Endocannabinoid Signaling in Autism

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Abstract Autism spectrum disorder (ASD) is a complex behavioral condition with onset during early childhood and a lifelong course in the vast majority of cases. To date, no behavioral, genetic, brain imaging, or electrophysiological test can specifically validate a clinical diagnosis of ASD. However, these medical procedures are often implemented in order to screen for syndromic forms of the disorder (i.e., autism comorbid with known medical conditions). In the last 25 years a good deal of information has been accumulated on the main components of the "endocannabinoid (eCB) system", a rather complex ensemble of lipid signals ("endocannabinoids"), their target receptors, purported transporters, and metabolic enzymes. It has been clearly documented that eCB signaling plays a key role in many human health and disease conditions of the central nervous system, thus opening the avenue to the therapeutic exploitation of eCB-oriented drugs for the treatment of psychiatric, neurodegenerative, and neuroinflammatory disorders. Here we present a modern view of the eCB system, and alterations of its main components in human patients and animal models relevant to ASD. This review will thus provide a critical perspective necessary to explore the potential exploitation of distinct elements of eCB system as targets of innovative therapeutics against ASD.

Key Words Autism spectrum disorder · endocannabinoid system · fragile X syndrome · metabolic regulation · reward system

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The Endocannabinoid System

Twenty-five years after the cloning and expression of a complementary DNA that encoded a G protein-coupled receptor, named type-1 cannabinoid (CB$_1$) receptor [1], there is a good deal of information on the main components of the so-called "endocannabinoid (eCB) system", as well as on its role in controlling cannabinergic signaling in human health and disease [2–4]. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the most active eCBs as yet identified, although this family of bioactive lipids includes other arachidonic acid (AA) derivatives with cannabimimetic properties (i.e., noladin ether, virodhamine, N-arachidonoyldopamine, to name but a few). The classical dogma that eCBs are synthesized and released "on demand" upon (patho)physiological stimuli has been recently revisited on the basis of unexpected evidence for intracellular reservoirs and transporters of eCBs. These new entities have been shown to drive intracellular trafficking of eCBs, adding a new dimension to the regulation of their biological activity [5]. To date, several metabolic routes have

been described for AEA biosynthesis [6, 7], yet the most relevant pathway is believed to begin with the transfer of AA from the sn-1 position of 1,2-sn-di-arachidonoyl-phosphatidylcholine to phosphatidylethanolamine, generating the AEA precursor N-arachidonoyl-phosphatidylethanolamine. The latter compound is next cleaved by a specific N-acyl-phosphatidylethanolamine (NAPE)-specific phospholipase D, which has been characterized in detail [8]. However, the degradation of AEA to AA and ethanolamine is mainly due to 2 fatty acid amide hydrolases (FAAH and FAAH-2) [9, 10]. When FAAH and FAAH-2 are inhibited, N-acyletanolamine-hydrolyzing acid amidase cleaves AEA in an alternate route [11, 12]. The main enzymes responsible for AEA metabolism are reported in Fig. 1.

Much like AEA, the biological activity of 2-AG is controlled through cellular mechanisms that include: 1) synthesis through rapid hydrolysis of inositol phospholipids by a specific phospholipase C to generate diacylglycerol that is then converted into 2-AG by a sn-1-specific diacylglycerol lipase (DAGL) [13]; and 2) degradation to AA and glycerol by a monoacylglycerol lipase (MAGL) [14], as schematically represented in Fig. 1. AEA and 2-AG can be also oxidized by cyclooxygenase-2, different lipoxygenase isozymes, as well as by cytochrome P450, to generate, respectively, prostaglandin–ethanolamides [15] and prostaglandin-glyceryl esters [16], hydroxy-anandamides and hydroxyleicosatetraenoyl-glycerols [17], and epoxy-eicosatrienoyl-glycerols [18].

An open question concerning eCB metabolism remains the transport of these compounds across the plasma membrane [19, 20]. As yet, the most accepted mechanisms are: 1) passive diffusion, which can be favored by the formation of AEA–cholesterol complexes, possibly in preferred microdomains called "lipid rafts" [21, 22]; 2) facilitated transport through a purported eCB membrane transporter [23]; and 3) endocytosis assisted by caveolins (reviewed in [24]). Once taken up, intracellular AEA reaches distinct sites, where distinct metabolic and signaling pathways take place. Heat shock protein 70, and albumin and fatty acid binding proteins 5 and 7 have been shown to act as eCB intracellular transporters, able to ferry AEA (and likely also 2-AG) within the cytoplasm to the nucleus and other destinations, including storage compartments like adiposomes [5, 25].

eCBs act principally through type-1 and type-2 (CB_1 and CB_2) cannabinoid receptors. Interestingly, CB_1 but not CB_2 resides within lipid rafts, and their interaction with these specialized microdomains influences signal transduction thereof [26]. Additionally, eCBs are also able to interact with non-CB_1/non-CB_2 targets, such as 1) the transient receptor potential vanilloid type 1 channel, which is activated by both AEA and 2-AG [27, 28]; 2) peroxisome proliferator-activated receptor-α and peroxisome proliferator-activated receptor-γ [29]; and 3) the orphan G protein-coupled receptor GPR55 [30, 31]. By interacting with these receptors, eCBs trigger a multiplicity of signaling pathways that are involved in both physiological and pathological conditions [32]. On a final note, the existence of compounds structurally related to eCBs, and collectively known as "eCB-like" substances, should be recalled because of their "entourage effect". These compounds potentiate eCB activity at their receptors by increasing binding affinity or by inhibiting eCB hydrolysis [33–35]. A schematic representation of eCBs, their molecular targets, biosynthetic and hydrolizing enzymes, and extra- and intracellular transporters, is depicted in Fig. 1.

Autism Spectrum Disorder: Clinical Traits, Neuropsychological Deficits, and Neuroanatomical Underpinnings

Autism spectrum disorder (ASD) is a complex behavioral condition with onset during early childhood and a lifelong course in the vast majority of cases. It is characterized by deficits in communication and social interaction, as well as by stereotypic behaviors, restricted patterns of interest, and abnormal sensory issues [36]. The diagnosis is based on clinical observation, substantiated by standardized testing of the patient with the Autism Diagnostic Observation Schedule-Generic [37], later revised into the Autism Diagnostic Observation Schedule-2 [38], and/or by parental interview with the Autism Diagnostic Interview-Revised [39]. To date, no behavioral, genetic, brain imaging, or electrophysiological test can specifically validate a clinical diagnosis of ASD, although these medical procedures are regularly implemented in order to screen for syndromic forms of the disorder (i.e., autism due to known medical conditions). Two essential features distinguish ASD from most other behavioral disorders: 1) an impressive clinical and pathogenetic heterogeneity, which has led to the designaton, by the term "autisms", of a set of neurodevelopmental disorders with early onset in life, sharing autism as a common feature, but produced through distinct processes [40]; 2) the distribution of autistic features as a dimensional continuum in the general population, which fully justifies referring to the "autism spectrum" rather than to a categorical distinction between "affected" and "unaffected" [41, 42]. As many as 1 in 68 (1.6 %) 8-year-old children receive an ASD diagnosis [43], with a male:female ratio of 4:1. Siblings of children already diagnosed with ASD have a significantly higher incidence of the same disorder, reported at 18.7 % in a prospective follow-up study [44], and ranging from 15 % to 25 % depending on sex and clinical severity. The prospective follow-up of these siblings later diagnosed with ASD has led to the observation that some behavioral abnormalities can appear very early on (e.g.,, sensory issues [e.g., extreme responses to certain sounds/textures, fascination with lights/spinning objects] are already present at 7 months of age), others emerge at 12–14 months (e.g., disengagement
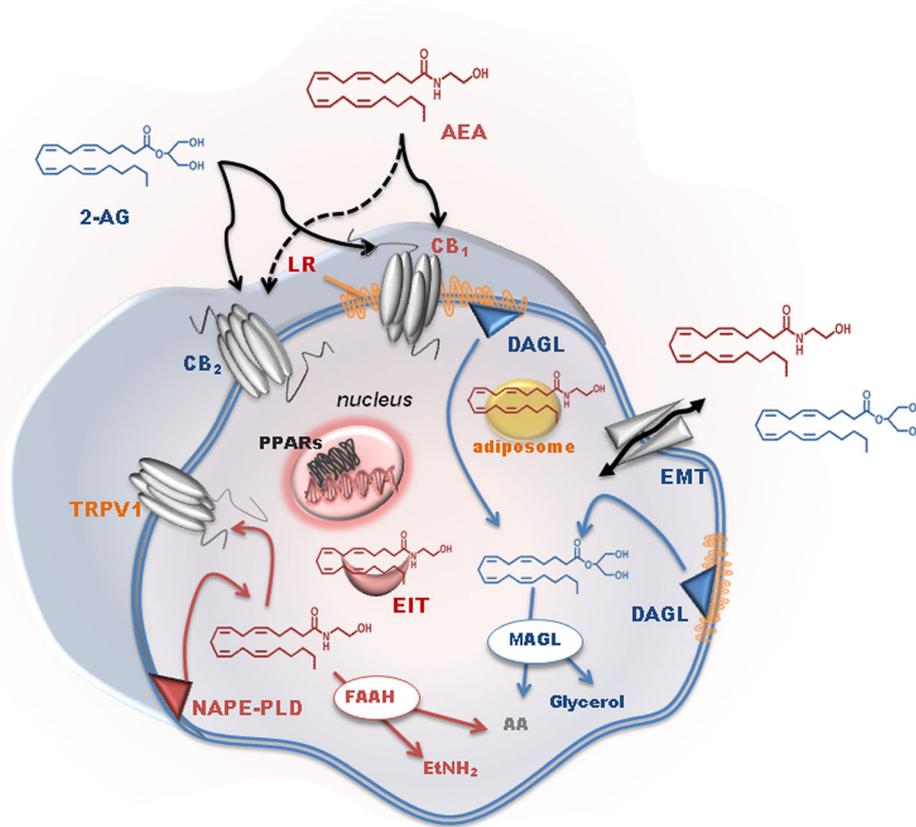
Fig. 1 Schematic representation of the main elements of the endocannabinoid (eCB) system. N-arachidonoylethanolamine (AEA) is mainly synthesized by the sequential activity of N-acyltransferase (not shown) and N-acylphosphatidyl-ethanolamine (NAPE)-specific phospholipase D (NAPE-PLD). The intracellular degradation of AEA is due to a fatty acid amide hydrolase (FAAH) that generates ethanolamine (EtNH$_2$) and arachidonic acid (AA). 2-Arachidonoylglycerol (2-AG) is released from membrane lipids through the activity of diacylglycerol lipase (DAGL), and can be hydrolyzed by a cytosolic monoacylglycerol lipase (MAGL), which releases glycerol and AA. Cyclooxygenase-2, lipoxygenase isozymes and cytochrome P450 were omitted for the sake of clarity. Extracellular eCBs can cross the plasma membrane through a purported eCB membrane transporter (EMT), and then they are trafficked within the cytoplasm through eCB intracellular transporters (EIT), which deliver them to their different targets or, alternatively, to storage organelles like adiposomes. Both AEA and 2-AG trigger several signal transduction pathways by acting at type-1 and type-2 cannabinoid receptors (CB$_1$ and CB$_2$, respectively), or at other non-CB$_1$/non-CB$_2$ targets, such as peroxisome proliferator-activated receptors (PPARs) in the nucleus. CB$_1$, but not CB$_2$, resides within specialized membrane microdomains enriched in cholesterol and sphingolipids, which are called lipid rafts (LR). AEA (and also 2-AG) can also translocate to the inner membrane leaflet, where it binds to transient receptor potential vanilloid type 1 (TRPV1) channels

of visual attention), while the bulk of more typical autistic abnormalities has an onset between 14 and 24 months [45–48]. Frequently, comorbid conditions include intellectual disability (65 %), seizures (30 %), and different forms of sleep problems [49–51]; less recognized, but equally impairing, are frequent psychiatric comorbidities, that include anxiety disorders, obsessive–compulsive disorders, and depression [52]. Altered neurodevelopment during early pregnancy represents the neuropathological cause of ASD [53, 54]. Postmortem studies have unveiled neuroanatomical and cytoarchitectonic abnormalities in the cerebellum, inferior olivary complex, deep cerebellar nuclei, hippocampus, amygdala, entorhinal cortex, fusiform gyrus, and anterior and posterior cingulate cortex, with thinner cortical minicolumns, excessive growth of the frontal lobes, and excessive dendritic spine density [55]. These abnormalities are suggestive of derangements occurring during the first/second trimester of pregnancy, namely reduced programmed cell death and/or increased cell proliferation, altered cell migration, abnormal cell differentiation with reduced neuronal body size, abnormal neurite sprouting, and pruning that result in atypical cell–cell wiring. In addition, neurodevelopmental mechanisms extending into late prenatal/postnatal life include reduced synapse formation and delayed myelination [40, 56, 57]. The latter result in abnormal neuronal wiring, which was previously believed to be characterized by long-range hypoconnectivity and local hyperconnectivity [58], but more recently has been shown to be a highly individualized mix of hyper- and hypoconnectivity specific to each single patient with ASD [59]. These abnormalities have been associated with deficits in multiple behavioral tasks that relate to social behavior, such as empathy, theory of mind, joint attention, and face and emotion

processing. Many of these observed behavioral features suggest a deficit in the social reward processing system in ASD, as we discuss in the following section. The neurocognitive phenotype in ASD stems from a complex and highly heterogeneous array of genetic and environmental causes, with patients ranging from "purely genetic" cases due to known ASD-causing chromosomal aberrations or mutations to "purely environmental" cases due to rare prenatal exposure to specific viral agents, drugs, and toxins [60–62]. In between these extremes, ASD for most cases fully qualifies for the definition of a "complex" disorder, whereby a host of rare and common genetic variants, often but not necessarily in conjunction with epigenetic factors [63], yield the neurodevelopmental abnormalities summarized above, resulting in autistic behaviors. Finally, neuroinflammation is also a frequent finding in postmortem brains of autistic individuals [64, 65]. It may represent a nonspecific consequence of insufficient neurite pruning and abnormal wiring of neural networks, resulting in elevated oxidative stress (possibly a common feature shared by several neurodevelopmental disorders) [66], but it could also stem from a broader immune dysfunction which, together with gastrointestinal disturbances and recurrent infections, collectively qualifies ASD as a systemic disorder [67–71].

Autism and Reward System

A deficit in theory of mind and empathy has commonly been suggested to underlie atypical social behavior in individuals with ASD [72]. A set of recent studies raises the possibility that some of these social behavioral deficits in ASD arise due to deficits in reward system functioning [73–75]. This hypothesis is supported by studies that report a lack of social motivation in children with autism [76, 77]. One rationale for the social motivation-based account of ASD relies on the following premise: if individuals with ASD do not find social stimuli rewarding, and hence do not attend to them as much as neurotypicals, then they are less likely to exhibit empathy toward them. An alternative formulation of the social motivation hypothesis suggests that the attention of individuals with and without ASD is drawn to social stimuli to a comparable extent, but individuals with ASD find social stimuli less rewarding, which leads to the observed deficits in empathy (Fig. 2).

Both of these possible accounts of the social motivation hypothesis are faced with a key question. Do people with ASD find social stimuli rewarding or do they have a domain-general dysfunction of the reward system? To test these possibilities, a number of studies have compared processing of social and nonsocial reward stimuli in people with and without ASD. An early study using a continuous performance task found no behavioral evidence for group differences in trials with monetary rewards *versus* nonrewards

[78]. Similarly, comparable behavioral performance in a reward-processing task using a variant of the go–no-go task was found in children and adults with and without ASD [79, 80]. In contrast, using a similar task, DeMurie et al. [81] demonstrated that children with ASD were slower in responding to social rewards than monetary rewards, but this was not specific to ASD. Overall, the behavioral evidence summarized above appears equivocal about any circumscribed deficit in social reward processing in ASD. Yet, a main group effect in the majority of these studies points toward a domain-general deficit in the reward system. In contrast to the behavioral studies using button-press responses, eyegaze tracking, electroencephalography, and functional magnetic resonance imaging studies suggest clear differences in processing of social rewards in ASD compared with typically developed controls [82]. Eyegaze tracking studies typically involve measuring gaze fixation patterns in response to social and nonsocial stimuli. Children and adults with ASD are found to look less at social stimuli than at nonsocial stimuli [83–85]. Gaze fixation patterns have often been used as proxy metrics related to reward processing [86], thus supporting the hypothesis of atypical processing of social rewards in ASD. Using electroencephalography in children with ASD, a recent study reported lower magnitude of a component related to reward anticipation (stimulus preceding negativity) in response to social *versus* nonsocial stimuli [87]. Similarly, functional magnetic resonance imaging studies revealed lower activity in the ventral striatum in response to social stimuli (neutral faces) in individuals with ASD [88]. The latter finding is consistent with the observation that a reduced ventral striatal response to happy faces was associated with lower self-reported empathy in individuals with and without ASD [89].

In sum, there is substantial evidence across different techniques to suggest atypical reward processing in ASD. Irrespective of their domain specificity, such functional differences in the reward circuit in ASD have important consequences for the processing of social stimuli. Atypical response to social rewards from an early age can result in deficits in learning about the social world, which, in turn, can lead to social behavioral impairments in adulthood.

Alterations of the eCB System in Autism

The complexities that make autism hard to understand, from the diagnostic criteria and clinical heterogeneity to the genetic/environmental causes that provoke communication and behavioral problems to the innovative therapies to be applied in order to give patients the best quality of life, encourage scientists to look at predictive biomarkers and/or therapeutic targets for the pharmacological management of this disorder [90, 91]. It is now clear that eCB system is altered in several neurodegenerative diseases and, very interestingly, that
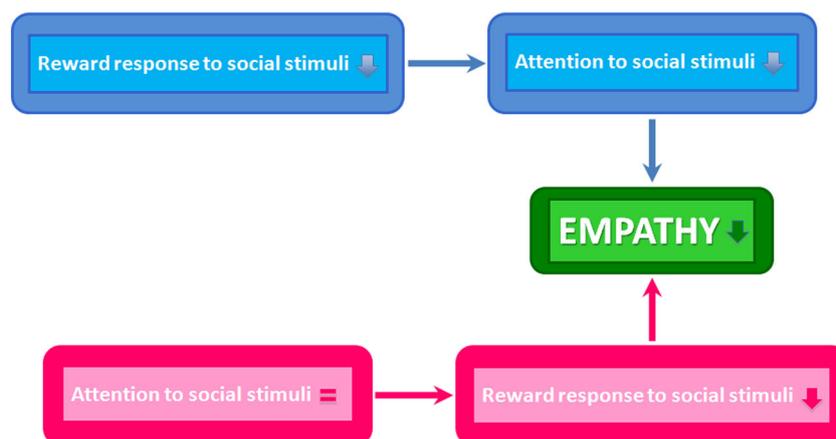
Fig. 2 Two possible routes through which atypical reward processing can lead to reduced empathy as seen in ASD. (Top panel) The first of these routes suggest that a lower reward response to social stimuli reduces the salience of social stimuli and hence how much attention they capture. The reduced empathy is therefore a product of this reduced attention to social stimuli. (Bottom panel) A second possible route suggests comparable salience for social and nonsocial stimuli in ASD, but lower value for social stimuli in ASD, leading to reduced empathy

distinct elements of the eCB system in peripheral blood mirror these perturbations, providing novel and noninvasive diagnostic tools for several neuroinflammatory diseases [92, 93]. In addition, the eCB system controls emotional responses [94], behavioral reactivity to context [95], and social interaction [96]. Thus, it can be hypothesized that alterations in this endogenous circuitry may contribute to the autistic phenotype. In this and the following sections, we critically discuss the evidence for this proposition from animal and human studies. Recent investigations have addressed the involvement of eCBs in autism, where, unfortunately, the role of these bioactive lipids remains poorly understood. Indeed, autism is uniquely human and there are only a few validated animal models (e.g., *fmr1* knockout mice, BTBR mice, and valproic acid-treated rats), that display autistic-like features. Fragile X syndrome (FXS) is an inherited disorder caused by mutations in *FMR1*, which is translated into the fragile X mental retardation 1 protein, which, in turn, plays a role in the development of synapses [97, 98]. Expansion mutations of *FMR1* produce autistic features in approximately 40 % of patients with FXS, and thus FXS provides a valuable model for identifying novel biomarkers/targets for autism and for dissecting the underlying neurochemical pathways [99]. In the first study addressing eCB system in FXS, it has been reported that the ablation *fmr1* gene causes a dysfunctional 2-AG metabolism, with increasing DAGL and MAGL activities in the striatum of $fmr1^{-/-}$ mutants, but unaltered striatal 2-AG levels [100]. According to a more recent study [101], stimulation of 2-AG signaling could be a useful treatment for mitigating FXS symptoms because it is able to normalize synaptic activity through type I metabotropic glutamate activation; additionally, genetic or pharmacological attenuation of CB$_1$-dependent signal transduction and blockade of the mammalian target of rapamycin pathway might provide alternative strategies to treat autistic patients [102].

A link between the eCB system and autism was put forward by Schultz [103], who proposed that acetaminophen, an antipyretic drug that is metabolized to a potent inhibitor of the purported eCB membrane transporter AM404, could trigger autism by activating CB receptors. In line with this, elevated levels of circulating AEA during pregnancy or in the first postnatal days might interefere with the neurodevelopment of offspring, and might increase the risk of delivering autistic children. Abnormalities in sociability and nociception tests, and alterations of distinct elements of eCB system have been reported in adolescent rats on valproic acid [104]. In particular, mRNA levels of the enzymes responsible for 2-AG metabolism (i.e., DAGL and MAGL), which is disrupted in the FXS model of autism [100], were altered in the cerebellum and hippocampus, whereas endogenous levels of 2-AG in the same regions remained at steady state [104]. Interestingly, the content of AEA, *N*-oleoylethanolamine (OEA), and *N*-palmitoylethanolamine (PEA), all of which are substrates of FAAH, were increased in the hippocampus following exposure to sociability tests, suggesting that a deficit in social play behaviors might be due to reduced AEA levels in critical brain areas [104]. Moreover, the same study documented a down-regulation of GPR55 and PPAR gene expression, supporting a role for these receptors in the cognitive mechanisms involved in autism [104]. Preliminary data also addressed CB$_2$ as a potential target for autism. Indeed, genomic studies have highlighted an upregulation of mRNA levels of the CB$_2$A, but not the CB$_2$B, isoform in the cerebellum of BTBR T+tF/J mice [105], which have an autism-like behavioral phenotype [106]. Also, an independent clinical study performed on young (3–9-year-old) children demonstrated that CB$_2$ is highly expressed, both at transcriptional and translational levels, in peripheral blood mononuclear cells of patients with autism, compared with matched healthy controls [107]. All the other elements of the eCB system remained unaltered, except for a

slight downregulation of NAPE–phospholipase D mRNA. According to a recent hypothesis on autism and inflammation [108], and in keeping with data on the key role of CB_2 in immune-related pathologies [109–111], it can be speculated that the increase in CB_2 expression may serve a compensatory role with respect to the inflammatory state associated with autism. Thus, the observed enhancement of CB_2 may be a negative feedback response aimed at counteracting the proinflammatory responses implicated in the pathogenesis of this neurobehavioral condition. In this context, it should be also recalled that AEA suppresses the release of proinflammatory cytokines from human lymphocytes through a CB_2-mediated mechanism [112]. The main alterations of the eCB system in human patients and animal models of autism are summarized in Tables 1 and 2, respectively. However, the paucity of the relevant human data and their largely correlational nature do not allow for a systematic comparison with the animal data.

Additionally, it is worth noting that plasma levels of polyunsaturated fatty acids (which are components of eCBs) are lower in patients with autism, and that 2 derivatives of docosahexaenoic acid and eicosapentaenoic acid (also components of eCBs) are able to activate both CB_1 and CB_2 receptors [113]. These data further suggest that a dysregulation of eCB signaling might be driven by diet, resulting in an imbalance of pro- and anti-inflammatory metabolites, and thus favoring the development of autism [114].

Suggested Roles of the eCB System in Autism

As discussed in the previous sections, there is phenotypic evidence across multiple levels that suggests a role for atypical reward system functioning in ASD. It is therefore vital to investigate in detail the eCB system in autism and related endophenotypes, also in view of its key role in modulating mesolimbic dopaminergic neurotransmission. The majority of studies on the eCB system in autism-related endophenotypes in humans have tested the role of the *CNR1*, which is strongly expressed in striatal structures implicated in processing

Table 1 Molecular markers of the endocannabinod (eCB) system in autism and related phenotypes in humans

Biological sample	Model	eCB alterations	Reference
Postmortem brain	Human	↓CB_1	[121]
PBMCs	Human	↓NAPE–PLD mRNA = FAAH, CB_1 mRNA ↑CB_2 mRNA and protein	[107]
Saliva	Human	CB_1 SNP	[116, 119]

PBMCs=peripheral blood mononuclear cells; CB^1 =type-1 cannabinoid receptor; NAPE–PLD=*N*-acylphosphatidyl-ethanolamine-specific phospholipase D; FAAH=fatty acid hydrolase; CB2=type-2 cannabinoid receptor; SNP=single nucleotide polymorphism

rewards [115]. The previous section presented an overview of largely animal studies that point to a role for eCB signaling in autism-relevant phenotypes. Further clues from both human and animal studies are discussed in the following section, and are summarized in Fig. 3.

The first clue comes from a human neuroimaging study that measured striatal response to social rewards (happy faces *vs* neutral faces). This investigation found that common single nucleotide polymorphisms in *CNR1* are associated with activity in the ventral striatal cluster in response to happy (but not to disgusted) faces [116], and later on it was replicated in an independent cohort [117]. In view of the central role of the ventral striatum in reward processing, it is reasonable to infer that variation in *CNR1* was linked to differences in sensitivity to social rewards such as happy faces. Another study in an independent sample used eyegaze tracking to show that the same *CNR1* polymorphisms were associated with greater gaze duration to happy faces, but again not to disgust faces [118]. A parallel population genetic study found a nominal association of the same *CNR1* genetic variations with trait empathy [119]. Individuals with ASD score low in trait empathy, and, consistent with this, a gene expression study in postmortem brains of individuals with autism had earlier reported a reduced expression of CB_1 [120, 121]. In sum, multiple indirect lines of evidence suggest a role for *CNR1* genetic variations in underlying social reward responsivity, a putative endophenotype for autism. These findings in human patients parallel observations in animal models that show a strong role for the eCB system in social play behavior, which is a proxy measure for social reward responsivity [122, 123].

A second clue for the role of eCB system in autism comes from observations in early neural development [124]. Autism is neurodevelopmental in nature, and atypical development of neural connectivity has been suggested to underlie its key phenotypic features [125]. A set of genes involved in neurodevelopmental processes that mediate the formation, stabilization, and pruning of synapses has been consistently associated with autism-related phenotypes in animal models [126–128]. Neuroligins (NLGN) represent a significant part of this set, and indeed several genes of NLGN family have been associated with autism [129]. In a mouse model, an autism-associated mutation in NLGN3 was found to be associated with deficits in social behavior and disrupted tonic eCB signaling [130, 131]. Evidence from this study and several others (reviewed in [124]) provides a potential causal bridge between atypical neural development and potential dysfunction of the eCB system in autism.

A third clue comes from the role of the eCB system in influencing circadian rhythm in animal models [132–134]. Autism has been associated with atypical sleep patterns and circadian rhythms [135]. Polymorphisms in *ASMT* (involved in melatonin synthesis), paralleled by reduced levels of circulating melatonin, have been reported in autism [136].

Table 2 Molecular markers of the eCB system in autism-related animal models

Biological sample	Model	eCBs alterations	Reference
Striatum	*fmr1*^{-/-} mouse	=2-AG levels	[100]
		↑DAGL and MAGL activity	
Cerebellum	Rat valproic acid	= AEA, OEA, PEA and 2-AG levels	[104]
		↓DAGLα mRNA	
Frontal cortex		= AEA, OEA, PEA and 2-AG levels	
		↓PPAR-α and ↓GPR55 mRNA	
Hippocampus		↑AEA, ↑OEA, ↑PEA content*	
		↓PPAR-γ and ↓GPR55 mRNA	
		↓MAGL mRNA	
		↑MAGL activity	
Cerebellum	BTBR T+tF/J mouse	↑CB$_2$A isoform mRNA	[105]

2-AG=2-arachidonoylglycerol; DAGL=diacylglycerol; MAGL=monacylglycerol lipase; AEA=anandamide; OEA=*N*-oleoylethanolamine; PEA=*N*-palmitoylethanolamine; PPAR=peroxisome proliferator-activated receptor

*After sociability tests

Interestingly, the eCB system also plays a role in regulating circadian rhythms [134], thus making it a putative target to examine using animal models of autism-related phenotypes.

The fourth clue comes from comorbidities commonly observed in autism. First of these is anxiety, which is highly comorbid with autism (42–56 %). The eCB system, and cannabidiol in particular, is known to mediate anxiety and related phenotypes [137, 138]. Anecdotal reports of cannabis use in autism suggest a reduction in anxiety-related symptoms. A potential role for the eCB system in ASD can thus also be mediated through its influence on the anxiety-related component of the disease. The second commonly occuring comorbidity in autism relevant to the current review is epilepsy (up to 30 %). The eCB system is studied intensively as targets for potential antiepileptic drugs [139]. It is therefore possible that a potential future drug acting on the eCB system is better able to ameliorate epilepsy-related comorbidities in ASD.

Conclusions

Accumulated evidence suggests that the eCB system constitutes a relatively less investigated piece of a puzzle that brings together 4 phenotypic features known to be atypical in autism: 1) social reward responsivity; 2) neural development; 3) circadian rhythm; and 4) anxiety-related symptoms. Therefore, the potential therapeutic exploitation of distinct elements of this system (e.g., receptor targets, biosynthetic and hydrolytic enzymes, and transmembrane/intracellular transporters)

Fig. 3 Four clues pointing to the role of the endocannabinoid (eCB) system in autism. The eCB system constitutes a relatively less investigated piece of a puzzle that brings together 4 phenotypic features known to be atypical in autism: 1) social reward responsivity; 2) neural development; 3) circadian rhythm; and 4) anxiety-related symptoms. fMRI = functional magnetic resonance imaging
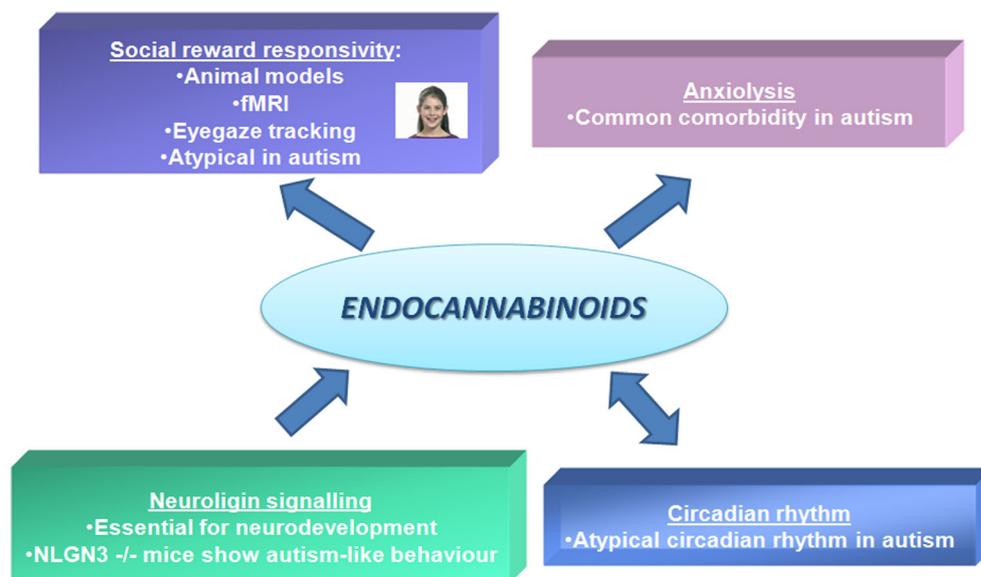
seems immense. As supported by the evidence presented in the previous sections in humans and animal models, any potential therapeutic approach is unlikely to involve a simple choice between activation *versus* inhibition of the eCB system to target specific features related to autism. Any such approach will need to be precisely tuned to the developmental timeline and to the specific pathogenetic underpinnings of autism in the single patient. Our understanding of eCB signaling in autism is still in its infancy compared with other disorders of the central nervous system or of peripheral tissues, where eCB-based therapies have already reached preclinical and clinical phases [4]. However, research in this field is rapidly evolving, and novel drugs able to hit specifically a distinct element of the eCB system are developed at a surprising speed [4]. Among them, those that target metabolic enzymes of eCBs, and, at the same time, key enzymes of oxidative pathways like cyclooxygenases seem to hold promise as next-generation therapeutics against human disorders with an inflammatory component [140], and therefore they will possibly result in also being beneficial for ASD. A second medium-term target could focus on the antiepileptic drugs that are being developed, focusing on the eCB system. These drugs could potentially ameliorate the epilepsy-related symptoms that commonly co-occur with ASD. On a final note, it seems of major interest that preliminary data, showing consistency between changes in distinct eCB system elements (i.e., CB$_2$) in animal models of ASD and in peripherabl blood mononuclear cells from young patients with ASD [106, 107], support a role for these elements in the (early) diagnosis of the disease. Future work should test expression profiles for key players of the eCB system in prospective samples to test the potential of these as diagnostic biomarkers. In this context, it should be recalled that easily accessible biomarkers of neurological disorders are highly searched for, and some of them have been already identified to hold promise in human neurodegenerative/neuroinflammatory diseases [141].

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