State of the art

Neurobiology of cannabinoid receptor signaling

Beat Lutz, PhD

The endocannabinoid system (ECS) is a highly versatile signaling system within the nervous system. Despite its widespread localization, its functions within the context of distinct neural processes are very well discernable and specific. This is remarkable, and the question remains as to how such specificity is achieved. One key player in the ECS is the cannabinoid type 1 receptor (CB_1), a G protein–coupled receptor characterized by the complexity of its cell-specific expression, cellular and subcellular localization, and its adaptable regulation of intracellular signaling cascades. CB_1 receptors are involved in different synaptic and cellular plasticity processes and in the brain's bioenergetics in a context-specific manner. CB_2 receptors are also important in several processes in neurons, glial cells, and immune cells of the brain. As polymorphisms in ECS components, as well as external impacts such as stress and metabolic challenges, can both lead to dysregulated ECS activity and subsequently to possible neuropsychiatric disorders, pharmacological intervention targeting the ECS is a promising therapeutic approach. Understanding the neurobiology of cannabinoid receptor signaling in depth will aid optimal design of therapeutic interventions, minimizing unwanted side effects.

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Introduction

The endocannabinoid system (ECS) comprises two cannabinoid receptors—CB_1 and CB_2 receptors—that belong to the family of seven transmembrane G protein-coupled receptors (GPCRs); the ligands for the cannabinoid receptors—the two major endocannabinoids (eCBs) anandamide (arachidonoylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG), both being derivatives of the fatty acid arachidonic acid; and the machinery for the synthesis and degradation of eCBs.[1,2] The ECS is evolutionarily well conserved in vertebrates,[3] is widely distributed in the body, and takes a central position in the regulation of a myriad of biological processes, both in neural and non-neural tissues.[4,5] It is intertwined with many neurotransmitter and lipid signaling systems, thereby integrated into broad functional networks.[6]

It appears that the ECS plays roles in the fine-tuning of physiological processes that keep the body in homeostatic set-points.[7-10] In humans, several polymorphisms in ECS components with associated neuropathophysiological processes have been described, suggesting they promote susceptibility toward development of neuropsychiatric disorders.[11] ECS dysregulation can also be induced by particular life factors, such as living under chronic stress,[12] or by metabolic factors, such as with obesity.[8] Pharmacological interventions targeting ECS activity aim to normalize such pathophysiological processes,[13-15] thereby rescuing the subject from unfavorable allostatic set-points.

Since the discovery of the ECS in the 1990s, this signaling system has attracted intense attention, especially as it aided understanding of the effects of phytocannabinoids.

Author affiliations: Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University, Mainz, Germany. **Address for correspondence:** Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University, Duesbergweg 6, 55128 Mainz, Germany (email: beat.lutz@uni-mainz.de)

Furthermore, detailing the various components of the ECS uncovered the fascinating complexity of how this signaling system acts in the functional network of the entire organism, in particular in the brain. This article presents a general overview of the neurobiology of the ECS. However, the vast literature on this subject makes it nearly impossible to touch on all aspects, and gaps are inevitable. Furthermore, due to space constraints, the discussion here focuses on the ECS of the brain in rodents and human, in particular the CB_1 receptor. However, the ECS is also widely involved in the regulation of peripheral immune, cardiovascular, metabolic, gastrointestinal, muscular, and peripheral nervous system processes,[16-19] which in turn can influence central nervous system (CNS) functions.[17,20]

Endocannabinoid mechanisms

The peculiarities of the cannabinoid receptors

CB_1 and CB_2 receptors[21] feature the many typical characteristics of GPCRs, making these receptors highly versatile and adaptable, for example, regarding ligand binding, intracellular signaling coupling, homo- and heterodimerization, and subcellular localization.[22] Here, the focus will be on the CB_1 receptor, the major cannabinoid receptor in the nervous system, but several aspects of CB_2 receptors will also be addressed. It is interesting to note that despite the ubiquitous occurrence of the CB_1 receptor in the nervous system, this signaling system appears to act in a highly specific manner in a given context. Several key features come into play and will be discussed below.

Receptor expression at the cellular level

Detailed expression analyses at the regional and cellular levels in the CNS revealed that the CB_1 receptor is present in virtually all brain regions and in all major cell types (*Figure 1*), ie, in neurons,[2] glial cells (astrocytes, oligodendrocytes),[23,24] and brain-resident immune cells (microglia).[25] The CB_1 receptor is also present in the neurons of the major neurotransmitter systems (glutamate, γ-aminobutyric acid [GABA], serotonin, noradrenalin, acetylcholine),[7] and possibly also in dopaminergic neurons. CB_1 receptor–expressing cells can also be classified according to the presence of peptidergic transmitters, such as corticotropin-releasing hormone,[26] cholecystokinin,[27,28] and somatostatin.[29] The expression levels can vary greatly, depending on the brain region and cell type. For example, the central amygdala contains very low levels of CB_1 receptor in only a few of the presumably GABAergic neurons.[30,31] On the other hand, very high levels of CB_1 receptor messenger RNA (mRNA) are detected in hippocampal and neocortical GABAergic interneurons.[27,28] Glutamatergic neurons generally contain rather low levels of CB_1 receptor.[32,33]

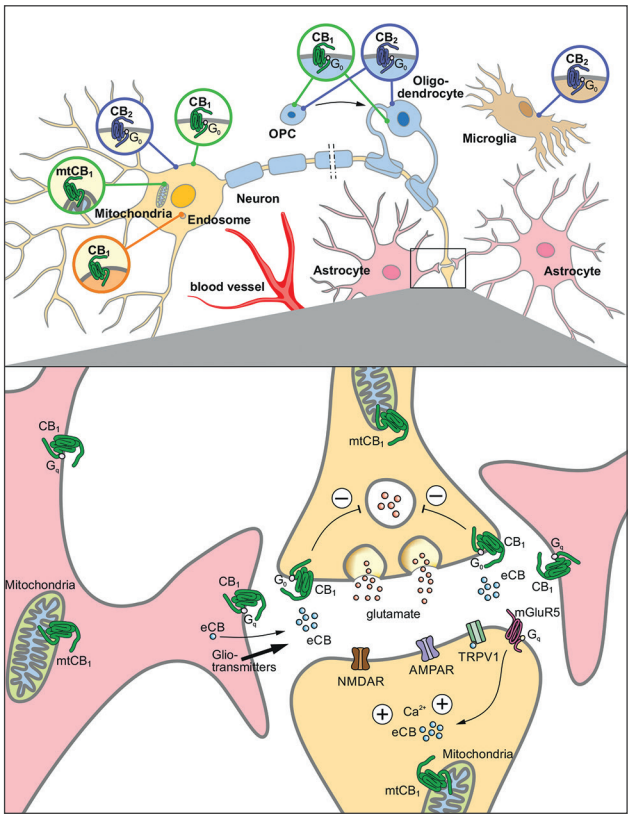

Figure 1. Expression of cannabinoid type 1 and type 2 (CB_1 and CB_2) receptors in neural tissue. The endocannabinoid system is present in neurons, astrocytes, oligodendrocytes, oligodendrocyte precursor cells (OPCs), and microglia. Functional CB_1 receptors are located on the plasma membrane, but also in mitochondria ($mtCB_1$) of neurons and astrocytes. Presynaptic CB_1 receptor suppresses neurotransmitter release, as shown here, at a glutamatergic synapse. For this process, postsynaptic increase of Ca^{2+} triggers the synthesis of endocannabinoids, which travel to the presynapse to activate CB_1 receptor. Astrocytic CB_1 receptor can regulate gliotransmitter release. AMPAR, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) type glutamate receptor; CB_1/CB_2, cannabinoid type 1/type 2 receptor; eCB, endocannabinoid; mGluR5, metabotropic glutamate receptor 5; $mtCB_1$, mitochondrial CB_1 receptor; NMDAR, NMDA (*N*-methyl-D-aspartate receptor) type glutamate receptor; OPC, oligodendrocyte precursor cell; TRPV1, transient receptor potential cation channel subfamily V member 1

Furthermore, the CB_1 receptor is barely detectable in astrocytes,[23,34] oligodendrocytes,[24,35,36] oligodendrocyte precursor cells (OPCs),[36,37] and adult neural stem cells (NSCs).[38,39] Specific functions of CB_1 receptor in these different populations and in many brain areas have been described in mouse by using conditional gene inactivation of CB_1 receptor in the respective cell types and/or brain regions, together with local pharmacological interventions.[7,17,40-42] Importantly, the relative abundance of CB_1 receptor does not indicate the importance of the receptor in a particular physiological process.[23,32]

CB_2 receptor expression has been predominantly described in peripheral immune cells[16] and brain-resident immune cells, the macrophages,[43] but was eventually also detected in neurons, a circumstance that has fueled many investigations on CB_2 receptor in neural functions.[44] In the CNS, CB_2 receptor expression was reported in cells such as activated microglia,[43] brain stem neurons,[45] hippocampal glutamatergic neurons,[46] and dopaminergic neurons of the ventral tegmental area.[47,48] CB_2 receptor transcripts are reported to be 100 to 200 times less abundant than CB_1 receptor mRNA, but are strongly upregulated in response to various insults, such as chronic pain, neuroinflammation, and stroke.[44,49] Genetic approaches, together with pharmacology and very specific and sensitive cellular detection methods of mRNA, paved the way for the recognition of CB_2 receptor in many neural functions.[44]

In summary, the mRNA encoding the two major receptors for eCBs is very widely expressed in the brain in many cell types, allowing the involvement in numerous physiological and pathophysiological processes. In the next paragraph, the subcellular location of the proteins will be discussed, preparing the ground for detailing the involvement of the receptors in particular cell-signaling processes.

Receptor expression at the subcellular level

Presynaptic localization: The dominant site of CB_1 receptor protein location is the presynapse, where the activation of CB_1 receptor can suppress presynaptic neurotransmitter release. This mechanism is very well detailed for glutamatergic and GABAergic synapses, whereby the eCB 2-AG is generated in the postsynaptic site and travels retrogradely to the presynaptic site to stimulate the CB_1 receptor. This can result in a short-term decrease in Ca^{2+} influx at the presynaptic terminal.[1,2,7,50] This signaling can typically lead to processes called depolarization-induced suppression of excitation (DSE) at the glutamatergic synapse, and depolarization-induced suppression of inhibition (DSI) at the GABAergic synapse. Most of the investigations have substantiated 2-AG as the retrograde eCB, consistent with the neuroanatomical configuration with postsynaptic synthesizing and presynaptic degrading enzymes.[50] For AEA, the situation is far from being understood. First, AEA synthesis machinery seems to be mainly located at the presynaptic site,[51] whereas the degrading machinery is at the postsynaptic site.[52] Secondly, besides the cannabinoid receptors, the postsynaptically acting transient receptor potential cation channel subfamily V member 1 (TRPV1) has to be considered as an AEA receptor as well.[53] Altogether, DSE and DSI appear to be mediated by 2-AG but not by AEA, as evidenced via studies that used a genetically induced decrease in 2-AG and AEA signaling in hippocampal glutamatergic neurons by overexpression of 2-AG–degrading enzyme monoacylglycerol lipase (MAGL)[54] and the AEA-degrading enzyme fatty acid amide hydrolase (FAAH),[55] respectively.

Postsynaptic localization: Postsynaptic CB_1 receptor has been reported in electrophysiological experiments in neocortical GABAergic[56] and glutamatergic neurons,[57] and in hippocampal glutamatergic neurons involving potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 1 (HCN1), possibly via a somatodendritic mechanism.[58] Based on immunohistological analysis, CB_1 receptor has been described to be intracellular, within somatodendritic endosomes, and until now not yet on the cell membrane.[59]

In summary, the neuronal CB_1 receptor is dominantly expressed at the presynapse; however, functional postsynaptic CB_1 receptor has also been reported. CB_2 receptor has been reported to be localized in the soma,[48] but to date, no immunostaining has revealed a presynaptic or dendritic localization.

Intracellular localization: Receptor trafficking is central to activity regulation and function of GPCRs. Receptors are synthesized in the cell soma and transported to the destination—for CB_1 receptors, this is mainly the axonal terminal—and finally integrated into the cellular membrane. Distinct sequences of the CB_1 receptor are involved in axonal trafficking,[60,61] whereby alterations in such sequences can lead to eCB-mediated electrophysiological and behavioral alterations.[62] Distribution of CB_1 receptor in the different

neuronal compartments can be influenced by the activation state of the receptor. For example, pharmacological activation of CB_1 receptor increases its presence in the somatodendritic endosomes and decreases the receptor's presence at the presynapse. This is explained by high internalization and retrograde transport of the CB_1 receptor. This process is very pronounced in hippocampus and neocortex, but absent in basal ganglia. On the other hand, treatment with CB_1 receptor antagonist leads to a decreased number of cell bodies with CB_1 receptor–containing endosomes. This observation suggests that under steady-state conditions, the CB_1 receptor is steadily activated and internalized into the soma.[59] Using high-resolution microscopy, long-term treatment with the psychoactive phytocannabinoid Δ^9-tetrahydrocannabinol (THC) was shown to lead to a decrease in CB_1 receptors located in the presynaptic membrane of hippocampal GABAergic interneurons. This internalization persists for many days after termination of THC treatment.[63] Along these lines, genetic deletion of the 2-AG–degrading enzyme MAGL also leads to β-arrestin–mediated internalization and desensitization of the CB_1 receptor,[64] an effect that is not observed in FAAH-deficient mice containing increased AEA levels.[65]

Apart from intracellular localization in the endosomal compartment in the context of receptor trafficking, CB_1 receptor was also detected at mitochondrial membranes of neurons and named $mtCB_1$ receptor.[66] Here it mediates the reduction in mitochondrial oxygen consumption upon stimulation by exogenous cannabinoids and eCBs, in the end decreasing the production of adenosine triphosphate (ATP). Subsequently, cannabinoids were shown to regulate complex I of the respiratory chain by modulation of mitochondrial protein kinase A (PKA) activity and downstream phosphorylation events.[41] Thus, the ECS directly regulates mitochondrial energy production via $mtCB_1$ receptor. Moreover, $mtCB_1$ receptor was shown in the same study to mediate the memory-impairing effects of cannabinoids. Recently, activation of astrocytic $mtCB_1$ was shown to inhibit glucose metabolism and lactate production, altering neuronal functions and behavioral responses in a social-interaction test.[67]

Despite the very widespread presence of the CB_1 receptor in the brain, its functions are amazingly specific

In summary, considering that eCBs are synthesized locally and that these lipid-signaling molecules diffuse and occupy a restricted three-dimensional space, generating a microdomain of eCB signaling, the precise localization of the cannabinoid receptors determines the downstream signaling. Research in recent years has established that functional CB_1 receptor is present both in the plasma membrane as well as in intracellular compartments, particularly in the outer mitochondrial membrane. Duration and intensity of eCB signaling is determined by the dynamics of eCB synthesis and degradation. Furthermore, it is thought that eCB signaling contains both tonic (constitutive) and phasic (short-term, "on-demand") components.[2,68] Thus, important hallmarks of eCB signaling are its temporal and spatial restrictions, which of course is also a common feature of "classical" neurotransmitter receptor systems. Next, the diversity of the intracellular signal transduction upon cannabinoid receptor activation will be addressed.

Intracellular signaling: Among the GPCRs, the CB_1 receptor appears to be most highly expressed in the brain, with protein levels in the same range as for the major components of the excitatory and inhibitory neurotransmitter systems, ie, for *N*-methyl-D-aspartate (NMDA) and $GABA_A$ receptors, respectively.[21] Intrinsically, GPCRs contain a rich repertoire for regulation of cellular processes upon ligand binding.[22,69,70] Many aspects of CB_1 receptor signaling have been reported[71,72]; however, we are far from understanding how CB_1 receptor signaling gains specificity depending on cellular context.

Dimerization: GPCRs contain the capacity to form homo- and heterodimers, ie, a particular GPCR interacts with the same type of receptor to form a homodimer, or a particular GPCR interacts with another type of receptor to form a heterodimer. Such dimerization processes are involved in signal integration upon receptor activation.[73] Moreover, dimerization processes are also implicated in the emergence of mental disorders, and this might also be the case for cannabinoid receptors.[74] Homodimers were reported for CB_1 receptors[75] and heterodimers reported for CB_1 receptor with several other GPCRs, including CB_2 receptor,[76,77] dopamine

D_2 receptor,[78,79] and serotonin 5-HT_{2A} receptor.[80] Heterodimerization was reported to lead to alterations in signaling. For example, CB_1 receptor/dopamine D_2–receptor heterodimers in cultured striatal neurons can change the intracellular signaling upon CB_1 receptor stimulation.[78] Along the same line, a recent investigation showed the heterodimerization of CB_1 receptor with the adenosine A_{2A} receptor in striatum, whereby the costimulation of both receptors reduces intracellular signaling.[81] CB_1/CB_2 receptor heterodimers have also been reported to enhance ligand binding of the phytocannabinoid cannabigerol.[82] Furthermore, heterodimerization was shown between cannabinoid receptor with non-GPCR. For example, heterodimerization between CB_2 receptor and the tyrosine kinase receptor HER2 is involved in the antitumor action of THC, whereby THC interrupts the dimer.[83] Altogether, these features make up a powerful tool for cell-type–specific fine-tuning of intracellular signaling. Obviously, the two dimerizing receptors must be in tight proximity within the same cell compartment, providing a constraint for activation of this mechanism.

G protein coupling: CB_1 receptor is typically coupled to $G_{i/o}$ proteins, leading to a decreased production of cyclic adenosine monophosphate (cAMP) and an inhibition of N- and P/Q-type Ca^{2+} channels, resulting in decreased Ca^{2+} influx upon stimulation.[1,21] Under particular circumstances, G_s coupling in striatal neurons was reported, in particular in concert with dopamine D_2 receptor signaling.[84-86] G_q coupling was reported in astrocytes.[87] $G_{\alpha z}$ was found as a CB_1 receptor–interacting G protein, detected from a biochemical pull-down proteomic experiment using hippocampal synaptosomes expressing tagged CB_1 receptor.[88] Furthermore, biochemical analysis of G proteins interacting upon CB_1 receptor stimulation in neocortical extracts revealed the presence not only of $G_{i/o}$, but also of $G_{\alpha z}$, $G_{\alpha 12/13}$, and $G_{\alpha q/11}$.[89] The coupling was dependent on the CB_1 receptor agonist used. Differences in the G protein coupling has been proposed to be a possible mechanism to explain the observation that low levels of CB_1 receptor proteins in glutamatergic hippocampal neurons show higher GTPγ-binding activity than the high CB_1 receptor content in GABAergic interneurons. However, these experiments were performed with whole hippocampal extracts without subcellular fractionation and represent an average over the entire tissue.[90] Furthermore, the regulator of G protein–signaling (RGS) proteins constitute an important intracellular component in the control of GPCR signaling.[91] For example, RGS proteins have been implicated in the interaction of CB_1 receptor with dopamine D_2 receptor regarding the regulation of eCB-mediated retrograde synaptic signaling of striatal neurons.[92]

Altogether, these observations suggest that CB_1 receptor signaling can depend on the availability of the intracellular G protein pool and on the specific ligand that activates the CB_1 receptor. The former parameter is obviously also influenced by the presence of other GPCRs in the same subcellular domain, by competing for the same G protein pool and RGSs. Furthermore, as dysregulation of ECS activity has been reported for pathophysiological processes, alterations in these different constituents (G proteins, RGS, other GPCRs), can also lead to altered CB_1 receptor signaling.

Signaling via β-arrestin: Binding of CB_1 receptor to β-arrestins is important for the internalization of the receptor upon ligand stimulation,[93] but β-arrestins are also signal transducers for GPCR intracellular pathways, such as extracellular-signal–regulated kinase (ERK), and c-jun terminal kinase (JNK).[94] So-called biased β-arrestin signaling upon ligand activation of GPCRs has been recognized as therapeutically relevant also for CB_1 receptor signaling.[93,95] Deletion of the CB_1 receptor phosphorylation site that is involved in β-arrestin binding was reported to lead to resistance to cannabinoid tolerance and hypersensitivity to cannabinoids.[96] CB_1 receptor can also activate the mammalian target of rapamycin (mTOR) pathway, eg, to regulate presynaptic protein synthesis in the context of long-term synaptic plasticity.[97] On the other hand, mTOR mediates the amnesic effects of THC via the CB_1 receptor.[98]

Receptor-interacting proteins: Furthermore, the presence of CB_1 receptor–interacting proteins can also contribute to the diversity of CB_1 receptor signaling. Here, the cannabinoid receptor interacting protein 1A (CRIP1A) is reported to influence CB_1 receptor agonist-induced regulation of excitatory neurotransmission,[99] to modulate which $G_{i/o}$ subtypes interact with CB_1 receptor, and to attenuate CB_1 receptor internalization via β-arrestin.[100]

Splice variants and posttranslational modifications: Lastly, for both CB_1 and CB_2 receptors, splice variants have been reported in rodents and human.[44,101-103] The in vivo significance of these variants has not yet been clarified, although differences in mRNA expression, receptor signaling, trafficking, and glycosylation have been reported.[101,102,104,105]

Along these lines, posttranslational modifications, such as phosphorylation for the β-arrestin binding site[96] and N-linked glycosylation, also have to be considered in the regulation of receptor activity. For example, reduction in glycosylation of the CB_1 receptor reduces the cell membrane expression of the receptor, but not ligand-binding affinity.[106]

In summary, in the CNS, the ECS constitutes an important mechanism for the fine-tuned regulation of synaptic transmission in numerous projections and local networks in most, if not all, brain regions. Its proposed function as a so-called circuit breaker, due to the retrograde mechanism of the suppression of neurotransmitter release, is certainly a central aspect of the ECS function, but the ECS is acting beyond this and might also be seen as an integrator of synaptic processes, enabling encoding of information.

Cellular plasticity in the adult brain

Generation of new neurons

Besides the involvement of the ECS in synaptic plasticity, eCB-regulated plasticity is also present at the cellular level (*Figure 2*). Adult neurogenesis, ie, the generation of newly born neurons from NSCs, is an important physiological process and is implicated in neuropsychiatric disorders.[107-109] In humans and rodents, the major sites of neurogenesis are the subgranular zone (SGZ) of the dentate gyrus of the hippocampus and the subventricular zone (SVZ) in the lateral ventricle. In the adult, neurogenesis occurs continuously, but the rate of proliferation and subsequent differentiation are tightly regulated and can be influenced by different intrinsic and extrinsic factors. Proliferation is increased, eg, by enriched environment and physical activity, and decreased, eg, upon stress, during depression, and over the course of aging.[107,108] The role of the ECS in adult neurogenesis is well documented, as reviewed in detail elsewhere.[110-112] Experiments performed in mice under home-cage conditions without behavioral challenges revealed that ubiquitous gene deficiency of CB_1 receptor[38] and impaired 2-AG signaling in mice with deficiency of the 2-AG synthesizing enzyme diacylglycerol lipase-α (DAGLα)[113,114] lead to decreased NSC proliferation. On the contrary, under basal conditions, ubiquitous CB_2 receptor deficiency did not lead to impairments in NSC proliferation.[115] Reduced proliferation was also observed in rodents treated with specific CB_1- and CB_2 receptor antagonists.[116]

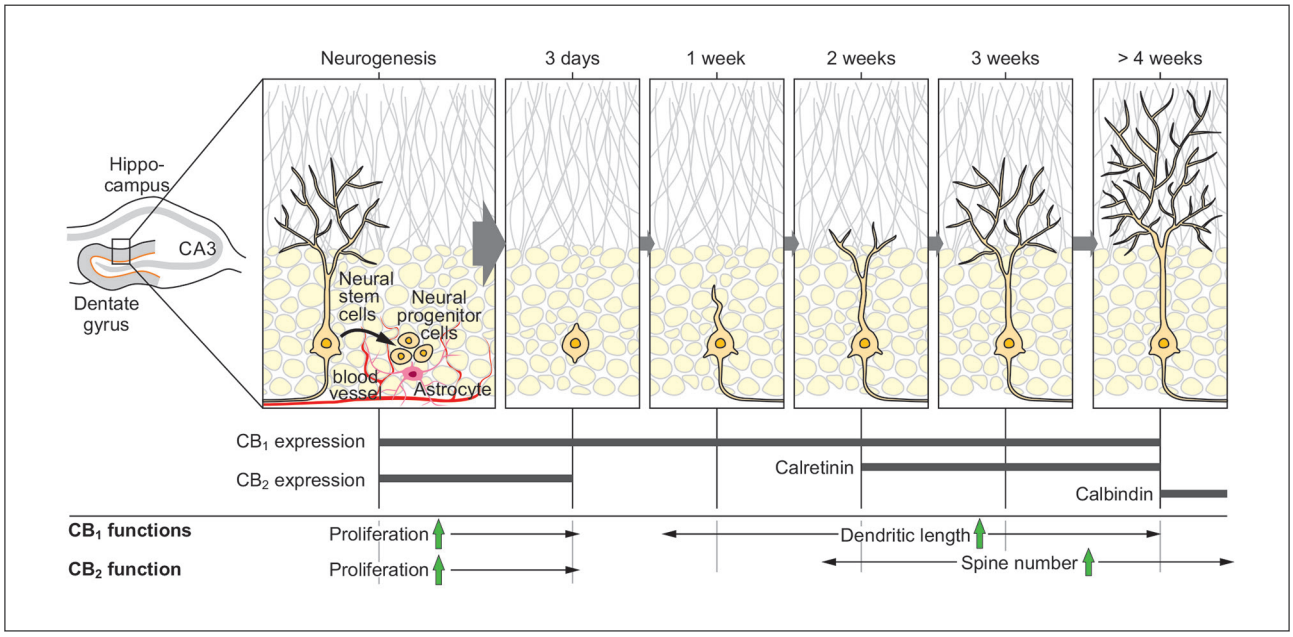

Figure 2. Neurogenesis in the subgranular zone of the adult brain hippocampus. Both cannabinoid type 1 and type 2 (CB_1 and CB_2) receptors are expressed in neural stem cells and neural progenitor cells and participate in the proliferation of neural stem cells. CB_1 receptor also acts later on in the differentiation of the newly generated neurons with regard to dendritic length and spine number. CB_1/CB_2, cannabinoid type 1/type 2 receptor

On the other hand, the enhancement of eCB signaling through the genetic inactivation of the AEA-degrading enzyme FAAH[38] and the pharmacological blockade of the 2-AG–degrading enzyme MAGL[117] led to increased NSC proliferation. Synthetic cannabinoid receptor agonist–stimulated neurogenesis appears to require both CB_1 and CB_2 receptors.[118] Furthermore, the phytocannabinoid THC can also stimulate neurogenesis.[119] Interestingly, the nonpsychotropic phytocannabinoid cannabidiol (CBD) was also reported to enhance neurogenesis,[120] possibly through facilitating eCB signaling.[121] Interestingly, CBD treatment was shown to increase AEA levels.[122]

Exercise is an efficient intervention for increasing adult neurogenesis. Pharmacological blockade of the CB_1 receptor has been shown to blunt exercise-induced increase in proliferation in the SGZ.[123] However, in another study, using CB_1 receptor–deficient mice, no genotype differences were observed in neurogenesis over a 6-week running period, but the CB_1 receptor–deficient mice showed reduced motivation to run.[124] These divergent results might be explained by the differences in the pharmacological versus genetic blockade of CB_1 receptor. Results from an investigation in a mouse model of Down syndrome are very different. Here, interestingly, pharmacological blockade of CB_1 receptor led to the alleviation of impaired cognitive performance, synaptic plasticity, and neurogenesis,[125] an effect possibly explained by the pathology of enhanced hippocampal CB_1 receptor expression and concomitant increased receptor function at excitatory terminals.

NSCs contain a functional ECS, including the expression of CB_1 and CB_2 receptors, and eCB-synthesizing and -degrading enzymes.[110] In vitro experiments suggest the involvement of phosphoinositide 3-kinase/protein kinase B (PI3K/PKB), ERK, and mTOR complex 1 (mTORC) pathways in eCB-dependent stimulation of proliferation of NSCs.[110,112,116] For the CB_2 receptor, it has emerged that this receptor is mainly important under pathophysiological conditions, whereby reduction in neurogenesis induced by damaging conditions (epilepsy, alcohol, stroke, neurodegenerative processes) can be alleviated by CB_2 receptor activation.[112]

It has to be recognized that the neurogenic niches are embedded into an environment that strongly affects proliferation and that also contains a functional ECS. Therefore, phenotypic outcomes after modulation of ECS activity depend on the cellular and temporal specificity of the targeting of the respective ECS components. A study using mice with ubiquitous loss of the CB_1 receptor and of FAAH revealed that eCB signaling controls neural progenitor differentiation in the adult brain by altering astroglial differentiation of newly born cells. The survival for the newly born cells was not changed.[38] The question arises whether CB_1 receptor expressed in NSCs is directly involved in the promotion of newly born neurons from NSCs. To this end, specific genetic loss of the CB_1 receptor in NSCs revealed decreased proliferation.[39] Furthermore, CB_1 receptor deficiency caused decreased dendritic branches and spine numbers in the differentiating neurons, reduced long-term potentiation (LTP) and short-term spatial memory, and increased depression-like behavior. Yet, no alteration in cell fate was observed, indicating that the effect on decreased astroglial differentiation observed in the ubiquitous CB_1 receptor–deficient mice was probably caused by the ECS changes around the NSC niche. The effect on dendritic branching observed on NSC-specific CB_1 receptor–deficient mice would also suggest a postsynaptic function of the CB_1 receptor that is required for maturation, but eventually, CB_1 receptor expression stops in terminally differentiated and integrated granule cells.

Generation of new oligodendrocytes

Another cell plasticity process is reported for the replenishment of oligodendrocytes from OPCs, which express the protein neural/glial antigen 2 (NG2).[126] Upon challenges of the adult brain, such as damaging of the myelin, OPCs are capable of proliferating and differentiating into mature oligodendrocytes, alleviating or even repairing the damage.[127] The ECS is functionally present in cultured OPCs and is required for maintaining proliferation, a process requiring PI3K/PKB/mTOR-signaling pathways, as inhibition of 2-AG synthesis and blockade of CB_1/CB_2 receptors seem to induce cell-cycle arrest.[37] The ECS is also present in mature oligodendrocytes.[24,35,36] CB_1/CB_2-agonist stimulation leads to increased myelin basic protein (MBP) expression.[128] Furthermore, in animal models of demyelination, stimulation of the ECS can alleviate various pathologies associated with demyelination.[129,130] Altogether, the ECS regulates cellular plasticity in the adult brain. Here, we focused on proliferation, but the ECS can also regulate various aspects of apoptosis and autophagy,[131] two important cellular events for maintaining homeostasis in the body.

Neurogenesis in the embryo and early postnatal brain development

Finally, it is important to mention that the ECS has numerous functions during embryogenesis and at postnatal stages when the brain develops to its final maturation state. The understanding of these processes gives us essential mechanistic insights into the detrimental effects of cannabis use during these stages of development.[132-134] As it is beyond the scope of this short presentation, the reader is referred to recent reviews on the ECS in neural development.[110,135]

Dysregulated endocannabinoid system

Functions of the ECS help to maintain homeostasis in the body (*Figure 3A*), for example, through regulation of the stress response, feeding, and energy metabolism, and for ensuring the excitatory/inhibitory balance in the nervous system. Considering the temporal and spatial activity of the ECS, it is no surprise that a dysregulated ECS can lead to new set-points, called allostasis, that might then be implicated in distinct neuropsychiatric disorders (*Figure 3A, B*). Human polymorphisms in genes of the ECS can be linked to particular phenotypes, as summarized in detail in a recent review,[11] suggesting a dysregulated ECS as origin of the observed altered behavior. A wealth of data is available for the phenotypes caused by the FAAH polymorphism rs324430 (C385A),[11] which leads to decreased protein stability of FAAH in the A allele, leading to increased AEA levels.[136] This point mutation was also introduced into the mouse genome, allowing for comparative studies in human and mouse.[137] These investigations indicate that the A allele promotes fear extinction, reduces anxiety,[137] and provides protection against stress-induced decreases in AEA.[138] On the other hand, the A allele of FAAH constitutes a risk factor for developing anxiety and depression from repetitive childhood trauma,[139] indicating the relevance of the developmental dynamics of ECS activity during childhood and adolescence. A recent case report describes a microdeletion in FAAH that led to increased AEA levels in blood and to insensitivity to pain.[140] Several polymorphisms in the CB_1 receptor gene (*CNR1*) have been described. None of them lead to overt biochemical changes in CB_1 receptor protein functions. However, these polymorphisms might contribute to the susceptibility for development of certain neuropsychiatric disorders,[11] although the data are not as clear as for the FAAH C385A polymorphism; further studies are needed to substantiate previous observations with independent cohorts.[11] Investigations on a CB_2 receptor polymorphism (*CNR2*) recently summarized in a meta-analysis suggested an association of a particular SNP (rn2501432) with depression.[141] Furthermore, a study revealed that the R allele of the *CNR2* Q63R polymorphism together with the A allele of the FAAH C385A polymorphism are associated with enhanced vulnerability to childhood trauma and to a later anxious and depressive phenotype.[142]

In humans, ECS activity can be monitored by measuring eCB levels in blood, saliva, hair, and cerebrospinal fluid. Expression of ECS genes can also be determined in the tissue post mortem. Numerous studies have reported alterations in eCB levels in patients suffering from various disorders, such as depression, posttraumatic stress disorder (PTSD), schizophrenia, anorexia nervosa, and Tourette syndrome.[11,143,144] Also, the course of therapy can be followed up by monitoring eCB levels, such as antidepressant treatment with electroconvulsive intervention.[145] In investigations with patients, drug treatments for the respective diseases constitute a serious confounding for eCB measurements. eCB can also be monitored in healthy subjects who are undergoing psychological tests and other challenges, and it is hoped that such monitoring, in combination with other biomarkers, would make possible the evaluation of potential predispositions toward development of distinct disorders.

In humans, as recently reviewed,[146] positron emission tomography (PET), a noninvasive method to determine ECS activity via radioactive tracers for cannabinoid receptors and ECS enzymes, has been applied to various disorders. Studies with alcohol-use disorders and schizophrenia are inconsistent, some reporting increased and others decreased CB_1 receptor binding. Data on aberrant CB_1 receptor binding in individuals with anorexia and PTSD are more coherent, but the data sets are still limited.

In summary, dysregulated ECS activity is reported in several neuropsychiatric disorders. This dysregulation may originate from genetic and/or epigenetic alterations in ECS components, or may be consequences of alterations of other signaling pathways, leading to changes in ECS activity.

The ECS as a therapeutic target

Given that dysregulated ECS activity can lead to allostatic set-points that could potentially represent pathological states, pharmacological treatment targeting ECS compo-

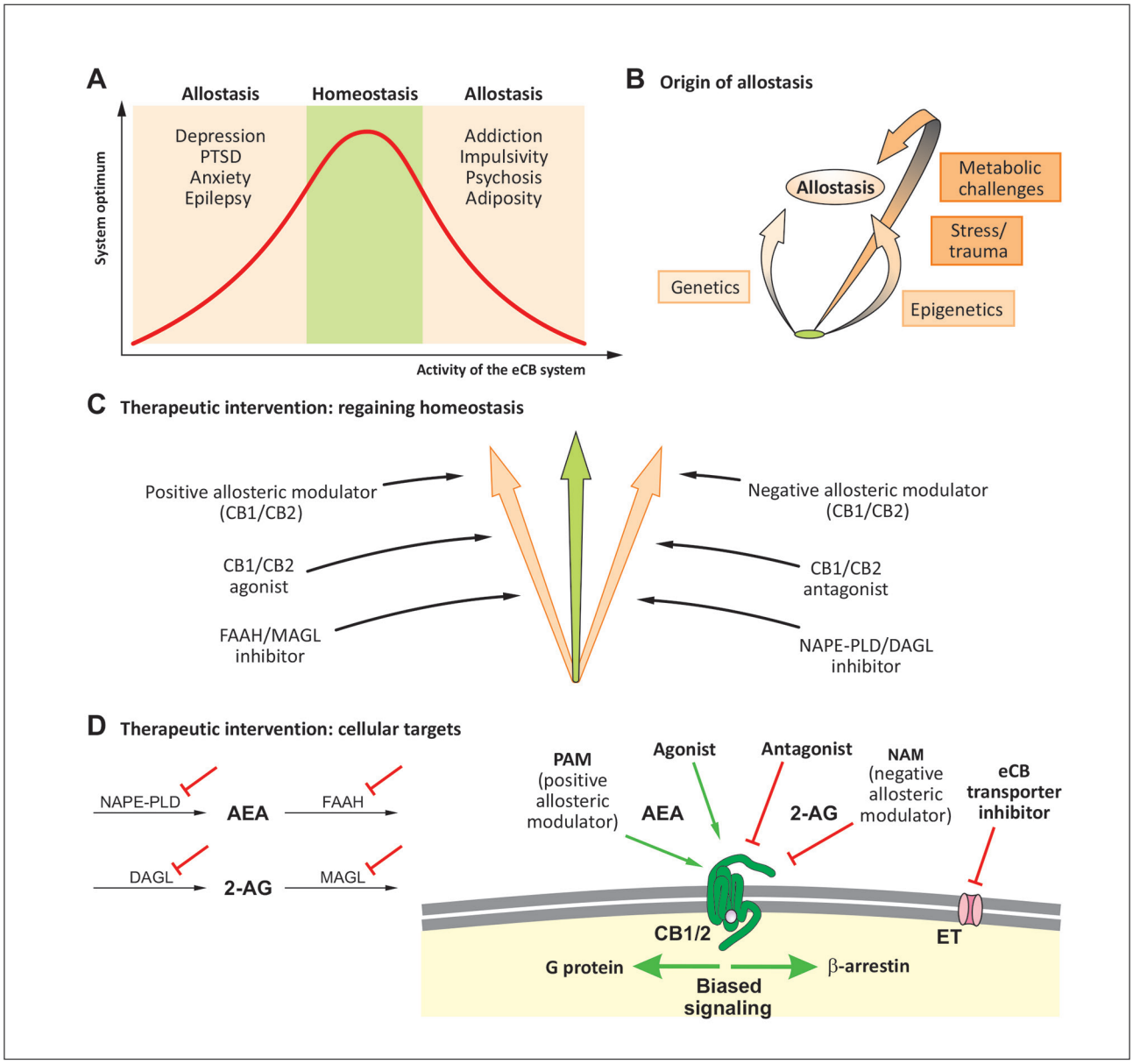

Figure 3. The endocannabinoid system is a homeostatic system. (A) It is proposed that various physiological processes need optimal ECS activity to maintain a homeostatic set-point. Aberrant ECS activity may lead to allostatic set-points, with the emergence of various diseases. (B) The shift from a homeostatic to an allostatic set-point may have different origins, such as genetic causes, but also life events and life history (eg, stress, trauma, metabolic challenges), acting, among others, via epigenetic mechanisms. (C) Pharmacological interventions aiming at regaining homeostasis by targeting different ECS components using different mechanistic approaches. (D) Pharmacological interventions targeting the different ECS components. Compounds acting on cannabinoid receptors with biased signaling effects toward G protein or β–arrestin pathways contain high potentials. 2-AG, 2-arachidonoylglycerol; AEA, anandamide (arachidonoylethanolamide); CB_1/CB_2, cannabinoid type 1/ type 2 receptor; DAGL, diacylglycerol lipase; eCB, endocannabinoid; ET, eCB transporter; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAM, negative allosteric receptor modulator; NAPE-PLD, *N*-acyl phosphatidylethanolamide-specific phospholipase D; PAM, positive allosteric receptor modulator; PTSD, posttraumatic stress disorder

nents is a promising strategy. A wealth of therapeutic applications has been investigated in preclinical animal models, but only in a few cases has translation to humans been achieved in clinical trials. The focus of the present discussion is on the modulation of the various components of the ECS (*Figure 3C, D*). Treatment options using phytocannabinoids are beyond the scope of this presentation, but information on this subject is found in other reviews.[147-149]

Complexity of eCB signaling molecules

Biosynthesis of eCBs occurs from membrane precursors, and eCB degradation products are precursors of eicosanoids.[1] Thus, eCB signaling is integrated into a lipid metabolism and signaling network. In consequence, modification of activity of eCB synthesizing and degrading enzymes may also alter other lipid signaling systems.[150] The synthesis and degradation machinery of AEA and 2-AG have different cellular and subcellular distributions, indicating differential functions.[50] Furthermore, AEA and 2-AG have different pharmacological profiles with regard to interaction with their receptors, CB_1 and CB_2 receptors, but can also activate other receptors, such as TRPV1 and $GABA_A$ receptors, respectively.[5,21] Furthermore, endogenous peptides called pepcans or hemopressin were recently characterized[151-153]; these can act on CB_1 and CB_2 receptors, thereby modifying biological processes.

Cannabinoid receptors

For many years, the focus has been on CB_1 receptor antagonism/inverse agonism. However, the failure of rimonabant (Acomplia) because of CNS side effects[154] stopped clinical applications. Nevertheless, convincing alternative strategies, in particular peripherally acting CB_1 receptor antagonists, have been developed in recent years and shown to be active without appreciable CNS side effects.[13,155] As peripheral organ systems interact with CNS functions, alleviation of dysregulated ECS activity in the periphery also has potential for beneficial therapeutic effects in dysregulated CNS functions.[17,18,20] As discussed above, the ECS is featured by its temporal and spatial specificity in signaling. Thus, both positive (PAM) and negative (NAM) allosteric receptor modulators constitute a promising alternative path compared with direct receptor agonism/antagonism.[156] Indeed, preclinical research has shown very promising efficacy using such compounds.[157,158] Recently, pregnenolone and non-metabolizable derivatives thereof have emerged as a promising NAM of CB_1 receptor.[133,159] The development of receptor ligands with bias toward distinct intracellular pathways, mainly G protein versus β-arrestin, also represents a very promising strategy to increase the therapeutic effects and diminish unwanted signaling side effects.[72] Pepcans (hemopressins) were reported to be a NAM for CB_1 receptor[151] and a PAM for CB_2 receptor.[160] A further strategy to enhance specificity is to generate dual-target drugs.[13] For example, a mainly peripherally acting hybrid CB_1/inducible nitric oxide synthase (iNOS) antagonist is effective in the treatment of experimental liver fibrosis,[161] and the fusion peptide between hemopressin and neuropeptide VF shows potent antinociceptive effects with reduced cannabinoid-related side effects.[162] Comparable approaches have been pursued for interfering with CB_2 receptor activity, although with slower progress.[163] Particularly in the field of neuroinflammation and neurodegeneration, CB_2 receptor agonism seems to be promising.[44] CB_2 receptor as a target is particularly attractive because of the lack of psychotropic activity, which is present in the case of CB_1 receptor agonism.

Catabolic and metabolic eCB enzymes

Knowing that eCBs cannot be stored in vesicles, that ECS activity is spatially and temporally regulated, and that AEA and 2-AG have different functions in the brain, the ability to pharmacologically interfere with biosynthesis and degradation of AEA and 2-AG, respectively, is useful. It also allows a possible increase in specificity at the site of action and may reduce side effects. Therefore, the inhibition of FAAH and MAGL separately, and the inhibition of both FAAH and MAGL together are the most investigated approaches in preclinical research and in clinical trials for various applications, such as pain, inflammation, anxiety, and depression-like behavior.[164-167] As for modulators of cannabinoid receptors, peripherally acting compounds may help avoid CNS-derived side effects.[167,168] FAAH inhibitors also have preclinical applications.[169,170] Whereas hopes are high that such compounds will be useful in humans, this has yet to be achieved. Clinical trials must be conducted with care so that setbacks can be avoided, a lesson learned, for example, in the clinical trial using the FAAH inhibitor BIA 10-2474, which contained nonspecific reactions toward serine hydrolases other than FAAH, revealed from incidences of clinical neurotoxicity.[171] Recently, new compounds inhibiting NAPE-PLD were reported.[172] Interestingly, CBD can alter levels of AEA and other *N*-acylethanolamines,[122] suggesting mechanisms via an indirect stimulation of the ECS, thereby possibly explaining the low level of side effects induced by CBD.

eCB membrane transporter

eCBs seem to be transported through a facilitated transporter across the plasma membrane. Despite the fact that to date no such protein has been cloned, drugs influencing the activity of such transporters have been found. The early generation of such compounds often did not have high efficacy and selectivity[173]; however, recently, a series of compounds have been developed that lack activity on cannabinoid receptor, eCB-degrading enzymes, and binding to fatty-acid binding protein.[174,175] Inhibition of such a transporter is expected to enhance the availability of extracellular eCBs, as eCBs cannot be transported into cells for degradation. Yet, it might also be argued that the export of eCBs is inhibited upon stimulated synthesis of eCBs, thereby increasing intracellular eCBs, leading, for example, to increased activation of mitochondrial CB_1 receptor ($mtCB_1$). Furthermore, because of the probable effect on both AEA and 2-AG levels, several different target receptors have to be considered (CB_1/CB_2 receptor, TRPV1, peroxisome proliferator-activated receptor-γ [PPARγ]). Altogether, the inhibition of eCB membrane transporters would influence multiple cellular signaling systems, but represents a promising pharmacological target.

In summary, the diversity of ECS signaling molecules and their interactions with various receptors, together with signaling complexity of the receptor systems, makes pharmacological intervention of the ECS a challenging task, containing a considerable degree of unpredictability in the outcome of the biological effects in the whole organism.

Future directions and concluding remarks

Understanding of the brain's ECS in its complexity at the mechanistic levels is very valuable for identifying promising disease states that can be optimally treated by modulating ECS activity. Given that eCB signaling is very widespread in the brain and intertwined with other signaling systems, the mechanistic insights will help minimize potentially unwanted side effects. Thus, it is important to understand ECS functions in the context of the entire organism. To this end, animal models, such as mice and rats, have been shown to be very suitable; owing to the evolutionary conservation of the ECS, these insights are expected to be transferrable to humans in many instances.

An important topic is the understanding of the integration of eCB signaling into the brain's complex network. For example, excellent recent studies investigated pathways from amygdala to nucleus accumbens in the context of depressive-like behavior,[176] and from amygdala to cortex regarding stress effects.[177] Here, genetic manipulations of the CB_1 receptor functions were investigated in a pathway-specific manner, and in fact, very distinct functions of this receptor were uncovered. Thus, again, despite the very widespread presence of CB_1 receptor in the brain, its functions are amazingly specific. In further developments, high-resolution and specific genetic manipulations can be included, such as intersectional targeting of cells and circuits,[178-180] and optogenetic-induced genetic manipulation.[181] Furthermore, with the advent of the clustered regularly interspaced short palindromic sequences (CRISPR)/CRISPR-associated protein (Cas) technology,[182,183] the introduction of mutations into the mouse or rat genome, in particular point mutations that were characterized in in vitro structure-function analyses or are present in humans as small nucleotide polymorphisms (SNPs),[11] opens the path to novel insights into the analysis of ECS components in the context of the entire organism.

Furthermore, SNPs in ECS components[11] can also be investigated using human organoids generated from induced pluripotent stem cells,[184,185] with the additional potential of genomic manipulation using CRISPR/Cas technology[186] and pharmacological interventions, for example, with cannabinoids,[187] together with state-of-the-art analyses, such as single-cell RNA-seq.[188] These approaches will further strengthen mechanistic insights into the roles of the ECS in psychiatric disorders. ■

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