Endocannabinoid system in the neurodevelopment of GABAergic interneurons: implications for neurological and psychiatric disorders

Abstract: In mature mammalian brains, the endocannabinoid system (ECS) plays an important role in the regulation of synaptic plasticity and the functioning of neural networks. Besides, the ECS also contributes to the neurodevelopment of the central nervous system. Due to the increase in the medical and recreational use of cannabis, it is inevitable and essential to elaborate the roles of the ECS on neurodevelopment. GABAergic interneurons represent a group of inhibitory neurons that are vital in controlling neural network activity. However, the role of the ECS in the neurodevelopment of GABAergic interneurons remains to be fully elucidated. In this review, we provide a brief introduction of the ECS and interneuron diversity. We focus on the process of interneuron development and the role of ECS in the modulation of interneuron development, from the expansion of the neural stem/progenitor cells to the migration, specification and maturation of interneurons. We further discuss the potential implications of the ECS and interneurons in the pathogenesis of neurological and psychiatric disorders, including epilepsy, schizophrenia, major depressive disorder and autism spectrum disorder.

Keywords: autism spectrum disorder; endocannabinoid system; epilepsy; GABAergic interneuron; major depressive disorder; neurological disorders; schizophrenia.

Introduction

Over the last 30 years, the identification of the endocannabinoid system (ECS) has opened a new field of interest to research the physiological role of the ECS in the central nervous system (CNS) and the treatment of neurological and psychiatric disorders using modulating components of the ECS. In the mature nervous system, the ECS has been recognized for its role in the modulation of synaptic plasticity and the integrity of neural networks (Basavarajappa et al. 2009; Kendall and Yudowski 2016; Lu and Mackie 2016). In addition to its role in the mature nervous system, the ECS also influences the development of the immature nervous system, which may have far-reaching consequences in adults (Diaz-Alonso et al. 2012; Galve-Roperh et al. 2009; Sun and Dey 2008; Younts and Castillo 2014; Zhou et al. 2014). Since ECS-related drugs are widely studied for their therapeutic potential and are being introduced into clinical applications (Basavarajappa et al. 2017; Cristina et al. 2020; Hernández-Cervantes et al. 2017; O’Brien and McDougall 2018), it is necessary to elaborate on the roles of ECS in development to avoid unwanted side effects or to exert certain therapeutic effects. Fortunately, over the last 15 years, the roles of the ECS on neurodevelopment and subsequent pathological implications have been gradually elucidated.
GABAergic interneurons belong to a group of diverse neurons that mainly project locally and target the somas, dendrites and axons of adjacent neurons. Interneurons are crucial in controlling specific features of local circuits and processing the information flow along with neural networks (Tremblay et al. 2016). The sophisticated functions of interneurons are underpinned by the orchestrated modulation of their neurodevelopment, since the right development gives rise to appropriate anatomical, morphological, neurochemical and electrophysiological properties of a certain subtype of interneurons which in turn guarantees that interneurons function in the right space at the right time. The development of interneurons is regulated by many factors. Furthermore, the ECS is emerging as an important regulator in the neurodevelopment of interneurons.

In this review, we provide a brief introduction of the ECS and the diversity of interneurons. We will focus on the subtle process of interneuron development, the role of the ECS in the neural stem/progenitor cells and the ECS-mediated modulation of interneuron development from aspects of migration, morphogenesis/specification and connectivity. Finally, we will discuss the implications of the ECS and interneurons in the pathogenesis of neurological and psychiatric disorders including epilepsy, schizophrenia, major depressive disorder and autism spectrum disorder. Species that were used in animal experiments cited by this review are all mouse, unless being particularly specified.

The ECS

The ECS is an important neuromodulatory system that plays a pivotal role in the CNS. The ECS participates in regulating responses to environmental or endogenous stimuli, modulating synaptic plasticity (Lu and Mackie 2016) and is involved in the development of the nervous system (Har-kany et al. 2008). The ECS is composed of cannabinoid receptors, endocannabinoids (eCBs) and related enzymes responsible for the synthesis and degradation of eCBs. The binding of eCBs to cannabinoid receptors underpins the biological activity of ECS. In this part of the review, components of the ECS will be briefly introduced, which help us better understand the functional roles of the ECS.

Cannabinoid receptors

Cannabinoid receptors belong to a family of receptors that mainly contain two specific subtypes, namely cannabinoid-1 (CB1) and cannabinoid-2 (CB2) receptors (Howlett et al. 2002). The CB1 and CB2 receptors are G protein-coupled receptors (GPCRs) that couple to G proteins of the Gi/o classes (Howlett et al. 2002). Both CB1 and CB2 receptors have been found in various species, including humans (De Jesús et al. 2006; Li et al. 2019), rodents (Han et al. 2012; Zoppi et al. 2014) and zebrafish (Suflan et al. 2019).

CB1 receptor

CB1 receptors are among the most abundant GPCRs in the CNS and are found in various locations. High protein levels of CB1 receptors are located in the medium spiny neurons, a group of GABAergic interneurons in the rat striatum (Matyas et al. 2006) and their messenger ribonucleic acid (mRNA) and protein are particularly enriched in both rat and mouse cholecystokinin (CCK) + GABAergic interneurons (Bodor et al. 2005; Marsicano and Lutz 1999; Tsou et al. 1999). In addition, electrophysiological and pharmacological studies have also indicated that functional CB1 receptors can be located in glutamatergic neurons in the cortex and hippocampus (Domenici et al. 2006). Notably, glutamatergic neurons in the cortex do not express particularly high levels of CB1 receptors when compared to other neuronal populations, such as GABAergic interneurons or neurons in the cerebellum and substantia nigra (Kano et al. 2009). In addition to the CNS, CB1 receptors are also found on peripheral nerve terminals and other organs or tissues such as the eye, testes, spleen and vascular endothelium. Moreover, the ECS outside the CNS is also involved in the development of a variety of peripheral organs (Galve-Roperh et al. 2013; Kendall and Yudowski 2016). Subcellularly, CB1 receptors are abundant in axon terminals and presynaptic compartments (Schmitz et al. 2016). Several studies have revealed that CB1 receptors are also present in the mitochondrial membranes of CA1 hippocampal neurons in mice (Bénard et al. 2012). These mitochondrial CB1 receptors directly modulate neuronal energy metabolism, contribute to endocannabinoid-dependent depolarization-induced suppression of inhibition (DSI) in the hippocampus, and participate in regulating learning and memory (Bénard et al. 2012; Djeungoue-Petga and Hebert-Chatelain 2017; Hebert-Chatelain et al. 2016; Jimenez-Blasco et al. 2020).

In the adult brain, CB1 receptors are well established for their retrograde regulatory role in synaptic transmission, also known as DSI and depolarization-induced suppression of excitation (DSE). DSI occurs in inhibitory synaptic transmission. Depolarization of postsynaptic neurons results in the release of eCBs that diffuse from the postsynaptic membrane and bind to CB1 receptors in the presynaptic terminals of interneurons. The activation of these presynaptic CB1 receptors inhibits the release of GABA (Howlett et al. 2004; Kano et al. 2009; Ohno-Shosaku
and Kano 2014). The counterpart of DSI is DSE, where eCBs released from the depolarized postsynaptic neurons act on CB1 receptors in excitatory presynaptic terminals and excitatory transmission is thus suppressed (Kano et al. 2009; Lu and Mackie 2016). The suppression effect of CB1 receptors is based on coupled G proteins. After being activated by ligands, CB1 receptors are coupled to pertussis toxin-sensitive Gi and G0 type proteins and thus inhibit adenylate cyclase activity, resulting in a rapid decrease in the level of intracellular cyclic adenosine monophosphate (cAMP) (Howlett et al. 2004). Notably, apart from being activated by receptor agonists, CB1 receptors are also known to mediate ligand-independent signaling (Howlett and Abood 2017; Mukhopadhyay et al. 2000). Investigation by Mukhopadhyay et al. demonstrated that CB1 receptors could be coimmunoprecipitated with its associated G protein, the Galpha (i/o) family and the CB1 receptor-juxtamembrane C-terminal domain could autonomously activate G (i/o) proteins (Mukhopadhyay et al. 2000). What’s more, this study also found that the peptide 401–407 from the CB1 receptor complex could exist in the absence of exogenous agonists (Mukhopadhyay et al. 2000). These results further expanded our knowledge about the signaling mechanism by CB1 receptors.

**CB2 receptor**

In contrast to CB1 receptors, CB2 receptors are expressed at a relatively low level in the CNS. CB2 receptors in the CNS are primarily localized in the human microvascular endothelium (Ramirez et al. 2012) and mouse microglia (Galan-Ganga et al. 2020). After tissue injury or during inflammation, the level of CB2 receptor increases up to 100-fold, suggesting a pivotal role of CB2 in the inflammation process in the CNS (Maresz et al. 2005). CB2 receptors have also been reported to be expressed in neurons, especially the dopamine (DA) neurons in the rat ventral tegmental areas (VTA) (Zhang et al. 2017). CB2 receptors were shown to be located in hippocampal interneurons (Li and Kim 2017). However, the distribution of CB2 in interneurons in other brain domains, and in different subclasses of interneurons still remains to be fully understood.

Similar to CB1 receptors, CB2 receptors also have peripheral distribution outside the CNS (Mackie 2008). Peripheral CB2 receptors are expressed in various immune cells, suggesting their important role in diseases relating to regulation of the immune system (Leleu-Chavain et al. 2013). Among the main human blood cell subpopulations, CB2 mRNA was detected in B-cells, natural killer cells, monocytes, polymorphonuclear neutrophil cells, T8 cells and T4 cells (Galiégue et al. 1995). In mice, CB2 receptors were also found in osteoblasts, osteocytes and osteoclasts with osteoclastic CB2 being essential for the maintenance of normal bone mass (Ofek et al. 2006).

**Endocannabinoids and their metabolic enzymes**

Both exogenous and endogenous ligands bind to CB1 receptors. Exogenous ligands include natural and synthetic compounds, such as the main active component of Cannabis sativa, Δ9-tetrahydrocannabinol (Δ9-THC) (Vinals et al. 2015), and synthetic ones such as JWH-015 (Murataeva et al. 2012) and WIN55,212-2 (Zhang et al. 2013). Endogenous ligands, also known as eCBs, are lipid-signaling molecules. To date, several eCBs have been identified including 2-arachidonoylglycerol (2-AG), arachidonylethanolamide (AEA), 2-arachidonoylglycerol ether, virodhamine, and N-arachidonyl-dopamine (Basavarajappa et al. 2009; Pacher et al. 2020). Among these eCBs, 2-AG and AEA are the two most widely studied. Endocannabinoids and their metabolism are quite complicated as a result of the promiscuity of mediators, the overlap with other pathways, and alternative metabolic processes (Cristino et al. 2020). In this section of the review, only the core metabolic enzymes will be introduced, which are widely investigated in neurodevelopmental studies. For a more comprehensive and detailed understanding of eCBs and their metabolism, please see reviews from Cristino et al. (2020), Iannotti et al. (2016), Muccioli (2010).

**2-AG and its metabolism**

The synthesis of eCBs is triggered on demand and usually depends on the increased intracellular level of calcium (Basavarajappa et al. 2009), activation of Gα11-coupled receptors, or both (Muccioli 2010). Two primary routes are responsible for the biosynthesis of 2-AG. First, phospholipase C (PLC) hydrolyzes membrane phospholipids to produce diacylglycerol (DAG), which is subsequently metabolized by diacylglycerol lipase (DAGL) to generate 2-AG (Basavarajappa 2007). DAGL can be subclassified into DAGLα and DAGLβ. DAGLα was reported to be the predominant enzyme for the synthesis of 2-AG in the endocannabinoid-mediated regulation of neurotransmission (Jain et al. 2013), DAGLβ may play a key role in synthesizing 2-AG during inflammatory responses (Hsu et al. 2012). The key intermediate DAG can be alternatively produced from phosphatidic acid by phosphatidic acid hydrolase (Bisogno et al. 1999b). The second synthetic pathway for 2-AG is mediated by phospholipase A1
(PLA1)-produced lysophospholipid which is hydrolyzed by lyso-PLC to generate 2-AG (Basavarajappa 2007).

The degradation of 2-AG is primarily mediated by monoacylglycerol lipase (MAGL), alpha/beta domain-containing hydrolase 6 (ABHD6), and alpha/beta domain-containing hydrolase12 (ABHD12) (Blankman et al. 2007). MAGL catalyzes 85% of the brain 2-AG hydrolysis into arachidonic acid while ABDH6 and ABDH12 catalyzes the remaining 15% of 2-AG, also into arachidonic acid (Blankman et al. 2007). In some conditions, 2-AG was also reported to be oxidized by cyclooxygenase-2 (COX-2) into prostaglandin (PG) H₂ glyceryl ester (Muccioli 2010).

Notably, fatty acid amide hydrolase (FAAH), the primary enzyme catalyzing the hydrolysis of AEA, was also reported to be deputed to the physiological inactivation of 2-AG (Di Marzo et al. 1998).

### AEA and its metabolism

Another eCB in the CNS is AEA. In the rat brain, the concentration of 2-AG is nearly 200-fold higher than that of AEA (Bisogno et al. 1999a). AEA primarily originates from N-arachidonoyl phosphatidyl ethanolamine (NAPE), the membrane phospholipid precursor of AEA (Liu et al. 2008). To date, several pathways have been identified in the synthesis of AEA. The major pathway to synthesize AEA is the hydrolysis of NAPE by NAPE-specific phospholipase D-like hydrolase (NAPE-PLD) (Okamoto et al. 2004). Other synthesis pathways for AEA include NAPE-PLC pathway where NAPE is cleaved by PLC followed by dephosphorylation by protein tyrosine phosphatase N22 (PTPN22) (Liu et al. 2006), NAPE dual-hydrolysis by alpha–beta hydrolase enzyme ABH4 followed by hydrolysis by glycerophosphodiesterase 1 (Simon and Cravatt 2010), and the N-acylated plasmalogen-type ethanolamine phospholipid-derived, NAPE-PLD-independent pathway (Tsuboi et al. 2013).

After being released into the synaptic cleft, AEA exerts its bioactivity on CB1 or CB2 receptors and then becomes inactivated rapidly (Di Marzo et al. 1994). The main enzyme catalyzing the degradation of AEA in the CNS is FAAH. FAAH is a membrane-bound homodimeric enzyme that degrades the AEA into arachidonic acid and ethanolamine (Cravatt et al. 1996). Alternative pathways to inactivate AEA include oxidation by COX-2 into PGH₂ ethanolamides (Alhouayek and Muccioli 2014; Van Dross 2009; Woodward et al. 2008; Yang et al. 2008) and degradation through N-acylthanolamine-hydrolyzing acid amidase (Tsuboi et al. 2005). Inhibition of FAAH may shunt AEA metabolism into these alternatives.

For a clear profile of the synthesis and degradation/inactivation of 2-AG and AEA, please refer to Table 1.

### Interneurons and their development

The functional cortical network consists of two major components: the glutamatergic excitatory neurons and the GABAergic inhibitory interneurons. Excitatory neurons are essential for long-range projections and information processing across different brain regions (Lodato et al. 2015). Inhibitory interneurons, which account for only 10–15% of all cortical neurons, play a crucial role in the modulation of neural circuits to ensure the coordination of neuronal activities and that the excitatory information is in check (Gupta et al. 2000). Cortical GABAergic interneurons demonstrate an enormous variety of subtypes in terms of morphology, biochemical characteristics, input and output connectivities, and electrophysiological profiles (Fishell and Rudy 2011; Markram et al. 2004; Riedemann 2019). Importantly, the dysfunction of interneurons and anomalies in their development has been reported to be the main cause of numerous neurological disorders (Jacob 2016; Morishita et al. 2015; Palop and Mucke 2016). In addition to the cerebral cortex, interneurons have also been detected in other parts of the brain, including the hippocampus (Bird et al. 2018), striatum (Rapanelli et al. 2017), thalamus (Jager et al. 2016) and cerebellum (Sotelo 2015). In the next part of this study, we will summarize some of the most important findings related to interneuron development and function.

#### Table 1: The main pathways of biosynthesis and degradation/inactivation of AEA and 2-AG.

<table>
<thead>
<tr>
<th>Endocannabinoids</th>
<th>Synthesis</th>
<th>Degradation/inactivation</th>
</tr>
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<tbody>
<tr>
<td>2-AG</td>
<td>PLC-DAGL, PLA1-lyso-PLC</td>
<td>MAGL, ABDH6/ABDH12, FAAH, COX-2</td>
</tr>
<tr>
<td>AEA</td>
<td>NAPE-PLD, NAPE-PLC</td>
<td>FAAH, COX-2</td>
</tr>
</tbody>
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2-AG, 2-arachidonoylglycerol; AEA, arachidonylethanolamine; PLC, phospholipase D; PLD, phospholipase D; PLC, phospholipase C; DAGL, diacylglycerol lipase; ABDH6, alpha/beta domain-containing hydrolase 6; ABDH12, alpha/beta domain-containing hydrolase 12; FAAH, fatty acid amidase hydrolase; COX-2, cyclooxygenase-2.
review, the diversity and development of interneurons will be introduced.

**Interneuron diversities**

Interneurons can be classified depending on their morphological, molecular and electrophysiological features. Although there is still no clear consensus on the exact number of interneuron types, their classification using neurochemical markers has become widely accepted. In general, cortical interneurons can be classified based on markers of the calcium-binding protein parvalbumin (PV), neuropeptide somatostatin (SST) and ionotropic serotonin receptor 5HT3aR (Tremblay et al. 2016). This classic classification based on specific molecular markers is consistent with recent interneuron taxonomy based on transcriptomics (Huang and Paul 2019). Notably, the expression pattern of cannabinoid receptors is distinct in different interneuron subtypes (Figure 1).

**PV interneurons**

PV interneurons are the most abundant interneuron subtype, accounting for about 40% of all cortical interneurons (Tremblay et al. 2016). PV interneurons can be further subdivided into basket cells, chandelier cells and “multipolar bursting cells” according to their morphology.

PV basket cells are the most common type of interneuron and are mainly present in L2–L6 (Kubota 2014). PV basket cells are characterized by their proximal “basket-like” terminals, with multipolar dendritic arbor and highly branching axonal arbor (Fishell and Rudy 2011; Kubota 2014). PV basket cells mainly project on the soma and dendrites of cortical pyramidal cells and other PV interneurons (Fishell and Rudy 2011) to inhibit local populations (Freund et al. 1983), while they also have interlaminar and intercolumnar projections to influence neighboring laminar and column (Bortone et al. 2014; Jiang et al. 2015; Thomson and Lamy 2007). PV basket cells have fast firing frequency and temporally precise

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**Figure 1:** Interneuron diversity in the cortex and the distribution of CB1 receptors. (A) A diagram of the main classes of interneuron in the mouse cortex. Interneurons can be categorized according to their expression of neurochemical markers, that is, parvalbumin (PV), somatostatin (SST), and the serotonin receptor 3A (5HT3aR). Each type of interneuron can be further subtyped by their morphology and physiological properties. (B) A schematic diagram of the distribution of CB1 receptors in interneurons of different forebrain areas. CCK, cholecystokinin; PV, parvalbumin; SST, somatostatin; VIP, vasoactive intestinal polypeptide.
electrophysiological properties, which make them have precise and fast inhibition of their postsynaptic targets (Rossignol et al. 2013).

Chandelier cells, also known as axo-axonic neurons, are characterized by their unique shape of candlestick-like projections (Tremblay et al. 2016). Localized in L2 and L5/6 of the cortex (Taniguchi et al. 2013), chandelier cells are well established for their stereotyped postsynaptic targets on the initial axon segment of pyramidal cells (Ascoli et al. 2008). Interestingly, in addition to exerting hyperpolarizing effects on postsynaptic targets (Glickfeld et al. 2009), chandelier cells have also been reported to depolarize postsynaptic pyramidal cells (Szabadics et al. 2006), depending on the activity state of the postsynaptic target—chandelier cells could activate quiescent pyramidal neurons but inhibit active ones (Woodruff et al. 2011).

Multipolar bursting cells (MBCs) are PV interneurons that are significantly different from basket cells and chandelier cells. In brief, MBCs have large round or oval cell bodies and several visible thick dendrites (Blatow et al. 2003). MBCs unidirectionally innervate basket cells and are reciprocally connected with layer 2/3 pyramidal cells (Blatow et al. 2003; Caputi et al. 2009). The synapses of MBCs to MBCs and MBCs to pyramidal cells exhibit paired-pulse facilitation (Blatow et al. 2003).

**SST interneurons**

SST interneurons account for about 30% of cortical interneurons and can be divided into two subtypes according to their morphology: Martinotti cells and non-Martinotti cells (Wamsley and Fishell 2017). Martinotti cells are mainly present in L2/3 and L5/6 and are characterized by their axon arbor on the distal dendrites of pyramidal cells in L1 (Munoz et al. 2014). Martinotti cells can also target the basal dendrites of local pyramidal neurons and other interneurons such as PV interneurons and vasoactive intestinal polypeptide (VIP) interneurons (Ma et al. 2006). In contrast to Martinotti cells, non-Martinotti cells are mainly present in L4 and L5/6. Non-Martinotti cells extensively branch in L4 and target the dendrites of local PV interneurons, producing the effect of disinhibition of L4 pyramidal cells (Xu et al. 2013).

SST interneurons, whether Martinotti or non-Martinotti cells, share two major electrical properties. First, excitatory inputs on the SST interneurons are strongly facilitating (Beierlein et al. 2003) which enables SST interneurons to be recruited by even a single high-frequency pulse from one presynaptic cell. This is different from PV interneurons because the activation of PV interneurons requires many presynaptic cells to fire simultaneously (Kapfer et al. 2007; Sylwestrak and Ghosh 2012). Another electrophysiological feature of SST interneurons is muscarinic-mediated depolarization. After incubating with cholinergic agents, such as carbachol, the depolarization of SST interneurons is strong enough to produce spiking activities (Urban-Ciecko et al. 2018; Xu et al. 2013).

**5HT3aR interneurons**

Accounting for about 30% of all neocortical interneurons, 5HT3aR interneurons express functional 5HT3a and nicotinic receptors (Lee et al. 2010). 5HT3aR interneurons are believed to be more heterogeneous than PV and SST interneurons and they can be subgrouped by whether or not they express neuropeptide VIP. Most VIP interneurons reside in L2/3 (Pronneke et al. 2015). According to the expression of CCK or calretinin, VIP interneurons can be further grouped into VIP+ calretinin+ interneurons (50–70%) and VIP+ CCK+ interneurons (10–30%) (Tremblay et al. 2016). VIP+ calretinin+ interneurons have a bipolar-like morphology with a vertically oriented axon and frequently bifurcated dendrites (Bayraktar et al. 2000). The dendrites of these VIP+ calretinin+ interneurons are transalaminar, ascending through L1 up to the near meninges surface (Tremblay et al. 2016), while their axons, mostly confined to either a barrel- or septum-related column, preferentially form synapses on to SST interneurons (Jiang et al. 2015). The most prominent electrophysiological feature of VIP interneurons is their high input resistance, which means VIP interneurons are extremely sensitive to excitatory inputs to produce depolarization (Lee et al. 2010). After receiving supra-threshold depolarizing pulses, VIP interneurons manifest two firing patterns, the continuous adapting pattern where VIP interneurons fire continuously, and the irregular spiking pattern in which VIP interneurons fire AP followed by intermittent AP with irregular intervals (Goff and Goldberg 2019).

Non-VIP 5HT3aR interneurons account for approximately 60% of 5HT3aR interneurons, including neurogliaform cells (NGFCs) and non-VIP CCK+ interneurons (Tremblay et al. 2016). NGFCs, the major interneuron components of L1, have multipolar morphology with small, round soma, multiple short dendrites spreading radially and dense axonal plexus stretching spherically (Olah et al. 2007; Tremblay et al. 2016). Notably, NGFCs are the only interneurons found to exert GABA<sub>B</sub> responses following a single AP (Olah et al. 2007). This could be because the synapses of NGFCs have a small junctional area and large cleft distance (Szabadics et al. 2007). Another set of 5HT3aR interneurons is CCK+ basket interneurons, whether VIP-expressing or not. CCK+ VIP-expressing interneurons,
often referred to as small CCK basket cells, tend to exhibit small soma with multipolar, or bifurcated dendrites and they are largely found in L2. In contrast, CCK+ non-VIP interneurons are shown to have large basket cell morphology with larger somata and dendritic and axonal spans (Tremblay et al. 2016). Like PV basket interneurons, these CCK+ non-VIP basket cells also exert perisomatic inhibition to postsynaptic pyramidal cells (Foldy et al. 2007).

**Interneurons outside the neocortex**

Interneurons are also widely distributed outside the neocortex. In hippocampus, GABAergic inhibitory interneurons account for approximately 10–15% of the total local neuron population (Bezaire and Soltesz 2013). Hippocampus interneurons manifest a remarkable diversity of classification. Besides PV+ basket cells, PV+ chandelier cells, CCK+ basket cells and NGFCs, other types of interneurons have also been identified in the hippocampus, including bistratified cells, oriens lacunosum-molecular interneurons, ivy cells and interneuron selective interneurons (Pelkey et al. 2017). Interneuron circuits in the hippocampus serve as scaffolding for rapid information learning by replaying, reversing, refining and regulating spike sequences (Nicola and Clopath 2019). In the dentate gyrus of the hippocampus, PV+ interneurons together with granule cells (GCs) establish a powerful and abundant lateral inhibition network where a GCs inhibit neighboring GCs via PV+ interneurons (Espinoza et al. 2018; Turi et al. 2019). Whether this kind of lateral inhibition is involved in pathogenesis of hippocampal circuit-related diseases, such as epilepsy or status epilepticus (Seinfeld et al. 2016), remains to be determined.

Apart from the hippocampus, interneurons in the amygdala, striatum and cerebellum are also reported to play important roles. In the amygdala, PV+ and SST+ interneurons have been reported to regulate the circuits for fear learning and conditioning (Krabbe et al. 2018; Wolff et al. 2014). Recent studies have found that Fluoxetine, a well-known antidepressant, could alter the structure of both interneurons, calretinin-basket cells (Marsicano and Lutz 1999). The high expression of CB1 receptors in cortical CCK+ interneurons has also been verified in rat somatosensory cortex (Bodor et al. 2005) and dorsolateral prefrontal cortex of macaque monkey (Eggan et al. 2010). In addition to be expressed in cortical CCK+ interneurons, CB1 receptors have also been shown to be expressed within SST-expressing and VIP-expressing interneurons in rat neocortical slices (Hill et al. 2007). Another study in rat medial prefrontal cortex demonstrated that CB1 receptors are colocalized with calbindin-expressing interneurons while double labeling of CB1 with PV or calretinin revealed no colocalization, which is consistent with results from Marsicano and Lutz (Marsicano and Lutz 1999; Wedzony and Chocyk 2009).

In the hippocampus, similar to the cortex, most CB1-expressing interneurons are proved to be CCK+/PV−/calretinin-basket cells (Marsicano and Lutz 1999). In addition, study in rat also found the co-expression of CB1 receptors and SST (Zou and Kumar 2015). CB1 receptors are important in the modulation of GABA release from CCK+ basket cells
in hippocampus, as was demonstrated by experimental studies that the GABA release from the CCK+ basket cells in CA1 elicited by CCK could be blocked by AM251 (10 μM), a CB1 receptor antagonist (Lee and Soltesz 2011). In the amygdala, studies in both rats and mice demonstrated that high levels of functional CB1 receptors were expressed in CCK-expressing basket cells while none of the PV+, SST+, or calretinin+ interneurons expressed high levels of CB1 receptors (Marsicano and Lutz 1999; McDonald and Mascagni 2001; Rovira-Esteban et al. 2019). In the striatum, on the contrary, most PV+ interneurons (86.5%), more than one-third (39.2%) of cholinergic interneurons are labeled for CB1 receptors (Fusco et al. 2004), while interneurons of calretinin+, as was suggested in rats (Fusco et al. 2004), or CCK+ interneurons, as was suggested in mice (Marsicano and Lutz 1999), are devoid of CB1 receptors. Notably, in CB1 knockout mice, PV immunoreactivity within individual neurons in the dorsolateral striatum, primary motor cortex (MI), and prefrontal cortex decreased significantly and the density of PV+ neurons was significantly lower in the striatum but not in the cortical regions (Fitzgerald et al. 2011). This result suggests that CB1 receptors might have important roles in the neurodevelopment of PV-containing cortical and striatal interneurons. In the thalamus, CB1 receptors are expressed at axon terminals on the PV+ neuronal projections from the ventral zona incerta to the posterior complex of the thalamus and they could mediate nociceptive behaviors (Wang et al. 2020).

**Neurodevelopment of interneurons**

The development of interneurons is a sophisticated process under the control of intrinsic and extrinsic signals. The neurodevelopment of diverse cortical interneurons and the underlying regulatory mechanisms were elaborated in two recent reviews (Lim et al. 2018; Wamsley and Fishell 2017). In this part of the review, the neurodevelopment process of interneurons will be briefly discussed to better understand the role of the ECS in interneuron development.

The origins of rodents and primates telencephalon interneurons are progenitors within several subpallium progenitor domains. These domains comprise the lateral, medial and caudal ganglionic eminences (LGE, MGE, and CGE, respectively) which localize at the floor of lateral ventricles (Liu et al. 2013), and the preoptic region, mainly including the preoptic area (POA) and the preoptic-hypothalamic (POH) border domain (Lim et al. 2018). Cortical interneurons originate from three domains, the MGE, CGE, and the POA (Hu et al. 2017). MGE is the origin of SST+ and PV+ cortical interneurons, with SST+ interneuron being generated from the dorsal MGE while PV+ interneurons from both the dorsal and ventral MGE (Lunden et al. 2019). The CGE is the origin of cortical 5HT3aR interneurons expressing VIP, reelin, or NPY (Lunden et al. 2019). The POA gives rise to the NPY+/SST− interneurons in the superficial layers in the neocortex (often at the boundary between layer I and layer II) and also the hippocampus (Gelman et al. 2009). The LGE, on the other hand, was proved to be the origin of interneurons in striatum and olfactory bulb (Stenman et al. 2003; Yun et al. 2003). The POH is the most caudal progenitor domain in the preoptic region, characterized by its lack of expression of the homeobox transcription factor Nkx2-1 (Flames et al. 2007). This Nkx2-1 lacking domain of POH is the origin of NGFCs and multipolar NPY+ interneurons (Lim et al. 2018).

After becoming postmitotic, interneurons undergo several periods of migration until they reach their final position within a specific layer of the cortex. The migration process is divided into two consecutive phases: tangential migration and radial migration. Tangential migration is used by interneurons to migrate from their original sites, the MGE, CGE, LGE and POA, to the pallium. Interneurons tangentially migrate through three different routes: a superficial route through the marginal zone (MZ), a deep route overlapping the subventricular zone (SVZ), and the subplate where a smaller fraction of interneurons migrate (Tanaka and Nakajima 2012). When appropriate, they disperse radially to integrate within the targeted laminar layer (Wamsley and Fishell 2017). Several factors influence the radial migration and laminar distribution of interneurons. Their interaction with cortical pyramidal cells is one of these factors (Lodato et al. 2011a). Once reaching their targeted layers, interneurons project axons to their synaptic targets, receive both local and long-range inputs, and exhibit different morphology and specific neurochemical markers (Wamsley and Fishell 2017). Several chemoattractant cues also guide the migration of interneurons. Neurogenin1 (NRG1) and its receptor, ErbB4 expressed by interneurons, is the controlling signal of interneuron tangential migration (Flames et al. 2004; Villar-Cerviño et al. 2015). As chemo-attractants, NRG1/ErbB4 controls the tangential migration of interneurons from the MGE toward the striatum (Marín 2013; Villar-Cerviño et al. 2015). Brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT4), acting through TrkB signaling, also control the tangential migration of interneurons (Polleux et al. 2002). As for tangential migration, NRG3 expressed by the developing pyramidal cells guides the radial migration and laminar allocation of interneurons within the developing cortical plate (Bartolini et al. 2017). Shortly after becoming postmitotic, the diversity of interneurons is already patent in their diverse transcriptional
programs which subsequently guide their further differentiation in the developing cortex (Mi et al. 2018). Single-cell RNA transcriptomics revealed that in the ganglionic eminences, there are distinct types of progenitor cells and newborn neurons with temporally and spatially restricted transcriptional patterns that lead to different classes of interneurons in the adult cerebral cortex (Mi et al. 2018). Similarly, another single-cell RNA sequencing study also demonstrated that upon becoming postmitotic, progenitors in ganglionic eminences diverge and differentiate into transcriptionally distinct states including an interneuron precursor state, and there existed shared sources of transcriptional heterogeneity between adult interneurons and their precursors (Mayer et al. 2018). These results suggested that the fate acquisition of different interneuron subtypes occurred during the postmitotic stage, a very early time point during interneuron development. Elimination of excess cells via postnatal programmed cell death is essential for the establishment of neural circuits and the integration of interneurons within (Kim and Sun 2011). It has been demonstrated that two waves of programmed cell death occurred during the neurodevelopment process. The first wave occurs during embryonic stages, primarily affecting progenitor populations to eliminate excess stem/progenitor cells (Kim and Sun 2011; Wong and Marín 2019). The second larger wave occurs during early postnatal stage and ultimately determines the final amount of cortical neurons (Wong and Marín 2019). Programmed cell death during development occurs in a cell type-specific manner and it depends on neuronal activity. As for interneurons, programmed cell death is determined intrinsically, as was suggested by the fact that during postnatal life about 40% developing cortical interneurons were eliminated through Bax-dependent apoptosis and was not affected by the cell-autonomous disruption of TrkB, the main neurotrophin receptor expressed by neurons of the CNS (Southwell et al. 2012). On the other hand, the survival of interneurons also depends on the activity of pyramidal cells during postnatal stage, that is, pyramidal cells regulate the survival of interneurons through the modulation of PTEN signaling which effectively drives interneuron death during this period (Wong et al. 2018). Considering the important roles of ECS in the maintenance of progenitor survival in the fetal brain (Maccarrone et al. 2014), it is reasonable to suppose that ECS plays regulatory roles in interneuron programmed cell death. Detailed studies are needed to verify this hypothesis concerning interneuron cell death.

The ECS and interneuron development

The ECS during neurodevelopment

During the development of the CNS, both the cannabinoid receptors and eCBs undergo dynamic temporal and spatial changes. In this section, the changing characteristics of the cannabinoid receptors and eCBs during neurodevelopment are discussed.

CB1 and CB2 receptors are expressed at a very early stage during embryonic development, even earlier than the formation of neural tubes (Sun and Dey 2008). Therefore they are important for blastocyst development and neural plate formation (Paria et al. 2001). For example, O-2545 (a watersoluble Δ⁹-THC analog) could bring about embryotoxic effects and interfere with neural plate formation in chick embryo (Psychoyos et al. 2008), suggesting a crucial regulatory role of ECS in the development of the nervous system.

The expression pattern of CB1 receptor mRNA in the developing mouse brain has been extensively studied. As early as embryonic day E11.5, CB1 receptor mRNA can be detected in the pallium, subpallium and developing spinal cord (Diaz-Alonso et al. 2012). Starting from E12.5, CB1 receptors are strongly expressed in reelin+ Cajal–Retzius cells, which play an important role in the regulation of radial glial cell morphology and the inside–out formation of the cortical plate (Vitalis et al. 2008). By E14.5, CB1 receptor mRNA is evident in the immature cortical plate and hippocampal primordium, peaks at approximately E16.5, and gradually declines in the late gestational embryo (Mulder et al. 2008). This period coincides with the tangential migration of interneurons (Lim et al. 2018). Notably, in rats, around the last days of the fetal period (gestational day 21) and the first days of the postnatal period (postnatal day 5), there is a transient abundant expression of CB1 receptor in regions where they are scarcely distributed in the adult brain, that is, the neuronal fiber-enriched areas, including the corpus callosum, anterior commissure, stria terminalis, fornix and white matter areas of the brainstem (Berrendero et al. 1998; Romero et al. 1997). In mice, the atypical location of CB1 in corticofugal and commissural fibers starts earlier (around E13) and progressively declines at the end of the first postnatal week (Diaz-Alonso et al. 2012). In adulthood, CB1 receptors are diffusely distributed throughout cortical laminas (particularly abundant in layers II–III, upper layer V, and layer VI) and the hippocampus, in excitatory
glutamatergic neurons (Katona et al. 2006), CCK+ GABAergic interneurons and calbindin+ interneurons (Bodor et al. 2005). Interestingly, PV+ fast-spiking interneurons in the nucleus accumbens, in contrast to those in the cortex and hippocampus, have been demonstrated to express CB1 receptors (Winters et al. 2012).

The levels of eCBs, 2-AG and AEA, are also finely regulated during development. 2-AG is the most abundant eCB in the embryonic period, while AEA levels peak in the perinatal stages (Galve-Roperh et al. 2009). DAGLα and DAGLβ, the 2-AG synthesizing enzymes, are detected during neurodevelopment. DAGLα is expressed during early neuronal development of chicks throughout the nerve cord in both the proliferative compartment and the intermediate and marginal zones, while DAGLβ is detectable at later stages of neurodevelopment in the developing axonal tracts (Watson et al. 2008). MAGL, the 2-AG degrading enzyme, is expressed in central and peripheral axons of the fetal nervous system by embryonic day 12.5 in mice (Keimpema et al. 2010). The expression of NAPE-PLD, the synthesizing enzyme of AEA, is evident as early as preimplantation and blastocyst development (Berghuis et al. 2007). FAAH, the degrading enzyme of AEA, was reported to remain constant between E7.5 and E10.5, and elevated over 30 times in the adult mouse forebrain (Psychoyos et al. 2013). In addition to CB1 and CB2 receptors, several studies have demonstrated the role of the ECS in neural stem/progenitor cells. Neural progenitors from both CB1−/− and CB2−/− knockout mice demonstrated impaired neurosphere generation, proliferation and self-renewal ability (Aguado et al. 2005; Palazuelos et al. 2006). Moreover, exposure to AEA, which has a similar affinity to both CB1 and CB2 receptors, could significantly promote neural stem cell (isolated from C57/BL6 mice embryos at E13.5) differentiation into neurons and mildly decreased the percentage of GFAP-positive astrocytes (Compagnucci et al. 2013). This effect could be abolished by co-incubation with AM251 (selective CB1 receptor antagonist), but not AM630 (selective CB2 receptor antagonist) (Compagnucci et al. 2013). By contrast, another study demonstrated that in rat neural progenitor cell culture activation of CB1 receptors by WIN55,212-2 increases progenitor proliferation and differentiation into astroglial cells in vitro (Aguado et al. 2006). The discrepancy between the above results might be due to differences between species, i.e. mouse or rat. Together these results suggest the indispensable role of CB1 receptors in the differentiation of neural stem cells. A recent study found that increased levels of 2-AG also caused bursts of neuroblast motility, as demonstrated by longer movement distances, lower frequency of turning and reduced neuron–neuron contacts. This effect of 2-AG was controlled by mGluR5/TRPC3 activity possibly through NRG1/ErbB4 pathways (Turunen et al. 2018).

Apart from the above mentioned in vitro studies, in vivo studies have also demonstrated the role of ECS in neural progenitor development (Díaz-Alonso et al. 2012). CB1 receptors regulate the proliferation of dorsal telencephalic progenitors by sustaining the transcriptional activity of the Pax6-Brachyury complex (mTORC1) pathway (Díaz-Alonso et al. 2015). In addition, CB1 receptors also contribute to the differentiation of corticospinal motor neuron differentiation through the Ctip2/Satb2 transcriptional regulation axis (Díaz-Alonso et al. 2012). As for migration, it was reported that disrupting CB1 receptors in mice would impair the migration of developing pyramidal neurons and cause cortical malformations (Díaz-Alonso et al. 2017), indicating that CB1 receptor is strictly required for appropriate pyramidal neuron migration in the developing cortex. Taken together, both in vitro and in vivo studies suggest the indispensable role of the ECS in both the proliferation and self-renewal of neural stem/progenitor cells, the facilitating effect of eCBs on neural stem/progenitor cell differentiation, and their important role in the migration of neural stem/progenitor cells.

### The ECS in neural stem/progenitor cells

Several studies have demonstrated the expression of the ECS in neural stem/progenitor cells by observing the in vitro aggregation, that is, the neurospheres. Neurospheres can produce AEA and 2-AG with 2-AG being 50–100 times more abundant than AEA (Aguado et al. 2005). Both CB1 and CB2 receptors are expressed in neurospheres and the level of CB1 receptors is significantly higher than that of CB2 (Compagnucci et al. 2013). In addition to CB1 and CB2 receptors, metabolizing enzymes of eCBs are also detected in neural stem cells. The AEA degrading enzyme FAAH is expressed by actively dividing cells co-expressed with nestin (Aguado et al. 2005). DAGLα is dramatically downregulated when neural stem cells are differentiated toward the GABAergic neuronal phenotype, which is likely to be regulated by the transcription factor (TF) Sp1 (Walker et al. 2010).
The ECS and interneuron development

In addition to the role of the ECS in general neural development investigated using neural stem/progenitor cells, the fact that CB1 receptors were detected in GE primordium implicates its essential role in the development of interneurons. As early as E12.5, numerous intensely labeled CB1+ cells have been identified in the subpial area of GE and the MZ of the dorsal telencephalon, with a subcellular location in the cytoplasm of the cell bodies and proximal dendrites (Morozov et al. 2009). The exact role of the ECS in interneuron development is not fully understood. However, several experiments have helped elucidate the role to some extent by using genetically modified mouse observations or chemical compound applications. To date, it has been demonstrated that the ECS is implicated in various steps in interneuron development (Figure 2). In the following part of the review, we will specifically focus on the role of the ECS in interneuron development, based on evidences from both in vivo and in vitro studies.

The ECS and interneuron generation

Disturbance of the ECS will affect the right generation of certain kinds of interneurons. In CB1−/− mice, decreased levels of PV within individual interneurons were detected in the primary motor cortex (M1), prefrontal cortex (PFC) and dorsolateral striatum. At the same time, a lower density of PV+ neurons was observed in the striatum but not in the cortical regions (Fitzgerald et al. 2011). In the dentate hilus of the hippocampus, the number of neuropeptide Y-immunoreactive interneurons is significantly lower in CB1−/− mice than in CB1+/+ mice, but there was no difference in the number of PV+ and CCK+ interneurons (Rogers et al. 2016). Furthermore, it has been suggested that CB1 receptors are a marker of prospective CCK+ interneurons in the hippocampus before they express identifiable interneuron markers (Morozov et al. 2009). While migrating through the neocortex, CB1+ cells do not express other interneuron markers. Upon arrival at their destination in the hippocampus, these CB1+ cells obtain Dlx, GABA and CCK (Morozov et al. 2009).

These studies together point to the potential essential role of the ECS in the generation of specific lines of interneurons, although the effect varies with different brain regions and cell types. Based on these results, we cannot assert the exact mechanism by which ECS influences the generation of interneurons. Currently, there is still no direct evidence demonstrating the role of the ECS in interneuron proliferation. The ECS might regulate the proliferation of

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**Figure 2:** The neurodevelopment process of interneurons and the roles of the ECS. Interneurons originate from the neurogenic domains in ganglionic eminences (GCs) and the preoptic regions. Neural stem cells (NSCs) first proliferate to expand the cell group and then gradually differentiate. Some of them are then specified into interneuron progenitors (IPs). IPs migrate tangentially and radially to their appropriate sites while the differentiation into interneuron moves on and the axon/dendrites grow to search for the targets. The processes of immature interneurons form synapses with targets and those who cannot integrate into the network properly will be eliminated by programmed cell death. The proper development of interneurons ensures the proper neural network wiring. The ECS is one of the important regulating factors. Direct evidences have demonstrated that the ECS plays an essential role in the neurosphere formation and differentiation in neural stem/progenitor cells, and the migration of immature interneuron progenitors. The ECS is also important in physiological functions of mature interneurons. As for proliferation of interneuron progenitors, the specification of certain type of interneuron, and programmed cell death, several investigations suggested that the ECS probably play roles in these events. However, further detailed studies are needed to directly verify these roles of the ECS. GCs, ganglionic eminences; ECS, endocannabinoid system.
interneuron progenitors or adjust the interneuron differentiation, migration, or both, because the aforementioned phenotypes in the CBI<sup>−/−</sup> mice might be attributed to either alteration in progenitor proliferation, or interneuron differentiation or migration, or both. More studies are needed to reveal the fundamental mechanisms involved.

**The ECS and interneuron migration**

Upon becoming postmitotic, interneurons undergo a long journey of tangential migration and radial migration until they reach their destination in the cortex, basal ganglions, or other regions (Lim et al. 2018). The role of the ECS on the migration of interneurons has been investigated in several studies. In one study, CBI receptors activated by AEA induced chemotaxis effects in rat CCK<sup>+</sup> interneurons (Berghuis et al. 2005). In addition, this AEA-induced and CBI receptor-dependent migratory response also modulated the BDNF-induced migration of interneurons in an additive fashion (Berghuis et al. 2005). Notably, the migratory effects of AEA were mediated through the coupling of the CBI receptor/TrkB complex and thus the downstream pathway of TrkB signaling (Berghuis et al. 2005). In another study, CBI receptors and doublecortin (DCX) were found to be co-expressed in postmitotic migrating neurons located in the mantle zone of GE and in the developing rat pallium (Saez et al. 2014). DCX is a microtubule-associated protein necessary for correct tangential migration and laminar allocation in developing telecephalons in both mice and rats (Francis et al. 1999). Its coexpression with CBI receptors indicates the possible role of CBI receptors in neuronal migration (Saez et al. 2014). In the prelate/MZ, the superficial migrating zone of interneurons, CNR1 was shown to be strongly expressed on GAD-67 positive interneurons at E15.5 (Antypa et al. 2011). This observation further indicated that CBI receptors are essential regulating factors in interneuron migration. As expected, prenatal exposure to WIN 55,212-2, a CB1 and CB2 receptor agonist, significantly increased the number of GABA<sup>+</sup> cells in the MZ (Saez et al. 2014). These investigations paved the way for further studies to confirm the role of the ECS on interneuron migration and relative molecular mechanisms during neural development.

**The ECS and interneuron maturation**

Appropriate morphogenesis and maturation of interneurons are critical for the correct integration into the local neural circuits, thus ensuring coordinated neural functions. The establishment of interneuron morphology and synaptogenesis is under the control of the interaction between external stimuli and intrinsic regulation networks (Wamsley and Fishell 2017). This is a complex process involving multiple signaling molecules and pathways such as ion channels (Chittajallu et al. 2017), intracellular calcium signals (Easton et al. 2016) and the activation of certain TFs (Le et al. 2017).

The role of the ECS in the maturation of interneurons has been investigated. First, CBI receptors have been reported to participate in the neuritogenesis and axonal pathfinding of GABAergic interneurons. CBI receptors are preferentially expressed on axons and axonal growth cones of GABAergic interneurons in the mouse cortex during the late gestation period as they are undergoing tangential and radial migration (Berghuis et al. 2007). In GABAergic interneuron cultures, early growth cones of quiescent axons expressed CBI receptors on the leading filopodial tips. A gradual enrichment of CBI receptors on the motile filopodial tips at the leading edge of the growth cones was associated with morphological growth cone differentiation (Berghuis et al. 2007). Meanwhile, the synthetic CBI receptor agonist WIN55,212-2 could induce growth cone repulsion, growth cone collapse and neurite retraction of GABAergic interneurons in a CBI receptor-dependent manner. This chemotropism effect is mediated by the activation of serine-threonine kinase Rho kinase and subsequent increase in the GTP-bound activity of RhoA (Berghuis et al. 2007), which controls cytoskeleton dynamics and integrity in axonal growth cones (Jaffe and Hall 2005; Rajnicek et al. 2006). In another study, it was found that eCBs could suppress the BDNF-dependent morphogenesis of interneurons and this suppression was abolished by Src kinase inhibition in vitro (Berghuis et al. 2005), suggesting that eCBs use TrkB receptor-dependent signaling pathways to regulate subtype selective interneuron specification. In addition, an in vivo study using GABA-CBI-KO mice found that a lack of CBI receptor-mediated endocannabinoid signals led to impaired postsynaptic target selection of cortical interneurons (Berghuis et al. 2007). Taken together, these observations demonstrate the critical role of the ECS in the specification, axon growth and morphogenesis, and synaptic integration of interneurons.

ECS alterations also contribute to other aspects of interneuron function associated with the wiring of neural network. Cannabinoid signaling has been reported to be an important determinant of dendritic dopamine D2 receptor (D2R) distribution and mitochondrial availability in PV<sup>+</sup> fast-spiking interneurons (Fitzgerald et al. 2012). In the prelimbic PFC of the CBI<sup>−/−</sup> mice, the cytoplasmic D2R was significantly decreased in medium PV-labeled dendrites and concomitantly increased in the small dendrites (Fitzgerald et al. 2012). Moreover, the number of mitochondria
in these PV-labeled dendrites was significantly lower in CB1\(^{-/-}\) mice (Fitzgerald et al. 2012). Considering the important regulatory role of D2R in neural circuits (Kenny et al. 2013; Ott and Nieder 2017; Tomasella et al. 2018), the alteration of D2R distribution due to CB1 receptor malfunction might have potential pathological implications.

Electrophysiological properties, as the fundamental functional elements of neural circuits, have also been shown to be influenced by ECS-mediated interneuron development. In mice devoid of CB1 receptors in GABAergic neurons (CB1\(^{+/+}\)Dlx5/6-Cre, also referred to as GABA-CB1-KO), the frequency of spontaneous IPSCs in CA1 pyramidal neurons was enhanced while tonic inhibition, paired-pulse facilitation and long-term potentiation in the hippocampus were not affected (Albayram et al. 2016). This study provided direct electrophysiology evidence of the role of the ECS in neural circuit development. In addition, by using the same strain of mice, it was found that the retrograde CB1-dependent short-term control of GABAergic transmission in the hippocampus entirely relies on the expression of CB1 on GABAergic interneurons, as was evidenced by DSI being abolished in GABA-CB1-KO mice (Monory et al. 2006). A recent study found that the cell-autonomous slow self-inhibition in regular spiking non-pyramidal cells (SHT3aR interneurons, preferentially synapse onto other interneurons) (Tremblay et al. 2016) of layer 2/3 in the somatosensory cortex was abolished in CB2 receptor knockout mice, rather than CB1 receptor knockout mice (Stumpf et al. 2018). This study showed that the alteration in CB2 receptor also has implications in interneuron electrophysiological properties. However, we should especially notice that the phenotypic alterations of CB1 or CB2 knockout mice could be the composite results of three aspects, (1) lacking the CB1 or CB2 functions exclusively manifested in the adulthood, (2) developmental alterations due to CB1 or CB2 deficiency, (3) compensatory mechanisms responding to the CB1 or CB2 knockout during development (Bello et al. 2017). Thus the interpretation of these results should be extremely cautious, i.e. the alterations exhibited in the adulthood are only partially attributed to ECS-mediated interneuron development.

**External cannabinoids exposure and interneuron development**

Above we discussed the role of the ECS in interneuron development by elucidating the functions of endogenous cannabinoid components. To better understand the role of the ECS in interneuron development, in the following section, we would like to further discuss the effects of external cannabinoid exposure on interneuron development and functional alterations. Marijuana use is becoming increasingly prevalent among adults living in the United States of America (USA). The prevalence of past-month marijuana use was reported to be 3.85% in pregnant women in the USA (Brown et al. 2017). Study in rats showed that external cannabinoids can be transferred from mother to offspring through the blood-placental barrier (Hutchings et al. 1989). Prenatal exposure to these chemical compounds always cause adverse effects on fetal neurodevelopment both in animal models and human (Calvignon et al. 2014), such as subtle but persistent changes in higher-level cognition and psychological well-being (Grant et al. 2018). Specifically, in utero exposure to exogenous cannabinoids was reported to affect interneuron development and the function of inhibitory circuits.

As previously described, prenatal exposure to WIN55-212,2 significantly increased the number of migrating GABAergic interneurons through the cortical MZ (Saëz et al. 2014). Another report showed that prenatal exposure to THC could significantly reduce the number of CCK+ interneurons in the CA1 region of the hippocampus (de Salas-Quiroga et al. 2020). In the remaining CCK+ interneurons, dendritic complexity and the overall dendritic length were also significantly decreased (Vargish et al. 2017). Moreover, THC exposure during embryonic development reduced the power of CA1 region \( \theta \) (4–12 Hz) and \( \gamma \) (30–60 Hz) oscillations (typical of exploratory behavior) and significantly alter the modes of sharp-wave ripple (a major hippocampal high-frequency oscillation recorded during immobility and sleep that is crucial for memory and learning). This could result in spatial learning deficits due to aberrant circuits caused by persistent reduction of CCK+ basket cells (de Salas-Quiroga et al. 2020). Notably, it has also been verified by recent work that CCK deficiency could diminish the power of theta oscillations during exploratory behavior and alterations in spatial learning and memory in adult mice (Del Pino et al. 2017). The consistency between the two above mentioned observations emphasized the importance of ECS-mediated CCK+ interneuron development to functional outcomes in adulthood.

In addition to prenatal exposure, cannabis exposure during adolescence also has implications for the development of interneurons. The human brain remains in a state of active and experience-guided development through childhood and adolescence until approximately 21 years of age (Gogtay et al. 2004). An animal study conducted in rats found that adolescent exposure to THC from postnatal day (PND) 35 to 45 could result in reduced basal GABA levels within the adult PFC and decreased expression of GAD67 in PV and CCK expressing interneurons in the PFC. These alterations may be related to long-term behavioral deficits.
characterized by recognition memory deficits, social withdrawal and altered emotional reactivity (Zamberletti et al. 2014). Cannabis seems to particularly exert its effects in a specific time window during adolescence. In rats, a history of WIN55,212-2 exposure during early (PND 35–40) or mid (PND 40–45) adolescence, but not in late adolescence (PND 50–55) or adulthood (PND 75–80) resulted in reduced prefrontal GABAergic transmission onto layer V pyramidal neurons and a state of frequency-dependent prefrontal disinhibition (Cass et al. 2014). This result restates the important role of the cannabinoid system in interneuron neurodevelopment during adolescence. Together with the prenatal exposure observations, these results suggest the fundamental influence of the cannabinoid system on the appropriate shaping and function of the interneuron circuit.

Endocannabinoid, interneuron development and pathophysiological implications

The important role of the ECS in the development of interneuron and neural circuits indicates that alterations in cannabinoid signaling, either hyper- or hypofunction, may contribute to the alterations in the neural network and consequently exert long-lasting functional anomalies. For example, deficiency of mice hippocampal CB1 receptors, especially in dopamine D1 receptor positive interneurons, has been suggested to disturb the consolidation of recognition memory (Busquets-Garcia et al. 2018; Oliveira da Cruz et al. 2020). Genetic polymorphisms of cannabinoid receptors can cause subtle changes during neurodevelopment by influencing signaling duration or strength and later influence synaptic transmission and excitatory/inhibitory balance. In human patients, polymorphisms of CNR1, which encodes CB1 receptors, were reported to be associated with schizophrenia (Martínez-Gras et al. 2006), social reward response and autism (Chakrabarti et al. 2006), depression (Kong et al. 2019), and anxiety (Peiro et al. 2020). Genetic variation of CNR2, the gene encoding CB2 receptor, is also believed to be associated with bipolar disorder (Minocci et al. 2011) and depressive syndrome (Onaivi et al. 2008). The polymorphisms in the DAGLα have also been reported to be associated with FCD in human patients (García-Rincón et al. 2018).

In the following part of the review, we will discuss the role of interneurons and the ECS in the pathogenesis of neurodevelopmental disorders, including epilepsy, schizophrenia, major depressive disorders and autism spectrum disorder. Although currently there is no direct evidence that explains the relationship between ECS-mediated interneuron development and the above disorders, a number of studies have focused on the ECS or interneuron development respectively. Thus, considering the crucial role of the ECS in the development of interneurons, it is reasonable to speculate that the ECS-mediated development of interneurons is key for maintaining the proper function of the nervous system. Dysregulation of interneuron ECS during development might result in neurodevelopmental disorders and further research will shed new light on this field.

Epilepsy and seizure

Epilepsy is one of the most common neurological disorders, affecting more than 70 million people worldwide (Thijs et al. 2019). Epilepsy is characterized by a predisposition to generate spontaneous epileptic seizures and is complicated with numerous cognitive and psychosocial consequences (Fisher et al. 2014). Epilepsy is believed to result from an imbalance between the excitatory and inhibitory activity of neuronal circuits which subsequently become likely to exert hypersynchronous and excessive oscillation and abnormal neural processing (Fisher et al. 2005).

The ECS plays a critical role in epilepsy. While CB1 knockout mice were reported to have a reduced seizure threshold in the kainic acid (KA) model of temporal lobe epilepsy (Marsicano et al. 2003), heterozygous and homozygous CB2 receptor knockout mice also exhibited increased susceptibility to pentylenetetrazole (PTZ)-induced seizures (Shapiro et al. 2019). Moreover, CB1 and CB2 double knockout mice spontaneously develop seizure-like behavior (Rowley et al. 2017). In the diversity of rat epilepsy models, agonists of CB1 and CB2 receptors have been reported to exert anti-epileptic effects (Di Maio et al. 2015; Vinogradova and van Rijn 2015), and antagonists against CB1 and CB2 receptors could promote epilepsy development (Vinogradova et al. 2011; Wallace et al. 2003). The primary components of the ECS, the eCBs and cannabinoid receptors, are altered during epilepsy. In rat model of pilocarpine-induced seizures, the levels of 2-AG were reported to increase significantly within the hippocampus (Wallace et al. 2003). In mice model of KA-induced seizure, KA administration rapidly increased the hippocampal levels of AEA (Marsicano et al. 2003). In addition to eCBs, CB1 receptors are also regulated after seizure attack. In the rat model of pilocarpine-induced temporal lobe epilepsy, one week after the initial seizure, there was a pronounced loss in CB1 receptor expression.
throughout the hippocampus (Falenski et al. 2009). These observations suggest the critical role of the ECS in the pathophysiology of epilepsy, and its antiepileptic therapeutic potential.

GABAergic interneuron development deficits have been widely reported to be associated with epileptogenesis. In mice, genetic reduction of cortical PV+ GABAergic interneurons at embryonic and perinatal stages resulted in increased anxiety levels, spontaneous seizure activity and higher susceptibility to pharmacologically induced convulsions in adulthood (Powell et al. 2003). In Dlx1-deficient mice, the adult brain demonstrates a reduction in the number of calretinin+ and SST+ interneurons, which was associated with a reduction of GABA-mediated IPSC in the neocortex and hippocampus, cortical dysrhythmia, generalized electrographic seizures and histological evidence of seizure-induced network reorganization (Cobos et al. 2005). Conditional ablation of COUP–TFI, a neurogenic transcription factor, leads to a decrease in late-born, CGE-derived, VIP+ and calretinin+ bipolar cortical interneurons in mice and a concurrent compensatory increase of the early-born MGE-derived, PV+ interneurons. This results in elevated resistance to pharmacologically induced seizures (Lodato et al. 2011b).

Focal cortical dysplasia (FCD) is characterized by developmental malformations of the cerebral cortex and is the most common brain structure lesion in children with drug-resistant focal epilepsies undergoing surgical therapy (Guerrini et al. 2015; Marsan and Baulac 2018). Heterogeneous disorganizations of the cerebral cortex structure have been identified in FCD, ranging from the disorganization or complete loss of the hexalaminar structure of the cortex, to the presence of novel and odd cell types in the cortex as well as heterotopic neurons observed in the subcortical white matter (Iliffland and Crino 2017). ECS has been reported to play a role in the pathogenesis of FCD. In human samples of cortical development malformation, prominent CB1 expression was detected in dysplastic neurons while reactive astrocytes were mainly stained with CB1 and microglia/macrophage were stained with CB2, suggesting the role of the ECS in the pathogenesis of FCD (Zurolo et al. 2010). Garcia-Rincon et al. (2018) found that in organotypic cultures from resection tissues of refractory epilepsy patients with FCD, CB1 receptors regulated the activity of PI3K/Akt/mTORC1 pathway, which contributed to FCD pathological features. In addition, Díaz-Alonso et al. (2017) found that in mice, CB1 receptors are required for the appropriate neurodevelopment of cortical pyramidal neurons, as is evidenced by the fact that CB1 acute silencing altered the morphology and radial migration of pyramidal neurons, leading to cortical malformations and increased seizure susceptibility in adulthood. These current studies suggest that the ECS contributes to cortex development and thus the pathogenesis of refractory epilepsy.

As is discussed above, the role of interneuron development in epilepsy as well as the role of ECS in epilepsy has been well established. However, we should notice that the phenotypes manifested in knockout mice are supposed to be the composite consequences of ECS-mediated interneuron development anomalies, or ECS malfunctions during adulthood, or both, which cannot be effectively distinguished in these studies. To address this dilemma, lineage-specific CB1 receptor expression-rescue strategy helps to distinguish these factors (de Salas-Quiroga et al. 2015; Ruehle et al. 2013). THC exposure during E12.5 to E16.5 could induce motor function deficits in the adulthood, as well as a transient decrease in the expression of CB1 receptors during embryonic stage which restored to normal at P2.5 (de Salas-Quiroga et al. 2015). Notably, by using lineage-specific restoration of CB1 receptors in a CB1-null background, the authors found that compared with the increased susceptibility in CB1-null mice, the seizure susceptibility induced by PTZ was partially restored in CB1-null mice, where CB1 receptors were specifically restored in forebrain GABAergic interneurons (de Salas-Quiroga et al. 2015). This study provides evidence that CB1 receptor-mediated interneuron development anomalies have a direct role in the pathogenesis of epilepsy. In addition, THC exposure during E10.5–E17.5 significantly downregulated the number of CA1 CCK+ interneurons and decreased the seizure latency in adult male mice rather than female ones. While in GABA-CB1-KO mice, these alterations were not observed, confirming CB1 receptors in the forebrain GABAergic interneurons mediated these dimorphic effects (de Salas-Quiroga et al. 2020).

**Schizophrenia**

Schizophrenia is a severe psychiatric disorder that affects approximately 1% of the world’s population, and is among the top 10 causes of long-term disability worldwide (Marder and Cannon 2019). Schizophrenia has a pronounced impact on both an individual’s health and wider society. The unemployment rate of patients with schizophrenia has been reported to be between 80 and 90% (Marwaha and Johnson 2004). Furthermore, the life expectancy of patients with schizophrenia has been reported as being reduced by 10–20 years (Chesney et al. 2014). Clinically, schizophrenia is characterized by (1) psychotic symptoms (positive symptoms) including delusions and hallucinations; (2) negative
symptoms such as impaired motivation, reduction in spontaneous speech and social withdrawal; and (3) impairments in cognitive functions such as working memory and cognitive control (Owen et al. 2016). One important characteristic of schizophrenia patients is that it is often first diagnosed in adolescence before the brain is completely mature (Owen et al. 2016). This suggests that neurodevelopment plays a role in the pathogenesis of schizophrenia.

Cannabis use is associated with the development of schizophrenia. In a 15-year follow-up of a cohort of 45,570 individuals, cannabis use was found to be an independent risk factor for developing schizophrenia (Andreason et al. 1987). The administration of Δ⁹-THC could exert both positive and negative symptoms of schizophrenia and alter perception ability in healthy human subjects (D’Souza et al. 2004). In mice, intraperitoneal administration of Δ⁹-THC induced acute psychotic-like states such as cognitive impairment, decreased prepulse inhibition, and reduced social interaction (Busquets-Garcia et al. 2017). In patients with schizophrenia, administration of Δ⁹-THC could exacerbate core psychotic and cognitive symptoms (D’Souza et al. 2005). All these results indicate a close relationship between cannabinoids and schizophrenia. Notably, schizophrenia-related symptoms appeared only when exposure occurred during adolescence but not during adulthood. For example, the decline of cognitive function exclusively occurred in adolescent-onset cannabis users and could not be fully restored after cessation of cannabis (Meier et al. 2012). Animal studies also confirmed that the cognitive impairment caused by cannabis could occur only when the administration happened in adolescence but not in adulthood (O’Shea et al. 2004; Schneider and Koch 2003). These findings suggest that ECS-mediated neurodevelopment may play a role in the pathogenesis of schizophrenia.

The PFC was reported to be one of the brain regions involved in schizophrenia where CB1 receptors demonstrate high density, as is suggested by autoradiography study in human (Glass et al. 1997). Schizophrenia is associated with multiple changes in the PFC, including changes of the ECS and interneuron circuits. First, the ECS in the PFC was altered in subjects with schizophrenia. It has been reported that in the dorsolateral PFC of patients with schizophrenia, the mRNA level of ABDH6 was elevated in those who were younger than 40 years old and had an illness duration shorter than 15 years (Volk et al. 2013). The expression of CB1 receptors decreased in schizophrenia patients in level of both mRNA and protein (Eggan et al. 2008). Second, interneurons in the PFC are altered in schizophrenia patients. The mRNA of PV per neuron was significantly decreased in layers III and IV of PFC area nine in patients with schizophrenia while the mRNA of calretinin was not altered (Hashimoto et al. 2003). In another study, mRNA expression of PV, CCK, SST, neuropeptide Y, and calretinin all demonstrated a reduction in patients (Fung et al. 2010). The discrepancy regarding calretinin might be attributed to the selection of different subjects and the sensitivity of different detection techniques. In addition, a deficit in the mRNA levels of GABA-synthesizing enzyme GAD67 was also detected in the PFC of patients with schizophrenia (Akbarian et al. 1995; Guidotti et al. 2000; Volk et al. 2000) and this decrease in GAD67 mRNA is particularly prominent in PV+ and CCK+ interneurons (Hashimoto et al. 2003). Lhx6, a gene that regulates the proliferation, migration and specification of cortical PV+ interneurons (Liodis et al. 2007), also demonstrates a significant deficit in GABA neurons in patients with schizophrenia and is accompanied by deficits in GAD67 (Volk et al. 2014, 2012). This observation indicates the important role of interneuron development in the pathophysiology of schizophrenia. It is reasonable to surmise that ECS-mediated interneuron development deficiency is essential for schizophrenia genesis because ECS contributes substantially to interneuron development. More detailed studies are needed to provide more evidence of ECS-mediated interneuron development in the pathogenesis of schizophrenia.

Major depressive disorder

Major depressive disorder (MDD) is a common psychological illness that severely impairs psychosocial functions and diminishes quality of life. Depression has a high prevalence, with a 12-month prevalence of approximately 6% (Kessler and Bromet 2013) and lifetime risk of 15–18% (Bromet et al. 2011). Typical symptoms of depression include anhedonia (impaired ability to experience pleasure), diurnal variation (worsening of depression symptoms during certain periods of the day), feelings of worthlessness and guilt, and other symptoms such as fatigue, insomnia and suicidal intention (Malhi et al. 2014; Malhi and Mann 2018).

The expression of CB1 receptors and eCBs has been reported to undergo various changes during depression. The level of CB1 receptors is consistently upregulated in the PFC in a variety of animal models of depression, including rat model of chronic mild stress model (Bortolato et al. 2007), rat model of chronic unpredictable stress model (Hill et al. 2008) and rat depression model of bilateral olfactory bulbectomy (Rodriguez-Gaztelumendi et al. 2009).
The up-regulation of CB1 in the PFC has also been verified in depressed suicide victims (Hungund et al. 2004). In other brain regions, such as the midbrain (Bortolato et al. 2007), hippocampus (Hill et al. 2008; Reich et al. 2009), hypothalamus (Hill et al. 2008), ventral striatum (Hill et al. 2008) and amygdala (Rubino et al. 2008), as are evidenced by rat depression models, the levels of CB1 receptors are decreased. Apart from CB1 receptors, the levels of eCBs also alter in depression. Furthermore, in rat models of depression, the level of 2-AG increase in the hypothalamus, midbrain (Hill et al. 2008) and thalamus (Bortolato et al. 2007) but decreases in the hippocampus (Hill et al. 2005), whereas the content of AEA decrease throughout the brain (Hill et al. 2008).

Studies have identified altered GABAergic function in depression and its implications in the pathophysiology of depression. In patients with major depression, proton magnetic resonance spectroscopy detected a significant reduction in GABA concentrations in the occipital cortex, whereas the glutamate levels were significantly increased, demonstrating the alteration of the excitatory-inhibitory neurotransmitter ratio (Sanacora et al. 2004). Decreased levels of GABA are also consistently identified in the PFC, amygdala and anterior cingulate cortex of patients with depression, which are all involved in cognition and emotion (Guilloux et al. 2012; Price et al. 2009; Rajkowska et al. 2007). In addition to GABA, the GABA-synthesizing enzyme GAD67 is also downregulated in the dorsolateral PFC in patients with depression (Karolewicz et al. 2010). Notably, following antidepressant treatment with the selective serotonin reuptake inhibitor citalopram (Bhagwagar et al. 2004), transcranial magnetic stimulation (Dubin et al. 2016), electroconvulsive therapy (Sanacora et al. 2003) and cognitive behavioral therapy (Sanacora et al. 2006), there is an increase in GABA levels in patients with depression. These observations suggest that GABAergic inhibition may be the underlying mechanism of depression.

Several lineages of GABAergic interneurons are altered in depression. The density of calbindin+ interneurons is decreased in the PFC, occipital cortex, and orbitofrontal cortex and the size of calbindin+ interneurons is reduced in the PFC, but not in the occipital cortex in human patients (Maciag et al. 2010; Rajkowska et al. 2007). However, there is no difference in the density and size of PV+ interneurons in the dorsolateral PFC and orbitofrontal cortex between patients with depression and healthy controls (Rajkowska et al. 2007). Moreover, there is a reduction in SST in the anterior cingulate cortex across all cortical layers (Seney et al. 2015; Tripp et al. 2011) and also in the lateral, basolateral and basomedial nuclei of the amygdala in patients with MDD (Douillard-Guilloux et al. 2017). Low SST has a causal role in mood-related phenotypes of depression. In mice lacking SST, the novelty suppressed feeding test revealed significantly elevated depressed behavioral emotionality. In addition, a high basal plasma level of corticosterone and reduced gene expression of BDNF, cortistatin, and GAD 67 was found (Lin and Sibille 2015), all of which are features of human depression (Malhi and Mann 2018). The impairment of interneurons in depression could alter the integrity of internal information transferring and decrease the coding of external information. This may manifest in depression as the shifting of attention from an external to internal focus and symptoms of rumination (Fee et al. 2017).

Although currently there is no direct evidence of the causal relationship between ECS-regulated interneuron development and depression, there still exist some clues. HU210, a potent synthetic cannabinoid, could promote the proliferation of cultured embryonic hippocampal neural stem/progenitor cells and also neurogenesis in the hippocampal dentate gyrus of adult mice, which is related with antidepressant-like effects (Jiang et al. 2005). This result suggests the role of ECS-mediated neurodevelopment in the pathogenesis of depression. Several recent studies have also provided clues about the pathogenic roles of ECS-mediated neurodevelopment in depression. Results from the Maternal Health Practices and Child Development Study (MHPCD), a longitudinal study focusing in utero exposure to cannabis and its consequences, demonstrated that in utero cannabis exposure was associated with a higher rate of depression in adolescence (McLemore and Richardson 2016). In young human subjects of 18–25 years old, marijuana users were highly associated with increased depressive symptoms, decreased fun-seeking and reward response (Wright et al. 2016). A recent meta-analysis also confirmed that adolescent cannabis consumption was associated with an increased risk of developing depression and suicidal behavior later in life (Gobbi et al. 2019). In addition, prenatal co-exposure to tobacco and cannabis led to a significant lower and flatter cortisol response during kindergarten age (Eiden et al. 2020), suggesting alterations in hypothalamic–pituitary–adrenal axis that is highly involved in the pathogenesis in depression (Malhi and Mann 2018). These aforementioned studies demonstrated that interfering with ECS early in life had a long-term consequence to develop depression later in life, suggesting ECS-mediated neurodevelopment could have an important role in the pathogenesis of depression. Future
animal studies with more detailed pathological and molecular analysis would provide more information. Conditional CB1 receptor knockout mice, combined with lineage-specific rescue strategy, as is mentioned in Section 4.1 could be of great help.

**Autism spectrum disorder**

Autism spectrum disorder (ASD) is a pervasive, heterogeneous, and complex neurodevelopment disorder (Masi et al. 2017). Its presentation is fairly multifaceted with signs and symptoms quite different from one another, but it is also characterized by two core features, social communication deficits, and restricted, repetitive motor-sensory behaviors, irrespective of culture, race, ethnicity or socioeconomic status (Lord et al. 2018). The prevalence of ASD has been increasing. In Asia, the average prevalence before 1980 was 1.9 per 10,000, while between 1980 and 2010, the prevalence rose to 14.8 per 10,000 (Xiang and Carrie 2010). Another meta-analysis which included studies from 2008 to 2018 found that the ASD prevalence in Asia was as high as 36 per 10,000 (Qiu et al. 2020). While in the USA, between 2014 and 2016, the prevalence of ASD was as high as 247 per 10,000 in children and adolescents aged 3–17 years old (Xu et al. 2018). Such a high prevalence, combined with social communication deficits in ASD patients, causes high economic and social burden on both the family and the society.

The etiology of ASD is commonly believed to be a genetic predisposition combined with environmental influences (Newshaifer et al. 2007). The role of the ECS in the pathogenesis of ASD has been widely investigated in both animal and human studies. In rats, perinatal exposure to THC from gestational day 15 to postnatal day 9 inhibited social interaction and play behavior in the adolescent offspring and induced anxiogenic-like profile in the adult offspring (Trezza et al. 2008). Notably, a recent study demonstrated that the social interaction impairment caused by prenatal exposure to WIN55,212-2 is sex dependent with male rats affected and female rats spared (Bara et al. 2018). However, another study where cannabinoid receptor agonist CP 55940 was administered at age 4 days (perinatal), 30 days (adolescent) and 56 days (young adult) for 21 consecutive days to rats showed that treatment at all three ages could impair social interaction 1 month after the treatment (O’Shea et al. 2006). This result might suggest that ECS-mediated neurodevelopment, as is evidenced by treatment in perinatal and adolescent stage, as well as the adult functions of the ECS, as is evidenced by treatment in young adult, together contribute to the pathogenesis of ASD.

Human studies also provide consistent results. A recent retrospective study found that maternal cannabis exposure significantly increased the risk of ASD in the offspring, with the incidence of ASD diagnosis being 4.00 per 1000 person-years among children with exposure compared to 2.42 among unexposed children (Corsi et al. 2020). Another study found that young adolescent cannabis users had greater reactivity in the bilateral amygdala to angry faces than neutral faces, an effect that was not observed in their abstinent peers, and activity levels in the right temporoparietal junction and bilateral dorsolateral prefrontal cortex did not discriminate between the two face conditions in cannabis users, but did differ in controls (Spechler et al. 2015). Since amygdala is highly involved in emotion control (Gallagher and Chiba 1996), hypersensitivity of amygdala to signals of threat may cause further social interaction problems. Together, these human researches highly indicate the role of ECS-mediated neurodevelopment in the etiology of ASD, and at the same time, suggest that cannabis use during pregnancy should be avoided to lower the risk of ASD of the children.

Interneuron functional and developmental aberrancies have been detected in ASD (Di et al. 2020; Lunden et al. 2019). In the postmortem brain of autistic subjects, GAD65 and GAD67, the enzymes responsible for GABA synthesis, are downregulated (Collins et al. 2006; Yip et al. 2007). In a recent postmortem study, it was found that in patients with ASD, the density of calretinin+ interneurons in the caudate was lower (35%) than controls with unaltered size of the caudate (Adorjan et al. 2017). In a Shank3B−/− mouse model of ASD, in vivo population calcium imaging in vibrissa primary somatosensory cortex (vS1) revealed increased spontaneous and stimulus-evoked firing in pyramidal neurons but reduced activity in interneurons. In addition, selective deletion of Shank3 in vS1 interneurons led to pyramidal neuron hyperactivity and increased stimulus sensitivity in the vibrissa motion detection task (Chen et al. 2020). This study provided evidence that cortical GABAergic interneuron dysfunction plays a crucial role in the sensory hyperreactivity in a Shank3 mouse model of ASD and further supported the important role of GABAergic interneuron in the etiology of ASD. More recently, parvalbumin hypothesis was raised, which posited that decreased PV level is causally related to the etiology of ASD (Filice et al. 2020). Since the ECS plays important roles in the development of...
interneurons, ECS-mediated interneuron development aberrancy could be a potential etiology of ASD. More experiment studies are urgently needed to verify this hypothesis.

**Conclusions**

Neurodevelopmental biology research has provided us with much information about the development of interneurons and the relative role of the ECS. During the early stage of neurodevelopment, ECS modulates the migration, morphogenesis of interneuron progenitors and the formation of appropriate connectivity in local circuits. Prenatal and adolescent exposure to cannabinoids has long-term effects on both the structure and function of neural circuits. We here summarize and emphasize the potential influence of the disturbed ECS on interneuron development in neurological and psychiatric disorders. However, there still remains knowledge gap regarding how the ECS regulates the neurodevelopment of interneurons, such as whether the ECS can crosstalk with Dlx signals, the critical transcription factors in modulation of interneuron development. Further studies on these areas will definitely help improve our understanding of neurodevelopment, promote reasonable medical use of ECS-related drugs and further inspire novel drugs or treatment for relevant neurological and psychiatric disorders in the long run.

**Author contributions:** All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

**Research funding:** Our work was supported by the National Natural Science Foundation (Grant No. 82071529).

**Conflict of interest statement:** None declared.

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