138.

Chakrabarti, B., Persico, A., Battista, N., Maccarrone, M., 2015. Endocannabinoid Signaling in Autism. Neurotherapeutics 12, 837-847.

Chan, W.Y., Kohsaka, S., Rezaie, P., 2007. The origin and cell lineage of microglia: new concepts. Brain Res Rev 53, 344-354.

Chaste, P., Leboyer, M., 2012. Autism risk factors: genes, environment, and gene-environment interactions. Dialogues Clin Neurosci 14, 281-292.

Chess, S., 1971. Autism in children with congenital rubella. J Autism Child Schizophr 1, 33-47.

Choi, G.B., Yim, Y.S., Wong, H., Kim, S., Kim, H., Kim, S.V., Hoeffer, C.A., Littman, D.R., Huh, J.R., 2016. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. Science 351, 933-939.

Chomiak, T., Turner, N., Hu, B., 2013. What We Have Learned about Autism Spectrum Disorder from Valproic Acid. Patholog Res Int 2013, 712758.

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R.D., Shanahan, F., Dinan, T.G., Cryan, J.F., 2013. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Mol Psychiatry 18, 666-673.

Codagnone, M.G., Podesta, M.F., Uccelli, N.A., Reines, A., 2015. Differential Local Connectivity and Neuroinflammation Profiles in the Medial Prefrontal Cortex and Hippocampus in the Valproic Acid Rat Model of Autism. Dev Neurosci 37, 215-231.

Connor, S.A., Hoeffer, C.A., Klann, E., Nguyen, P.V., 2011. Fragile X mental retardation protein regulates heterosynaptic plasticity in the hippocampus. Learn Mem 18, 207-220.

Cooper, R.E., Williams, E., Seegobin, S., Tye, C., Kuntsi, J., Asherson, P., 2017. Cannabinoids in attention-deficit/hyperactivity disorder: A randomised-controlled trial. Eur Neuropsychopharmacol 27, 795-808.

Couch, D.G., Tasker, C., Theophilidou, E., Lund, J.N., O'Sullivan, S.E., 2017. Cannabidiol and palmitoylethanolamide are anti-inflammatory in the acutely inflamed human colon. Clin Sci (Lond) 131, 2611-2626.

Crawley, J.N., 2012. Translational animal models of autism and neurodevelopmental disorders. Dialogues Clin Neurosci 14, 293-305.

Cristiano, C., Lama, A., Lembo, F., Mollica, M.P., Calignano, A., Mattace Raso, G., 2018. Interplay Between Peripheral and Central Inflammation in Autism Spectrum Disorders: Possible Nutritional and Therapeutic Strategies. Front Physiol 9, 184.

Croen, L.A., Braunschweig, D., Haapanen, L., Yoshida, C.K., Fireman, B., Grether, J.K., Kharrazi, M., Hansen, R.L., Ashwood, P., Van de Water, J., 2008. Maternal mid-pregnancy autoantibodies to fetal brain protein: the early markers for autism study. Biol Psychiatry 64, 583-588.

Croen, L.A., Qian, Y., Ashwood, P., Zerbo, O., Schendel, D., Pinto-Martin, J., Daniele Fallin, M., Levy, S., Schieve, L.A., Yeargin-Allsopp, M., Sabourin, K.R., Ames, J.L., 2019. Infection and Fever in Pregnancy and Autism Spectrum Disorders: Findings from the Study to Explore Early Development. Autism Res 12, 1551-1561.

Cunningham, C.L., Martinez-Cerdeno, V., Noctor, S.C., 2013. Microglia regulate the number of neural precursor cells in the developing cerebral cortex. J Neurosci 33, 4216-4233.

de Magistris, L., Familiari, V., Pascotto, A., Sapone, A., Frolli, A., Iardino, P., Carteni, M., De Rosa, M., Francavilla, R., Riegler, G., Militerni, R., Bravaccio, C., 2010. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J Pediatr Gastroenterol Nutr 51, 418-424.

de Magistris, L., Picardi, A., Siniscalco, D., Riccio, M.P., Sapone, A., Cariello, R., Abbadessa, S., Medici, N., Lammers, K.M., Schiraldi, C., Iardino, P., Marotta, R., Tolone, C., Fasano, A.,

Pascotto, A., Bravaccio, C., 2013. Antibodies against food antigens in patients with autistic spectrum disorders. Biomed Res Int 2013, 729349.

de Souza, D.F., Wartchow, K.M., Lunardi, P.S., Brolese, G., Tortorelli, L.S., Batassini, C., Biasibetti, R., Goncalves, C.A., 2015. Changes in Astroglial Markers in a Maternal Immune Activation Model of Schizophrenia in Wistar Rats are Dependent on Sex. Front Cell Neurosci 9, 489.

Delpech, J.C., Madore, C., Nadjar, A., Joffre, C., Wohleb, E.S., Laye, S., 2015. Microglia in neuronal plasticity: Influence of stress. Neuropharmacology 96, 19-28.

Depino, A.M., 2015. Early prenatal exposure to LPS results in anxiety- and depression-related behaviors in adulthood. Neuroscience 299, 56-65.

Devinsky, O., Cilio, M.R., Cross, H., Fernandez-Ruiz, J., French, J., Hill, C., Katz, R., Di Marzo, V., Jutras-Aswad, D., Notcutt, W.G., Martinez-Orgado, J., Robson, P.J., Rohrback, B.G., Thiele, E., Whalley, B., Friedman, D., 2014. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. Epilepsia 55, 791-802.

Devinsky, O., Cross, J.H., Laux, L., Marsh, E., Miller, I., Nabbout, R., Scheffer, I.E., Thiele, E.A., Wright, S., Cannabidiol in Dravet Syndrome Study, G., 2017. Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. N Engl J Med 376, 2011-2020.

Devinsky, O., Marsh, E., Friedman, D., Thiele, E., Laux, L., Sullivan, J., Miller, I., Flamini, R., Wilfong, A., Filloux, F., Wong, M., Tilton, N., Bruno, P., Bluvstein, J., Hedlund, J., Kamens, R., Maclean, J., Nangia, S., Singhal, N.S., Wilson, C.A., Patel, A., Cilio, M.R., 2016. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. Lancet Neurol 15, 270-278.

Devinsky, O., Nabbout, R., Miller, I., Laux, L., Zolnowska, M., Wright, S., Roberts, C., 2019. Long-term cannabidiol treatment in patients with Dravet syndrome: An open-label extension trial. Epilepsia 60, 294-302.

Devinsky, O., Patel, A.D., Thiele, E.A., Wong, M.H., Appleton, R., Harden, C.L., Greenwood, S., Morrison, G., Sommerville, K., Group, G.P.A.S., 2018. Randomized, dose-ranging safety trial of cannabidiol in Dravet syndrome. Neurology 90, e1204-e1211.

Deykin, E.Y., MacMahon, B., 1979. Viral exposure and autism. Am J Epidemiol 109, 628-638.

Di Marzo, V., Bifulco, M., De Petrocellis, L., 2004. The endocannabinoid system and its therapeutic exploitation. Nat Rev Drug Discov 3, 771-784.

Dinan, T.G., Cryan, J.F., 2017. The Microbiome-Gut-Brain Axis in Health and Disease. Gastroenterol Clin North Am 46, 77-89.

Dipasquale, V., Cutrupi, M.C., Colavita, L., Manti, S., Cuppari, C., Salpietro, C., 2017. Neuroinflammation in Autism Spectrum Disorders: Role of High Mobility Group Box 1 Protein. Int J Mol Cell Med 6, 148-155.

Doenyas, C., 2018. Gut Microbiota, Inflammation, and Probiotics on Neural Development in Autism Spectrum Disorder. Neuroscience 374, 271-286.

Dolen, G., Osterweil, E., Rao, B.S., Smith, G.B., Auerbach, B.D., Chattarji, S., Bear, M.F., 2007. Correction of fragile X syndrome in mice. Neuron 56, 955-962.

Donovan, A.P., Basson, M.A., 2017. The neuroanatomy of autism - a developmental perspective. J Anat 230, 4-15.

Dowling, O., Chatterjee, P.K., Gupta, M., Tam Tam, H.B., Xue, X., Lewis, D., Rochelson, B., Metz, C.N., 2012. Magnesium sulfate reduces bacterial LPS-induced inflammation at the maternal-fetal interface. Placenta 33, 392-398.

Eftekharian, M.M., Ghafouri-Fard, S., Noroozi, R., Omrani, M.D., Arsang-Jang, S., Ganji, M., Gharzi, V., Noroozi, H., Komaki, A., Mazdeh, M., Taheri, M., 2018. Cytokine profile in autistic patients. Cytokine 108, 120-126.

El-Ansary, A., Al-Ayadhi, L., 2012. Neuroinflammation in autism spectrum disorders. J Neuroinflammation 9, 265.

Enstrom, A.M., Onore, C.E., Van de Water, J.A., Ashwood, P., 2010. Differential monocyte responses to TLR ligands in children with autism spectrum disorders. Brain Behav Immun 24, 64-71.

Esnafoglu, E., Ayyildiz, S.N., Cirrik, S., Erturk, E.Y., Erdil, A., Dagli, A., Noyan, T., 2017. Evaluation of serum Neuron-specific enolase, S100B, myelin basic protein and glial fibrilliary acidic protein as brain specific proteins in children with autism spectrum disorder. Int J Dev Neurosci 61, 86-91.

Estes, M.L., McAllister, A.K., 2015. Immune mediators in the brain and peripheral tissues in autism spectrum disorder. Nat Rev Neurosci 16, 469-486.

Fatemi, S.H., Aldinger, K.A., Ashwood, P., Bauman, M.L., Blaha, C.D., Blatt, G.J., Chauhan, A., Chauhan, V., Dager, S.R., Dickson, P.E., Estes, A.M., Goldowitz, D., Heck, D.H., Kemper, T.L., King, B.H., Martin, L.A., Millen, K.J., Mittleman, G., Mosconi, M.W., Persico, A.M., Sweeney, J.A., Webb, S.J., Welsh, J.P., 2012. Consensus paper: pathological role of the cerebellum in autism. Cerebellum 11, 777-807.

Fernandez de Cossio, L., Guzman, A., van der Veldt, S., Luheshi, G.N., 2017. Prenatal infection leads to ASD-like behavior and altered synaptic pruning in the mouse offspring. Brain Behav Immun 63, 88-98.

Filiano, A.J., Gadani, S.P., Kipnis, J., 2015. Interactions of innate and adaptive immunity in brain development and function. Brain Res 1617, 18-27.

Finegold, S.M., Molitoris, D., Song, Y., Liu, C., Vaisanen, M.L., Bolte, E., McTeague, M., Sandler, R., Wexler, H., Marlowe, E.M., Collins, M.D., Lawson, P.A., Summanen, P., Baysallar, M., Tomzynski, T.J., Read, E., Johnson, E., Rolfe, R., Nasir, P., Shah, H., Haake, D.A., Manning, P., Kaul, A., 2002. Gastrointestinal microflora studies in late-onset autism. Clin Infect Dis 35, S6-S16.

Fiorentino, M., Sapone, A., Senger, S., Camhi, S.S., Kadzielski, S.M., Buie, T.M., Kelly, D.L., Cascella, N., Fasano, A., 2016. Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. Mol Autism 7, 49.

Fowlie, G., Cohen, N., Ming, X., 2018. The Perturbance of Microbiome and Gut-Brain Axis in Autism Spectrum Disorders. Int J Mol Sci 19.

Franco, R., Fernandez-Suarez, D., 2015. Alternatively activated microglia and macrophages in the central nervous system. Prog Neurobiol 131, 65-86.

Friedman, D., French, J.A., Maccarrone, M., 2019. Safety, efficacy, and mechanisms of action of cannabinoids in neurological disorders. Lancet Neurol 18, 504-512.

Gandal, M.J., Haney, J.R., Parikshak, N.N., Leppa, V., Ramaswami, G., Hartl, C., Schork, A.J., Appadurai, V., Buil, A., Werge, T.M., Liu, C., White, K.P., CommonMind, C., Psych, E.C., i, P.-B.W.G., Horvath, S., Geschwind, D.H., 2018. Shared molecular neuropathology across major psychiatric disorders parallels polygenic overlap. Science 359, 693-697.

Ghaziuddin, M., Tsai, L.Y., Eilers, L., Ghaziuddin, N., 1992. Brief report: autism and herpes simplex encephalitis. J Autism Dev Disord 22, 107-113.

Gogolla, N., Leblanc, J.J., Quast, K.B., Sudhof, T.C., Fagiolini, M., Hensch, T.K., 2009. Common circuit defect of excitatory-inhibitory balance in mouse models of autism. J Neurodev Disord 1, 172-181.

Goines, P., Haapanen, L., Boyce, R., Duncanson, P., Braunschweig, D., Delwiche, L., Hansen, R., Hertz-Picciotto, I., Ashwood, P., Van de Water, J., 2011. Autoantibodies to cerebellum in children with autism associate with behavior. Brain Behav Immun 25, 514-523.

Golan, H.M., Lev, V., Hallak, M., Sorokin, Y., Huleihel, M., 2005. Specific neurodevelopmental damage in mice offspring following maternal inflammation during pregnancy. Neuropharmacology 48, 903-917.

Gomis-Gonzalez, M., Busquets-Garcia, A., Matute, C., Maldonado, R., Mato, S., Ozaita, A., 2016. Possible Therapeutic Doses of Cannabinoid Type 1 Receptor Antagonist Reverses Key Alterations in Fragile X Syndrome Mouse Model. Genes (Basel) 7.

Graciarena, M., Seiffe, A., Nait-Oumesmar, B., Depino, A.M., 2018. Hypomyelination and Oligodendroglial Alterations in a Mouse Model of Autism Spectrum Disorder. Front Cell Neurosci 12, 517.

Grossman, A.W., Aldridge, G.M., Lee, K.J., Zeman, M.K., Jun, C.S., Azam, H.S., Arii, T., Imoto, K., Greenough, W.T., Rhyu, I.J., 2010. Developmental characteristics of dendritic spines in the dentate gyrus of Fmr1 knockout mice. Brain Res 1355, 221-227.

Guloksuz, S.A., Abali, O., Aktas Cetin, E., Bilgic Gazioglu, S., Deniz, G., Yildirim, A., Kawikova, I., Guloksuz, S., Leckman, J.F., 2017. Elevated plasma concentrations of S100 calcium-binding protein B and tumor necrosis factor alpha in children with autism spectrum disorders. Braz J Psychiatry 39, 195-200.

Hagerman, R., J., Hagerman, P., J., 2002. Fragile X Syndrome: Diagnosis, Treatment, and Research, 3rd ed.

Hao, L.Y., Hao, X.Q., Li, S.H., Li, X.H., 2010. Prenatal exposure to lipopolysaccharide results in cognitive deficits in age-increasing offspring rats. Neuroscience 166, 763-770.

Hornig, M., Bresnahan, M.A., Che, X., Schultz, A.F., Ukaigwe, J.E., Eddy, M.L., Hirtz, D., Gunnes, N., Lie, K.K., Magnus, P., Mjaaland, S., Reichborn-Kjennerud, T., Schjolberg, S., Oyen, A.S., Levin, B., Susser, E.S., Stoltenberg, C., Lipkin, W.I., 2018. Prenatal fever and autism risk. Mol Psychiatry 23, 759-766.

Hsueh, P.T., Wang, H.H., Liu, C.L., Ni, W.F., Chen, Y.L., Liu, J.K., 2017. Expression of cerebral serotonin related to anxiety-like behaviors in C57BL/6 offspring induced by repeated subcutaneous prenatal exposure to low-dose lipopolysaccharide. PLoS One 12, e0179970.

Hughes, H.K., Rose, D., Ashwood, P., 2018. The Gut Microbiota and Dysbiosis in Autism Spectrum Disorders. Curr Neurol Neurosci Rep 18, 81.

Inga Jacome, M.C., Morales Chacon, L.M., Vera Cuesta, H., Maragoto Rizo, C., Whilby Santiesteban, M., Ramos Hernandez, L., Noris Garcia, E., Gonzalez Fraguela, M.E., Fernandez Verdecia, C.I., Vegas Hurtado, Y., Siniscalco, D., Goncalves, C.A., Robinson-Agramonte, M.L., 2016. Peripheral Inflammatory Markers Contributing to Comorbidities in Autism. Behav Sci (Basel) 6.

Iovene, M.R., Bombace, F., Maresca, R., Sapone, A., Iardino, P., Picardi, A., Marotta, R., Schiraldi, C., Siniscalco, D., Serra, N., de Magistris, L., Bravaccio, C., 2017. Intestinal Dysbiosis and Yeast Isolation in Stool of Subjects with Autism Spectrum Disorders. Mycopathologia 182, 349-363.

Jayaraj, R.L., Azimullah, S., Beiram, R., Jalal, F.Y., Rosenberg, G.A., 2019. Neuroinflammation: friend and foe for ischemic stroke. J Neuroinflammation 16, 142.

Jeste, S.S., Geschwind, D.H., 2014. Disentangling the heterogeneity of autism spectrum disorder through genetic findings. Nat Rev Neurol 10, 74-81.

Jiang, H.Y., Xu, L.L., Shao, L., Xia, R.M., Yu, Z.H., Ling, Z.X., Yang, F., Deng, M., Ruan, B., 2016. Maternal infection during pregnancy and risk of autism spectrum disorders: A systematic review and meta-analysis. Brain Behav Immun 58, 165-172.

Jiang, N.M., Cowan, M., Moonah, S.N., Petri, W.A., Jr., 2018. The Impact of Systemic Inflammation on Neurodevelopment. Trends Mol Med 24, 794-804.

Jonakait, G.M., 2007. The effects of maternal inflammation on neuronal development: possible mechanisms. Int J Dev Neurosci 25, 415-425.

Jung, K.M., Sepers, M., Henstridge, C.M., Lassalle, O., Neuhofer, D., Martin, H., Ginger, M., Frick, A., DiPatrizio, N.V., Mackie, K., Katona, I., Piomelli, D., Manzoni, O.J., 2012. Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. Nat Commun 3, 1080.

Jyonouchi, H., Geng, L., Ruby, A., Zimmerman-Bier, B., 2005. Dysregulated innate immune responses in young children with autism spectrum disorders: their relationship to gastrointestinal symptoms and dietary intervention. Neuropsychobiology 51, 77-85.

Kalkman, H.O., Feuerbach, D., 2017. Microglia M2A Polarization as Potential Link between Food Allergy and Autism Spectrum Disorders. Pharmaceuticals (Basel) 10.

Kang, D.W., Adams, J.B., Coleman, D.M., Pollard, E.L., Maldonado, J., McDonough-Means, S., Caporaso, J.G., Krajmalnik-Brown, R., 2019. Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. Sci Rep 9, 5821.

Kang, D.W., Adams, J.B., Gregory, A.C., Borody, T., Chittick, L., Fasano, A., Khoruts, A., Geis, E., Maldonado, J., McDonough-Means, S., Pollard, E.L., Roux, S., Sadowsky, M.J., Lipson, K.S., Sullivan, M.B., Caporaso, J.G., Krajmalnik-Brown, R., 2017. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. Microbiome 5, 10.

Kaplan, J.S., Stella, N., Catterall, W.A., Westenbroek, R.E., 2017. Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. Proc Natl Acad Sci U S A 114, 11229-11234.

Karhson, D.S., Krasinska, K.M., Dallaire, J.A., Libove, R.A., Phillips, J.M., Chien, A.S., Garner, J.P., Hardan, A.Y., Parker, K.J., 2018. Plasma anandamide concentrations are lower in children with autism spectrum disorder. Mol Autism 9, 18.

Karimi, P., Kamali, E., Mousavi, S.M., Karahmadi, M., 2017. Environmental factors influencing the risk of autism. J Res Med Sci 22, 27.

Kataoka, S., Takuma, K., Hara, Y., Maeda, Y., Ago, Y., Matsuda, T., 2013. Autism-like behaviours with transient histone hyperacetylation in mice treated prenatally with valproic acid. Int J Neuropsychopharmacol 16, 91-103.

Kealy, J., Greene, C., Campbell, M., 2020. Blood-brain barrier regulation in psychiatric disorders. Neurosci Lett 726, 133664.

Kelly, R., Joers, V., Tansey, M.G., McKernan, D.P., Dowd, E., 2020. Microglial Phenotypes and Their Relationship to the Cannabinoid System: Therapeutic Implications for Parkinson's Disease. Molecules 25.

Kentner, A.C., Khoury, A., Lima Queiroz, E., MacRae, M., 2016. Environmental enrichment rescues the effects of early life inflammation on markers of synaptic transmission and plasticity. Brain Behav Immun 57, 151-160.

Kerr, D.M., Downey, L., Conboy, M., Finn, D.P., Roche, M., 2013. Alterations in the endocannabinoid system in the rat valproic acid model of autism. Behav Brain Res 249, 124-132.

Kerr, D.M., Gilmartin, A., Roche, M., 2016. Pharmacological inhibition of fatty acid amide hydrolase attenuates social behavioural deficits in male rats prenatally exposed to valproic acid. Pharmacol Res 113, 228-235.

Khan, A.A., Shekh-Ahmad, T., Khalil, A., Walker, M.C., Ali, A.B., 2018. Cannabidiol exerts antiepileptic effects by restoring hippocampal interneuron functions in a temporal lobe epilepsy model. Br J Pharmacol 175, 2097-2115.

Kim, Y.S., Leventhal, B.L., 2015. Genetic epidemiology and insights into interactive genetic and environmental effects in autism spectrum disorders. Biol Psychiatry 77, 66-74.

Kirsten, T.B., Bernardi, M.M., 2017. Prenatal lipopolysaccharide induces hypothalamic dopaminergic hypoactivity and autistic-like behaviors: Repetitive self-grooming and stereotypies. Behav Brain Res 331, 25-29.

Kirsten, T.B., Chaves-Kirsten, G.P., Chaible, L.M., Silva, A.C., Martins, D.O., Britto, L.R., Dagli, M.L., Torrao, A.S., Palermo-Neto, J., Bernardi, M.M., 2012. Hypoactivity of the central dopaminergic system and autistic-like behavior induced by a single early prenatal exposure to lipopolysaccharide. J Neurosci Res 90, 1903-1912.

Kirsten, T.B., Lippi, L.L., Bevilacqua, E., Bernardi, M.M., 2013. LPS exposure increases maternal corticosterone levels, causes placental injury and increases IL-1Beta levels in adult rat offspring: relevance to autism. PLoS One 8, e82244.

Kirsten, T.B., Queiroz-Hazarbassanov, N., Bernardi, M.M., Felicio, L.F., 2015. Prenatal zinc prevents communication impairments and BDNF disturbance in a rat model of autism induced by prenatal lipopolysaccharide exposure. Life Sci 130, 12-17.

Knuesel, I., Chicha, L., Britschgi, M., Schobel, S.A., Bodmer, M., Hellings, J.A., Toovey, S., Prinssen, E.P., 2014. Maternal immune activation and abnormal brain development across CNS disorders. Nat Rev Neurol 10, 643-660.

Koyama, R., Ikegaya, Y., 2015. Microglia in the pathogenesis of autism spectrum disorders. Neurosci Res 100, 1-5.

Lai, M.C., Lombardo, M.V., Baron-Cohen, S., 2014. Autism. Lancet 383, 896-910.

Lammert, C.R., Lukens, J.R., 2019. Modeling Autism-Related Disorders in Mice with Maternal Immune Activation (MIA). Methods Mol Biol 1960, 227-236.

Lattanzi, S., Brigo, F., Cagnetti, C., Trinka, E., Silvestrini, M., 2018. Efficacy and Safety of Adjunctive Cannabidiol in Patients with Lennox-Gastaut Syndrome: A Systematic Review and Meta-Analysis. CNS Drugs 32, 905-916.

Lau-Zhu, A., Fritz, A., McLoughlin, G., 2019. Overlaps and distinctions between attention deficit/hyperactivity disorder and autism spectrum disorder in young adulthood: Systematic review and guiding framework for EEG-imaging research. Neurosci Biobehav Rev 96, 93-115.

Lee, J.L.C., Bertoglio, L.J., Guimaraes, F.S., Stevenson, C.W., 2017. Cannabidiol regulation of emotion and emotional memory processing: relevance for treating anxiety-related and substance abuse disorders. Br J Pharmacol 174, 3242-3256.

Lehrman, E.K., Wilton, D.K., Litvina, E.Y., Welsh, C.A., Chang, S.T., Frouin, A., Walker, A.J., Heller, M.D., Umemori, H., Chen, C., Stevens, B., 2018. CD47 Protects Synapses from Excess Microglia-Mediated Pruning during Development. Neuron 100, 120-134 e126.

Lenz, K.M., Nelson, L.H., 2018. Microglia and Beyond: Innate Immune Cells As Regulators of Brain Development and Behavioral Function. Front Immunol 9, 698.

Leweke, F.M., Piomelli, D., Pahlisch, F., Muhl, D., Gerth, C.W., Hoyer, C., Klosterkotter, J., Hellmich, M., Koethe, D., 2012. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl Psychiatry 2, e94.

Li, X., Chauhan, A., Sheikh, A.M., Patil, S., Chauhan, V., Li, X.M., Ji, L., Brown, T., Malik, M., 2009. Elevated immune response in the brain of autistic patients. J Neuroimmunol 207, 111-116.

Ligresti, A., De Petrocellis, L., Di Marzo, V., 2016. From Phytocannabinoids to Cannabinoid Receptors and Endocannabinoids: Pleiotropic Physiological and Pathological Roles Through Complex Pharmacology. Physiol Rev 96, 1593-1659.

Lin, Y.L., Lin, S.Y., Wang, S., 2012. Prenatal lipopolysaccharide exposure increases anxiety-like behaviors and enhances stress-induced corticosterone responses in adult rats. Brain Behav Immun 26, 459-468.

Luchicchi, A., Lecca, S., Melis, M., De Felice, M., Cadeddu, F., Frau, R., Muntoni, A.L., Fadda, P., Devoto, P., Pistis, M., 2016. Maternal Immune Activation Disrupts Dopamine System in the Offspring. Int J Neuropsychopharmacol 19.

Lunn, C.A., Reich, E.P., Bober, L., 2006. Targeting the CB2 receptor for immune modulation. Expert Opin Ther Targets 10, 653-663.

Maccarrone, M., Rossi, S., Bari, M., De Chiara, V., Rapino, C., Musella, A., Bernardi, G., Bagni, C., Centonze, D., 2010. Abnormal mGlu 5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. Neuropsychopharmacology 35, 1500-1509.

Madsen-Bouterse, S.A., Romero, R., Tarca, A.L., Kusanovic, J.P., Espinoza, J., Kim, C.J., Kim, J.S., Edwin, S.S., Gomez, R., Draghici, S., 2010. The transcriptome of the fetal inflammatory response syndrome. Am J Reprod Immunol 63, 73-92.

Mahmood, U., Ahn, S., Yang, E.J., Choi, M., Kim, H., Regan, P., Cho, K., Kim, H.S., 2018. Dendritic spine anomalies and PTEN alterations in a mouse model of VPA-induced autism spectrum disorder. Pharmacol Res 128, 110-121.

Malkova, N.V., Yu, C.Z., Hsiao, E.Y., Moore, M.J., Patterson, P.H., 2012. Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. Brain Behav Immun 26, 607-616.

Manduca, A., Lassalle, O., Sepers, M., Campolongo, P., Cuomo, V., Marsicano, G., Kieffer, B., Vanderschuren, L.J., Trezza, V., Manzoni, O.J., 2016a. Interacting Cannabinoid and Opioid Receptors in the Nucleus Accumbens Core Control Adolescent Social Play. Front Behav Neurosci 10, 211.

Manduca, A., Morena, M., Campolongo, P., Servadio, M., Palmery, M., Trabace, L., Hill, M.N., Vanderschuren, L.J., Cuomo, V., Trezza, V., 2015. Distinct roles of the endocannabinoids anandamide and 2-arachidonoylglycerol in social behavior and emotionality at different developmental ages in rats. Eur Neuropsychopharmacol 25, 1362-1374.

Manduca, A., Servadio, M., Damsteegt, R., Campolongo, P., Vanderschuren, L.J., Trezza, V., 2016b. Dopaminergic Neurotransmission in the Nucleus Accumbens Modulates Social Play Behavior in Rats. Neuropsychopharmacology 41, 2215-2223.

Marco, E.M., Laviola, G., 2012. The endocannabinoid system in the regulation of emotions throughout lifespan: a discussion on therapeutic perspectives. J Psychopharmacol 26, 150-163.

Martin-Moreno, A.M., Reigada, D., Ramirez, B.G., Mechoulam, R., Innamorato, N., Cuadrado, A., de Ceballos, M.L., 2011. Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. Mol Pharmacol 79, 964-973.

Martinez-Pinilla, E., Varani, K., Reyes-Resina, I., Angelats, E., Vincenzi, F., Ferreiro-Vera, C., Oyarzabal, J., Canela, E.I., Lanciego, J.L., Nadal, X., Navarro, G., Borea, P.A., Franco, R., 2017. Binding and Signaling Studies Disclose a Potential Allosteric Site for Cannabidiol in Cannabinoid CB2 Receptors. Front Pharmacol 8, 744.

Martinez, F.O., Gordon, S., 2014. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 6, 13.

Matta, S.M., Hill-Yardin, E.L., Crack, P.J., 2019. The influence of neuroinflammation in Autism Spectrum Disorder. Brain Behav Immun 79, 75-90.

McPartland, J.M., Duncan, M., Di Marzo, V., Pertwee, R.G., 2015. Are cannabidiol and Delta(9) -tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br J Pharmacol 172, 737-753.

Mead, J., Ashwood, P., 2015. Evidence supporting an altered immune response in ASD. Immunol Lett 163, 49-55.

Mecha, M., Carrillo-Salinas, F.J., Feliu, A., Mestre, L., Guaza, C., 2016. Microglia activation states and cannabinoid system: Therapeutic implications. Pharmacol Ther 166, 40-55.

Mecha, M., Feliu, A., Carrillo-Salinas, F.J., Rueda-Zubiaurre, A., Ortega-Gutierrez, S., de Sola, R.G., Guaza, C., 2015. Endocannabinoids drive the acquisition of an alternative phenotype in microglia. Brain Behav Immun 49, 233-245.

Mechoulam, R., Peters, M., Murillo-Rodriguez, E., Hanus, L.O., 2007. Cannabidiol--recent advances. Chem Biodivers 4, 1678-1692.

Melancia, F., Schiavi, S., Servadio, M., Cartocci, V., Campolongo, P., Palmery, M., Pallottini, V., Trezza, V., 2018. Sex-specific autistic endophenotypes induced by prenatal exposure to valproic acid involve anandamide signalling. Br J Pharmacol 175, 3699-3712.

Melancia, F., Trezza, V., 2018. Modelling fragile X syndrome in the laboratory setting: A behavioral perspective. Behav Brain Res 350, 149-163.

Meltzer, A., Van de Water, J., 2017. The Role of the Immune System in Autism Spectrum Disorder. Neuropsychopharmacology 42, 284-298.

Meyer, U., Nyffeler, M., Engler, A., Urwyler, A., Schedlowski, M., Knuesel, I., Yee, B.K., Feldon, J., 2006. The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. J Neurosci 26, 4752-4762.

Meyer, U., Nyffeler, M., Yee, B.K., Knuesel, I., Feldon, J., 2008. Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice. Brain Behav Immun 22, 469-486.

Mittal, M., Siddiqui, M.R., Tran, K., Reddy, S.P., Malik, A.B., 2014. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal 20, 1126-1167.

Molloy, C.A., Morrow, A.L., Meinzen-Derr, J., Schleifer, K., Dienger, K., Manning-Courtney, P., Altaye, M., Wills-Karp, M., 2006. Elevated cytokine levels in children with autism spectrum disorder. J Neuroimmunol 172, 198-205.

Morgan, J.T., Chana, G., Abramson, I., Semendeferi, K., Courchesne, E., Everall, I.P., 2012. Abnormal microglial-neuronal spatial organization in the dorsolateral prefrontal cortex in autism. Brain Res 1456, 72-81.

Morgan, J.T., Chana, G., Pardo, C.A., Achim, C., Semendeferi, K., Buckwalter, J., Courchesne, E., Everall, I.P., 2010. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol Psychiatry 68, 368-376.

Mukhopadhyay, P., Rajesh, M., Horvath, B., Batkai, S., Park, O., Tanchian, G., Gao, R.Y., Patel, V., Wink, D.A., Liaudet, L., Hasko, G., Mechoulam, R., Pacher, P., 2011. Cannabidiol protects against hepatic ischemia/reperfusion injury by attenuating inflammatory signaling and response, oxidative/nitrative stress, and cell death. Free Radic Biol Med 50, 1368-1381.

Nagarkatti, P., Pandey, R., Rieder, S.A., Hegde, V.L., Nagarkatti, M., 2009. Cannabinoids as novel anti-inflammatory drugs. Future Med Chem 1, 1333-1349.

Nakagawa, Y., Chiba, K., 2016. Involvement of Neuroinflammation during Brain Development in Social Cognitive Deficits in Autism Spectrum Disorder and Schizophrenia. J Pharmacol Exp Ther 358, 504-515.

Napoli, I., Neumann, H., 2009. Microglial clearance function in health and disease. Neuroscience 158, 1030-1038.

Navarro, F., Liu, Y., Rhoads, J.M., 2016. Can probiotics benefit children with autism spectrum disorders? World J Gastroenterol 22, 10093-10102.

Navarro, F., Pearson, D.A., Fatheree, N., Mansour, R., Hashmi, S.S., Rhoads, J.M., 2015. Are 'leaky gut' and behavior associated with gluten and dairy containing diet in children with autism spectrum disorders? Nutr Neurosci 18, 177-185.

Nicolini, C., Fahnestock, M., 2018. The valproic acid-induced rodent model of autism. Exp Neurol 299, 217-227.

Nithianantharajah, J., Balasuriya, G.K., Franks, A.E., Hill-Yardin, E.L., 2017. Using Animal Models to Study the Role of the Gut-Brain Axis in Autism. Curr Dev Disord Rep 4, 28-36.

Onore, C., Careaga, M., Ashwood, P., 2012. The role of immune dysfunction in the pathophysiology of autism. Brain Behav Immun 26, 383-392.

Onore, C.E., Schwartzer, J.J., Careaga, M., Berman, R.F., Ashwood, P., 2014. Maternal immune activation leads to activated inflammatory macrophages in offspring. Brain Behav Immun 38, 220-226.

Osborne, A.L., Solowij, N., Babic, I., Huang, X.F., Weston-Green, K., 2017. Improved Social Interaction, Recognition and Working Memory with Cannabidiol Treatment in a Prenatal Infection (poly I:C) Rat Model. Neuropsychopharmacology 42, 1447-1457.

Paolicelli, R.C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., Giustetto, M., Ferreira, T.A., Guiducci, E., Dumas, L., Ragozzino, D., Gross, C.T., 2011. Synaptic pruning by microglia is necessary for normal brain development. Science 333, 1456-1458.

Pardo, C.A., Eberhart, C.G., 2007. The neurobiology of autism. Brain Pathol 17, 434-447.

Pardo, C.A., Farmer, C.A., Thurm, A., Shebl, F.M., Ilieva, J., Kalra, S., Swedo, S., 2017. Serum and cerebrospinal fluid immune mediators in children with autistic disorder: a longitudinal study. Mol Autism 8, 1.

Patel, A.B., Tsilioni, I., Leeman, S.E., Theoharides, T.C., 2016. Neurotensin stimulates sortilin and mTOR in human microglia inhibitable by methoxyluteolin, a potential therapeutic target for autism. Proc Natl Acad Sci U S A 113, E7049-E7058.

Patterson, P.H., 2009. Immune involvement in schizophrenia and autism: etiology, pathology and animal models. Behav Brain Res 204, 313-321.

Patterson, P.H., 2011. Maternal infection and immune involvement in autism. Trends Mol Med 17, 389-394.

Pekny, M., Wilhelmsson, U., Pekna, M., 2014. The dual role of astrocyte activation and reactive gliosis. Neurosci Lett 565, 30-38.

Pertwee, R.G., 2008. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol 153, 199-215.

Petrelli, F., Pucci, L., Bezzi, P., 2016. Astrocytes and Microglia and Their Potential Link with Autism Spectrum Disorders. Front Cell Neurosci 10, 21.

Pietropaolo, S., Bellocchio, L., Bouzon-Arnaiz, I., Yee, B.K., 2020. The role of the endocannabinoid system in autism spectrum disorders: Evidence from mouse studies. Prog Mol Biol Transl Sci 173, 183-208.

Piomelli, D., 2003. The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 4, 873-884.

Poleg, S., Golubchik, P., Offen, D., Weizman, A., 2019. Cannabidiol as a suggested candidate for treatment of autism spectrum disorder. Prog Neuropsychopharmacol Biol Psychiatry 89, 90-96.

Prata, J., Machado, A.S., von Doellinger, O., Almeida, M.I., Barbosa, M.A., Coelho, R., Santos, S.G., 2019. The Contribution of Inflammation to Autism Spectrum Disorders: Recent Clinical Evidence. Methods Mol Biol 2011, 493-510.

Prata, J., Santos, S.G., Almeida, M.I., Coelho, R., Barbosa, M.A., 2017. Bridging Autism Spectrum Disorders and Schizophrenia through inflammation and biomarkers - pre-clinical and clinical investigations. J Neuroinflammation 14, 179.

Premoli, M., Aria, F., Bonini, S.A., Maccarinelli, G., Gianoncelli, A., Pina, S.D., Tambaro, S., Memo, M., Mastinu, A., 2019. Cannabidiol: Recent advances and new insights for neuropsychiatric disorders treatment. Life Sci 224, 120-127.

Pretzsch, C.M., Freyberg, J., Voinescu, B., Lythgoe, D., Horder, J., Mendez, M.A., Wichers, R., Ajram, L., Ivin, G., Heasman, M., Edden, R.A.E., Williams, S., Murphy, D.G.M., Daly, E., McAlonan, G.M., 2019a. Effects of cannabidiol on brain excitation and inhibition systems; a randomised placebo-controlled single dose trial during magnetic resonance spectroscopy in adults with and without autism spectrum disorder. Neuropsychopharmacology 44, 1398-1405.

Pretzsch, C.M., Voinescu, B., Lythgoe, D., Horder, J., Mendez, M.A., Wichers, R., Ajram, L., Ivin, G., Heasman, M., Edden, R.A.E., Williams, S., Murphy, D.G.M., Daly, E., McAlonan, G.M., 2019b. Effects of cannabidivarin (CBDV) on brain excitation and inhibition systems in adults with and without Autism Spectrum Disorder (ASD): a single dose trial during magnetic resonance spectroscopy. Transl Psychiatry 9, 313.

Purcell, A.E., Jeon, O.H., Zimmerman, A.W., Blue, M.E., Pevsner, J., 2001. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. Neurology 57, 1618-1628.

Qin, M., Zeidler, Z., Moulton, K., Krych, L., Xia, Z., Smith, C.B., 2015. Endocannabinoid-mediated improvement on a test of aversive memory in a mouse model of fragile X syndrome. Behav Brain Res 291, 164-171.

Ransohoff, R.M., Perry, V.H., 2009. Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol 27, 119-145.

Reisinger, S., Khan, D., Kong, E., Berger, A., Pollak, A., Pollak, D.D., 2015. The poly(I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery. Pharmacol Ther 149, 213-226.

Ristori, M.V., Quagliariello, A., Reddel, S., Ianiro, G., Vicari, S., Gasbarrini, A., Putignani, L., 2019. Autism, Gastrointestinal Symptoms and Modulation of Gut Microbiota by Nutritional Interventions. Nutrients 11.

Rodriguez, J.I., Kern, J.K., 2011. Evidence of microglial activation in autism and its possible role in brain underconnectivity. Neuron Glia Biol 7, 205-213.

Ronovsky, M., Berger, S., Molz, B., Berger, A., Pollak, D.D., 2016. Animal Models of Maternal Immune Activation in Depression Research. Curr Neuropharmacol 14, 688-704.

Rossignol, D.A., Frye, R.E., 2012. A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. Mol Psychiatry 17, 389-401.

Roullet, F.I., Wollaston, L., Decatanzaro, D., Foster, J.A., 2010. Behavioral and molecular changes in the mouse in response to prenatal exposure to the anti-epileptic drug valproic acid. Neuroscience 170, 514-522.

Ruby, K., Falvey, K., Kulesza, R.J., 2015. Abnormal neuronal morphology and neurochemistry in the auditory brainstem of Fmr1 knockout rats. Neuroscience 303, 285-298.

Ruiz-Valdepenas, L., Martinez-Orgado, J.A., Benito, C., Millan, A., Tolon, R.M., Romero, J., 2011. Cannabidiol reduces lipopolysaccharide-induced vascular changes and inflammation in the mouse brain: an intravital microscopy study. J Neuroinflammation 8, 5.

Saito, V.M., Rezende, R.M., Teixeira, A.L., 2012. Cannabinoid modulation of neuroinflammatory disorders. Curr Neuropharmacol 10, 159-166.

Sales, A.J., Fogaca, M.V., Sartim, A.G., Pereira, V.S., Wegener, G., Guimaraes, F.S., Joca, S.R.L., 2019. Cannabidiol Induces Rapid and Sustained Antidepressant-Like Effects Through Increased BDNF Signaling and Synaptogenesis in the Prefrontal Cortex. Mol Neurobiol 56, 1070-1081.

Salgado, C.A., Castellanos, D., 2018. Autism Spectrum Disorder and Cannabidiol: Have We Seen This Movie Before? Glob Pediatr Health 5, 2333794X18815412.

Salter, M.W., Stevens, B., 2017. Microglia emerge as central players in brain disease. Nat Med 23, 1018-1027.

Sarn, N., Jaini, R., Thacker, S., Lee, H., Dutta, R., Eng, C., 2020. Cytoplasmic-predominant Pten increases microglial activation and synaptic pruning in a murine model with autism-like phenotype. Mol Psychiatry.

Schafer, D.P., Stevens, B., 2015. Microglia Function in Central Nervous System Development and Plasticity. Cold Spring Harb Perspect Biol 7, a020545.

Schumann, C.M., Hamstra, J., Goodlin-Jones, B.L., Lotspeich, L.J., Kwon, H., Buonocore, M.H., Lammers, C.R., Reiss, A.L., Amaral, D.G., 2004. The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. J Neurosci 24, 6392-6401.

Servadio, M., Manduca, A., Melancia, F., Leboffe, L., Schiavi, S., Campolongo, P., Palmery, M., Ascenzi, P., di Masi, A., Trezza, V., 2018. Impaired repair of DNA damage is associated with autistic-like traits in rats prenatally exposed to valproic acid. Eur Neuropsychopharmacol 28, 85-96.

Servadio, M., Melancia, F., Manduca, A., di Masi, A., Schiavi, S., Cartocci, V., Pallottini, V., Campolongo, P., Ascenzi, P., Trezza, V., 2016. Targeting anandamide metabolism rescues core and associated autistic-like symptoms in rats prenatally exposed to valproic acid. Transl Psychiatry 6, e902.

Servadio, M., Vanderschuren, L.J., Trezza, V., 2015. Modeling autism-relevant behavioral phenotypes in rats and mice: Do 'autistic' rodents exist? Behav Pharmacol 26, 522-540.

Sherwin, E., Bordenstein, S.R., Quinn, J.L., Dinan, T.G., Cryan, J.F., 2019. Microbiota and the social brain. Science 366.

Silote, G.P., Sartim, A., Sales, A., Eskelund, A., Guimaraes, F.S., Wegener, G., Joca, S., 2019. Emerging evidence for the antidepressant effect of cannabidiol and the underlying molecular mechanisms. J Chem Neuroanat 98, 104-116.

Siniscalco, D., Bradstreet, J.J., Antonucci, N., 2013a. Therapeutic role of hematopoietic stem cells in autism spectrum disorder-related inflammation. Front Immunol 4, 140.

Siniscalco, D., Sapone, A., Giordano, C., Cirillo, A., de Magistris, L., Rossi, F., Fasano, A., Bradstreet, J.J., Maione, S., Antonucci, N., 2013b. Cannabinoid receptor type 2, but not type 1, is up-regulated in peripheral blood mononuclear cells of children affected by autistic disorders. J Autism Dev Disord 43, 2686-2695.

Siniscalco, D., Schultz, S., Brigida, A.L., Antonucci, N., 2018. Inflammation and Neuro-Immune Dysregulations in Autism Spectrum Disorders. Pharmaceuticals (Basel) 11.

Smith, D.R., Stanley, C.M., Foss, T., Boles, R.G., McKernan, K., 2017. Rare genetic variants in the endocannabinoid system genes CNR1 and DAGLA are associated with neurological phenotypes in humans. PLoS One 12, e0187926.

Smith, S.E., Li, J., Garbett, K., Mirnics, K., Patterson, P.H., 2007. Maternal immune activation alters fetal brain development through interleukin-6. J Neurosci 27, 10695-10702.

Solek, C.M., Farooqi, N., Verly, M., Lim, T.K., Ruthazer, E.S., 2018. Maternal immune activation in neurodevelopmental disorders. Dev Dyn 247, 588-619.

Stella, N., 2009. Endocannabinoid signaling in microglial cells. Neuropharmacology 56 Suppl 1, 244-253.

Stern, D., Cho, M.T., Chikarmane, R., Willaert, R., Retterer, K., Kendall, F., Deardorff, M., Hopkins, S., Bedoukian, E., Slavotinek, A., Schrier Vergano, S., Spangler, B., McDonald, M., McConkie-Rosell, A., Burton, B.K., Kim, K.H., Oundjian, N., Kronn, D., Chandy, N., Baskin, B., Guillen Sacoto, M.J., Wentzensen, I.M., McLaughlin, H.M., McKnight, D., Chung, W.K., 2017. Association of the missense variant p.Arg203Trp in PACS1 as a cause of intellectual disability and seizures. Clin Genet 92, 221-223.

Straley, M.E., Van Oeffelen, W., Theze, S., Sullivan, A.M., O'Mahony, S.M., Cryan, J.F., O'Keeffe, G.W., 2017. Distinct alterations in motor & reward seeking behavior are dependent on the gestational age of exposure to LPS-induced maternal immune activation. Brain Behav Immun 63, 21-34.

Suzuki, K., Sugihara, G., Ouchi, Y., Nakamura, K., Futatsubashi, M., Takebayashi, K., Yoshihara, Y., Omata, K., Matsumoto, K., Tsuchiya, K.J., Iwata, Y., Tsujii, M., Sugiyama, T., Mori, N., 2013. Microglial activation in young adults with autism spectrum disorder. JAMA Psychiatry 70, 49-58.

Tanaka, M., Sackett, S., Zhang, Y., 2020. Endocannabinoid Modulation of Microglial Phenotypes in Neuropathology. Front Neurol 11, 87.

Tartaglia, N., Bonn-Miller, M., Hagerman, R., 2019. Treatment of Fragile X Syndrome with Cannabidiol: A Case Series Study and Brief Review of the Literature. Cannabis Cannabinoid Res 4, 3-9.

Tartaglione, A.M., Schiavi, S., Calamandrei, G., Trezza, V., 2019. Prenatal valproate in rodents as a tool to understand the neural underpinnings of social dysfunctions in autism spectrum disorder. Neuropharmacology 159, 107477.

Taylor, P.V., Veenema, A.H., Paul, M.J., Bredewold, R., Isaacs, S., de Vries, G.J., 2012. Sexually dimorphic effects of a prenatal immune challenge on social play and vasopressin expression in juvenile rats. Biol Sex Differ 3, 15.

Tetreault, N.A., Hakeem, A.Y., Jiang, S., Williams, B.A., Allman, E., Wold, B.J., Allman, J.M., 2012. Microglia in the cerebral cortex in autism. J Autism Dev Disord 42, 2569-2584.

Theoharides, T.C., Asadi, S., Patel, A.B., 2013. Focal brain inflammation and autism. J Neuroinflammation 10, 46.

Thiele, E.A., Marsh, E.D., French, J.A., Mazurkiewicz-Beldzinska, M., Benbadis, S.R., Joshi, C., Lyons, P.D., Taylor, A., Roberts, C., Sommerville, K., Group, G.S., 2018. Cannabidiol in patients with seizures associated with Lennox-Gastaut syndrome (GWPCARE4): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet 391, 1085-1096.

Thom, R.P., Keary, C.J., Palumbo, M.L., Ravichandran, C.T., Mullett, J.E., Hazen, E.P., Neumeyer, A.M., McDougle, C.J., 2019. Beyond the brain: A multi-system inflammatory subtype of autism spectrum disorder. Psychopharmacology (Berl) 236, 3045-3061.

Till, S.M., Asiminas, A., Jackson, A.D., Katsanevaki, D., Barnes, S.A., Osterweil, E.K., Bear, M.F., Chattarji, S., Wood, E.R., Wyllie, D.J., Kind, P.C., 2015. Conserved hippocampal cellular pathophysiology but distinct behavioural deficits in a new rat model of FXS. Hum Mol Genet 24, 5977-5984.

Trezza, V., Damsteegt, R., Manduca, A., Petrosino, S., Van Kerkhof, L.W., Pasterkamp, R.J., Zhou, Y., Campolongo, P., Cuomo, V., Di Marzo, V., Vanderschuren, L.J., 2012. Endocannabinoids in amygdala and nucleus accumbens mediate social play reward in adolescent rats. J Neurosci 32, 14899-14908.

Tsilioni, I., Patel, A.B., Pantazopoulos, H., Berretta, S., Conti, P., Leeman, S.E., Theoharides, T.C., 2019. IL-37 is increased in brains of children with autism spectrum disorder and inhibits human microglia stimulated by neurotensin. Proc Natl Acad Sci U S A 116, 21659-21665.

Vargas, D.L., Nascimbene, C., Krishnan, C., Zimmerman, A.W., Pardo, C.A., 2005. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 57, 67-81.

Vigli, D., Cosentino, L., Raggi, C., Laviola, G., Woolley-Roberts, M., De Filippis, B., 2018. Chronic treatment with the phytocannabinoid Cannabidivarin (CBDV) rescues behavioural

alterations and brain atrophy in a mouse model of Rett syndrome. Neuropharmacology 140, 121-129.

Voineagu, I., Wang, X., Johnston, P., Lowe, J.K., Tian, Y., Horvath, S., Mill, J., Cantor, R.M., Blencowe, B.J., Geschwind, D.H., 2011. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature 474, 380-384.

Wang, R., Tan, J., Guo, J., Zheng, Y., Han, Q., So, K.F., Yu, J., Zhang, L., 2018a. Aberrant Development and Synaptic Transmission of Cerebellar Cortex in a VPA Induced Mouse Autism Model. Front Cell Neurosci 12, 500.

Wang, S., Harvey, L., Martin, R., van der Beek, E.M., Knol, J., Cryan, J.F., Renes, I.B., 2018b. Targeting the gut microbiota to influence brain development and function in early life. Neurosci Biobehav Rev 95, 191-201.

Weber-Stadlbauer, U., Richetto, J., Labouesse, M.A., Bohacek, J., Mansuy, I.M., Meyer, U., 2017. Transgenerational transmission and modification of pathological traits induced by prenatal immune activation. Mol Psychiatry 22, 102-112.

Wegiel, J., Flory, M., Kuchna, I., Nowicki, K., Ma, S.Y., Imaki, H., Wegiel, J., Cohen, I.L., London, E., Brown, W.T., Wisniewski, T., 2014a. Brain-region-specific alterations of the trajectories of neuronal volume growth throughout the lifespan in autism. Acta Neuropathol Commun 2, 28.

Wegiel, J., Flory, M., Kuchna, I., Nowicki, K., Ma, S.Y., Imaki, H., Wegiel, J., Cohen, I.L., London, E., Wisniewski, T., Brown, W.T., 2014b. Stereological study of the neuronal number and volume of 38 brain subdivisions of subjects diagnosed with autism reveals significant alterations restricted to the striatum, amygdala and cerebellum. Acta Neuropathol Commun 2, 141.

Wei, D., Dinh, D., Lee, D., Li, D., Anguren, A., Moreno-Sanz, G., Gall, C.M., Piomelli, D., 2016a. Enhancement of Anandamide-Mediated Endocannabinoid Signaling Corrects Autism-Related Social Impairment. Cannabis Cannabinoid Res 1, 81-89.

Wei, D., Lee, D., Cox, C.D., Karsten, C.A., Penagarikano, O., Geschwind, D.H., Gall, C.M., Piomelli, D., 2015. Endocannabinoid signaling mediates oxytocin-driven social reward. Proc Natl Acad Sci U S A 112, 14084-14089.

Wei, D., Lee, D., Li, D., Daglian, J., Jung, K.M., Piomelli, D., 2016b. A role for the endocannabinoid 2-arachidonoyl-sn-glycerol for social and high-fat food reward in male mice. Psychopharmacology (Berl) 233, 1911-1919.

Wei, H., Alberts, I., Li, X., 2013. Brain IL-6 and autism. Neuroscience 252, 320-325.

Wei, H., Chadman, K.K., McCloskey, D.P., Sheikh, A.M., Malik, M., Brown, W.T., Li, X., 2012. Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. Biochim Biophys Acta 1822, 831-842.

Whiting, S.E., Carson, A.M., Clark, G.D., Schwartz, D.D., 2019. Case report of prenatal bilateral cerebellar infarction: implications for social-behavioral functioning. Clin Neuropsychol 33, 890-904.

Woodbury-Smith, M., Scherer, S.W., 2018. Progress in the genetics of autism spectrum disorder. Dev Med Child Neurol 60, 445-451.

Wu, H.F., Lu, T.Y., Chu, M.C., Chen, P.S., Lee, C.W., Lin, H.C., 2020. Targeting the inhibition of fatty acid amide hydrolase ameliorate the endocannabinoid-mediated synaptic dysfunction in a valproic acid-induced rat model of Autism. Neuropharmacology 162, 107736.

Xie, J., Huang, L., Li, X., Li, H., Zhou, Y., Zhu, H., Pan, T., Kendrick, K.M., Xu, W., 2017. Immunological cytokine profiling identifies TNF-alpha as a key molecule dysregulated in autistic children. Oncotarget 8, 82390-82398.

Xu, N., Li, X., Zhong, Y., 2015. Inflammatory cytokines: potential biomarkers of immunologic dysfunction in autism spectrum disorders. Mediators Inflamm 2015, 531518.

Xuan, I.C., Hampson, D.R., 2014. Gender-dependent effects of maternal immune activation on the behavior of mouse offspring. PLoS One 9, e104433.

Yamashita, Y., Fujimoto, C., Nakajima, E., Isagai, T., Matsuishi, T., 2003. Possible association between congenital cytomegalovirus infection and autistic disorder. J Autism Dev Disord 33, 455-459.

Yuskaitis, C.J., Beurel, E., Jope, R.S., 2010. Evidence of reactive astrocytes but not peripheral immune system activation in a mouse model of Fragile X syndrome. Biochim Biophys Acta 1802, 1006-1012.

Zamberletti, E., Gabaglio, M., Parolaro, D., 2017. The Endocannabinoid System and Autism Spectrum Disorders: Insights from Animal Models. Int J Mol Sci 18.

Zamberletti, E., Gabaglio, M., Piscitelli, F., Brodie, J.S., Woolley-Roberts, M., Barbiero, I., Tramarin, M., Binelli, G., Landsberger, N., Kilstrup-Nielsen, C., Rubino, T., Di Marzo, V., Parolaro, D., 2019a. Cannabidivarin completely rescues cognitive deficits and delays neurological and motor defects in male Mecp2 mutant mice. J Psychopharmacol 33, 894-907.

Zamberletti, E., Gabaglio, M., Woolley-Roberts, M., Bingham, S., Rubino, T., Parolaro, D., 2019b. Cannabidivarin Treatment Ameliorates Autism-Like Behaviors and Restores Hippocampal Endocannabinoid System and Glia Alterations Induced by Prenatal Valproic Acid Exposure in Rats. Front Cell Neurosci 13, 367.

Zerbo, O., Qian, Y., Yoshida, C., Grether, J.K., Van de Water, J., Croen, L.A., 2015. Maternal Infection During Pregnancy and Autism Spectrum Disorders. J Autism Dev Disord 45, 4015-4025.

Zhan, Y., Paolicelli, R.C., Sforazzini, F., Weinhard, L., Bolasco, G., Pagani, F., Vyssotski, A.L., Bifone, A., Gozzi, A., Ragozzino, D., Gross, C.T., 2014. Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. Nat Neurosci 17, 400-406.

Zhang, D., Hu, X., Qian, L., O'Callaghan, J.P., Hong, J.S., 2010. Astrogliosis in CNS pathologies: is there a role for microglia? Mol Neurobiol 41, 232-241.

Zhou, H., 2015. Region Specific Effects of Maternal Immune Activation on Offspring Neuroimmune Function. Open J Immunol 5, 51-63.

Zimmerman, A.W., Jyonouchi, H., Comi, A.M., Connors, S.L., Milstien, S., Varsou, A., Heyes, M.P., 2005. Cerebrospinal fluid and serum markers of inflammation in autism. Pediatr Neurol 33, 195-201.

Zou, S., Kumar, U., 2018. Cannabinoid Receptors and the Endocannabinoid System: Signaling and Function in the Central Nervous System. Int J Mol Sci 19.

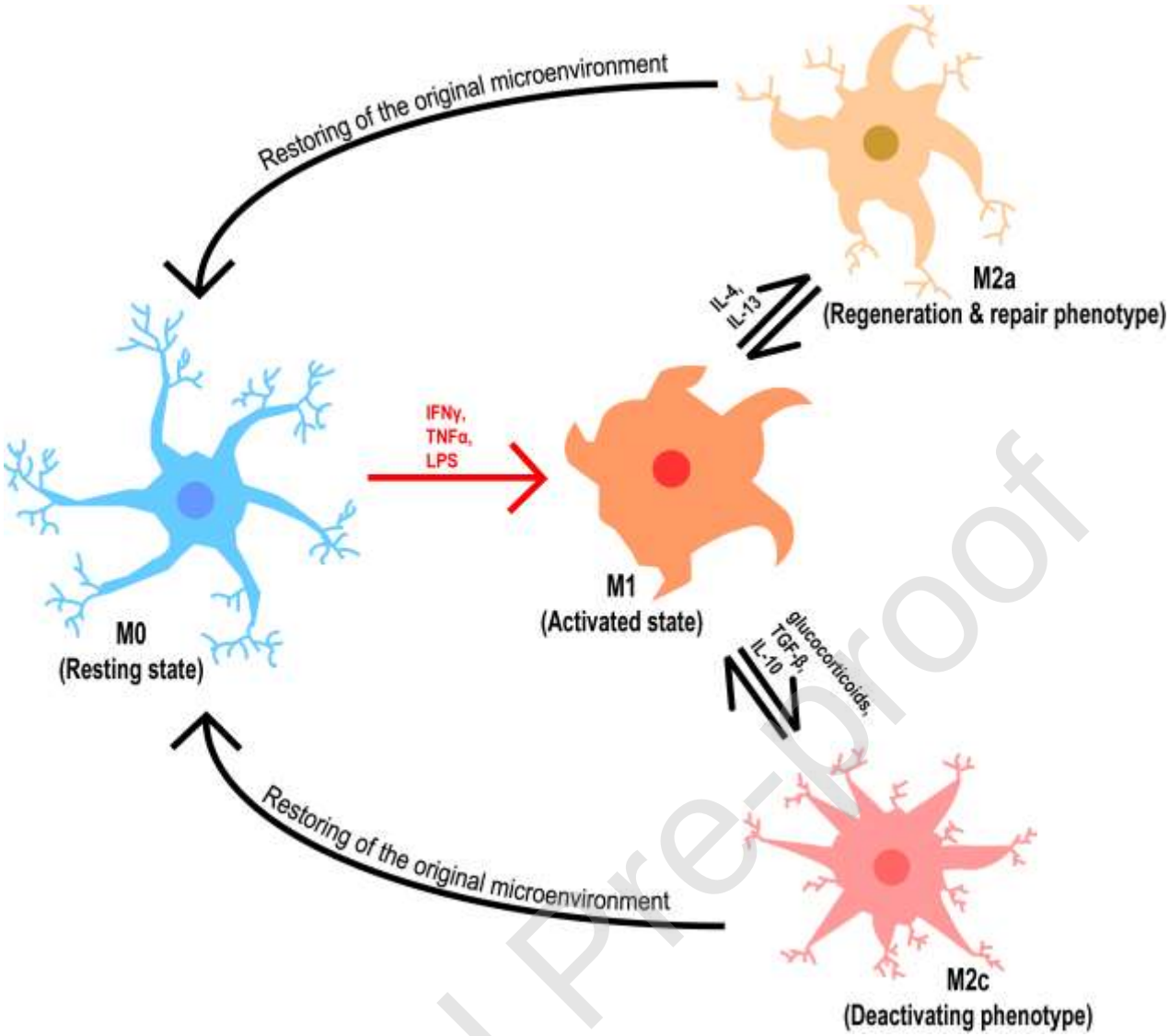

Figure 1. Schematic representation of alternative states of microglia.

The 'resting state' or M0 phase is characterized by a small soma with highly ramified processes, which constantly survey the local environment. After the detection of abnormalities, like infections or tissue damage (e.g. IFN-γ, TNF-α and LPS), microglia adopt an amoeboid morphology and retract its processes, assuming the 'classical activation state' or M1 phase. Microglia can also adopt an 'alternative activated state' or M2 phase, which is characterized by two different alternative states: M2a (induced by the cytokines IL-4 and IL-13) and M2c (induced by TGF-β, IL-10, and glucocorticoids in vitro). IFN-γ: interferon gamma; TNF-α: Tumor Necrosis Factor alpha; LPS: lipopolysaccharide; IL-4: interleukin 4; IL-13: interleukin 13; TGF-β: Transforming Growth Factor beta; IL-10: Interleukin 10.

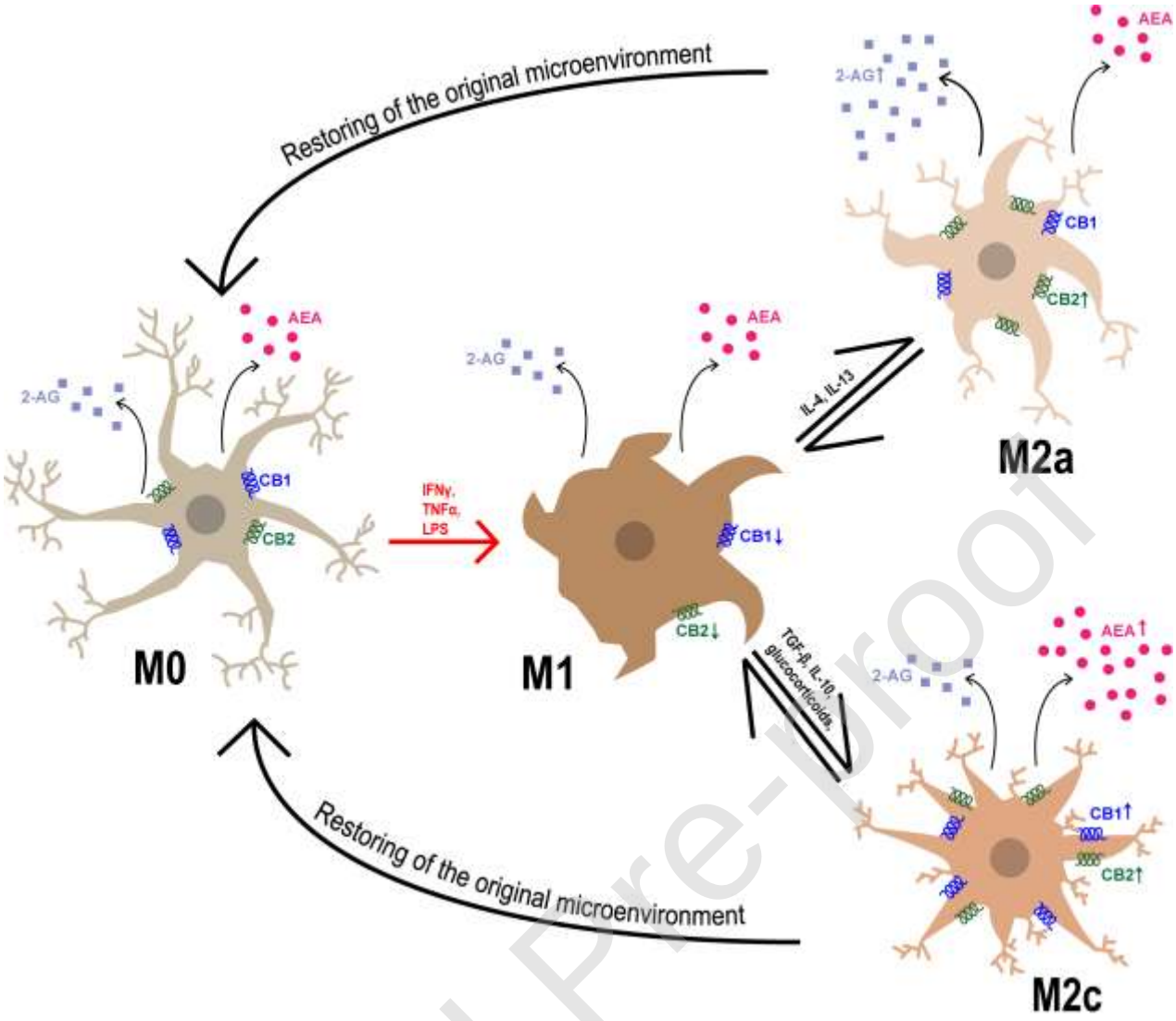

Figure 2. Proposed endocannabinoid regulatory pathway for microglia alternative states acquisition.

In the M0 phase (resting state), microglia synthesize 2-AG and AEA. Following stimulation by inflammatory agents, microglia polarize toward the M1 phase (activated state), with a down-regulation of CB1 and CB2 receptors. Finally, when assuming the M2a or the M2c phenotype, microglia show an increase of the expression of CB2 receptors or an increase of both CB2 and CB1 receptors, respectively. Furthermore, the M2a state is characterized by an increased synthesis of 2-AG, while the M2c state shows, instead, an increase of AEA synthesis.

2-AG: 2-arachidonoylglycerol; AEA: N-arachidonoylethanolamine (anandamide); CB1: Cannabinoid receptor type 1; CB2: Cannabinoid receptor type 2; IFN-γ: interferon gamma; TNF-α: Tumor Necrosis Factor alpha; LPS: lipopolysaccharide; IL-4: interleukin 4; IL-13: interleukin 13; TGF-β: Transforming Growth Factor beta; IL-10: Interleukin 10.