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 (CB₁), 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. CB₁ receptors are involved in different synaptic and cellular plasticity processes and in the brain’s bioenergetics in a context-specific manner. CB₁ 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.

Introduction

The endocannabinoid system (ECS) comprises two cannabinoid receptors—CB₁ and CB₂ 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 (arachidonylethanolamide, 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. The ECS is evolutionarily well conserved in vertebrates, 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. It is intertwined with many neurotransmitter and lipid signaling systems, thereby integrated into broad functional networks. It appears that the ECS plays roles in the fine-tuning of physiological processes that keep the body in homeostatic set-points. In humans, several polymorphisms in ECS components with associated neuropathophysiological processes have been described, suggesting they promote susceptibility toward development of neuropsychiatric disorders. ECS dysregulation can also be induced by particular life factors, such as living under chronic stress, or by metabolic factors, such as with obesity. Pharmacological interventions targeting ECS activity aim to normalize such pathophysiological processes, 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.
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 CB₁ receptor. However, the ECS is also widely involved in the regulation of peripheral immune, cardiovascular, metabolic, gastrointestinal, muscular, and peripheral nervous system processes, which in turn can influence central nervous system (CNS) functions.

Endocannabinoid mechanisms

The peculiarities of the cannabinoid receptors
CB₁ and CB₂ receptors 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. Here, the focus will be on the CB₁ receptor, the major cannabinoid receptor in the nervous system, but several aspects of CB₂ receptors will also be addressed. It is interesting to note that despite the ubiquitous occurrence of the CB₁ 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 CB₁ receptor is present in virtually all brain regions and in all major cell types (Figure 1), i.e., in neurons, glial cells (astrocytes, oligodendrocytes), and brain-resident immune cells (microglia). The CB₁ receptor is also present in the neurons of the major neurotransmitter systems (glutamate, γ-aminobutyric acid [GABA], serotonin, noradrenaline, acetylcholine), and possibly also in dopaminergic neurons. CB₁ receptor–expressing cells can also be classified according to the presence of peptidergic transmitters, such as corticotropin-releasing hormone, cholecystokinin, and somatostatin. The expression levels can vary greatly, depending on the brain region and cell type. For example, the central amygdala contains very low levels of CB₁ receptor in only a few of the presumably GABAergic neurons. On the other hand, very high levels of CB₁ receptor messenger RNA (mRNA) are detected in hippocampal and neocortical GABAergic interneurons. Glutamatergic neurons generally contain rather low levels of CB₁ receptor.
Furthermore, the CB₁ receptor is barely detectable in astrocytes, oligodendrocytes, and adult neural stem cells (NSCs). Specific functions of CB₁ receptor in these different populations and in many brain areas have been described in mouse by using conditional gene inactivation of CB₁ receptor in the respective cell types and/or brain regions, together with local pharmacological interventions. Importantly, the relative abundance of CB₁ receptor does not indicate the importance of the receptor in a particular physiological process.

CB₂ receptor expression has been predominantly described in peripheral immune cells and brain-resident immune cells, the macrophages, but was eventually also detected in neurons, a circumstance that has fueled many investigations on CB₂ receptor in neural functions. In the CNS, CB₂ receptor expression was reported in cells such as activated microglia, brain stem neurons, hippocampal glutamatergic neurons, and dopaminergic neurons of the ventral tegmental area. CB₂ receptor transcripts are reported to be 100 to 200 times less abundant than CB₁ receptor mRNA, but are strongly upregulated in response to various insults, such as chronic pain, neuroinflammation, and stroke.

Genetic approaches, together with pharmacology and very specific and sensitive cellular detection methods of mRNA, paved the way for the recognition of CB₂ receptor in many neural functions.

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 CB₁ receptor protein location is the presynapse, where the activation of CB₁ 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 CB₁ receptor. This can result in a short-term decrease in Ca⁺⁺ influx at the presynaptic terminal. 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. For AEA, the situation is far from being understood. First, AEA synthesis machinery seems to be mainly located at the presynaptic site, whereas the degrading machinery is at the postsynaptic site. 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. 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) and the AEA-degrading enzyme fatty acid amide hydrolase (FAAH), respectively.

**Postsynaptic localization:** Postsynaptic CB₁ receptor has been reported in electrophysiological experiments in neocortical GABAergic and glutamatergic neurons, and in hippocampal glutamatergic neurons involving potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 1 (HCN1), possibly via a somatodendritic mechanism. Based on immunohistological analysis, CB₁ receptor has been described to be intracellular, within somatodendritic endosomes, and until now not yet on the cell membrane.

In summary, the neuronal CB₁ receptor is dominantly expressed at the presynapse; however, functional postsynaptic CB₁ receptor has also been reported. CB₁ receptor has been reported to be localized in the soma, 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 CB₁ receptors, this is mainly the axonal terminal—and finally integrated into the cellular membrane. Distinct sequences of the CB₁ receptor are involved in axonal trafficking, whereby alterations in such sequences can lead to eCB-mediated electrophysiological and behavioral alterations. Distribution of CB₁ receptor in the different...
neuronal compartments can be influenced by the activation state of the receptor. For example, pharmacological activation of the 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$_3$ receptor antagonist leads to a decreased number of cell bodies with CB$_3$ receptor–containing endosomes. This observation suggests that under steady-state conditions, the CB$_3$ 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$_3$ receptors located in the presynaptic membrane of hippocampal GABAergic interneurons. This internalization persists for many days after termination of THC treatment.$^{61}$ Along these lines, genetic deletion of the 2-AG–degrading enzyme MAGL also leads to β-arrestin–mediated internalization and desensitization of the CB$_3$ 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-imparing 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.$^{67}$

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$_3$ 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$_3$ receptor signaling have been reported$^{71,72,73}$; however, we are far from understanding how CB$_3$ receptor signaling gains specificity depending on cellular context.

**Dimerization:** GPCRs contain the capacity to form homodimers, 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$_3$ receptors$^{75}$ and heterodimers reported for CB$_3$ receptor with several other GPCRs, including CB$_3$ receptor,$^{66,77}$ dopamine

---

**Despite the very widespread presence of the CB$_1$ receptor in the brain, its functions are amazingly specific**
State of the art

Neurobiology of cannabinoid receptor signaling - Lutz

D₃ receptor,78,79 and serotonin 5-HT₄ receptor.80 Heterodimerization was reported to lead to alterations in signaling. For example, CB₁ receptor/dopamine D₂ receptor heterodimers in cultured striatal neurons can change the intracellular signaling upon CB₁ receptor stimulation.79 Along the same line, a recent investigation showed the heterodimerization of CB₁ receptor with the adenosine A₂A receptor in striatum, whereby the costimulation of both receptors reduces intracellular signaling.81 CB₁/CB₂ 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 CB₁ 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: CB₁ receptor is typically coupled to G₁₅ proteins, leading to a decreased production of cyclic adenosine monophosphate (cAMP) and an inhibition of N- and P/Q-type Ca²⁺ channels, resulting in decreased Ca²⁺ influx upon stimulation.1,2,1 Furthermore, G₁₅ coupling in striatal neurons was reported, in particular in concert with dopamine D₁ receptor signaling.84-86 G₁₅ coupling was found as a CB₁ receptor–interacting G protein, detected from a biochemical pull-down proteomic experiment using hippocampal synaptosomes expressing tagged CB₁ receptor.88 Furthermore, biochemical analysis of G proteins interacting upon CB₁ receptor stimulation in neocortical extracts revealed the presence not only of G₁₅ but also of G₁₅, G₁₆₁₃, and G₁₆₁₁.89 The coupling was dependent on the CB₁ 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 CB₁ receptor proteins in glutamatergic hippocampal neurons show higher GTPγ-binding activity than the high CB₁ 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 CB₁ receptor with dopamine D₂ receptor regarding the regulation of eCB-mediated retrograde synaptic signaling of striatal neurons.92

Altogether, these observations suggest that CB₁ receptor signaling can depend on the availability of the intracellular G protein pool and on the specific ligand that activates the CB₁ 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 CB₁ receptor signaling.

Signaling via β-arrestin: Binding of CB₁ 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 extra-cellular-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 CB₁ receptor signaling.95,96 Deletion of the CB₁ receptor phosphorylation site that is involved in β-arrestin binding was reported to lead to resistance to cannabinoid tolerance and hypersensitivity to cannabinoids.96 CB₁ 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 CB₁ receptor.98

Receptor-interacting proteins: Furthermore, the presence of CB₁ receptor–interacting proteins can also contribute to the diversity of CB₁ receptor signaling. Here, the cannabinoid receptor interacting protein 1A (CRIP1A) is reported to influence CB₁ receptor agonist-induced regulation of excitatory neurotransmission,99 to modulate which G₁₅ subtypes interact with CB₁ receptor, and to attenuate CB₁ receptor internalization via β-arrestin.100

Splice variants and posttranslational modifications: Lastly, for both CB₁ and CB₂ receptors, splice variants have been reported in rodents and human.101,102,103 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 and N-linked glycosylation, also have to be considered in the regulation of receptor activity. For example, reduction in glycosylation of the CB₁ receptor reduces the cell membrane expression of the receptor, but not ligand-binding affinity.

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. 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. The role of the ECS in adult neurogenesis is well documented, as reviewed in detail elsewhere. Experiments performed in mice under home-cage conditions without behavioral challenges revealed that ubiquitous gene deficiency of CB₁ receptor and impaired 2-AG signaling in mice with deficiency of the 2-AG synthesizing enzyme diacylglycerol lipase-α (DAGLα) lead to decreased NSC proliferation. On the contrary, under basal conditions, ubiquitous CB₁ receptor deficiency did not lead to impairments in NSC proliferation. Reduced proliferation was also observed in rodents treated with specific CB₁- and CB₂ receptor antagonists.

![Figure 2](image-url)
On the other hand, the enhancement of eCB signaling through the genetic inactivation of the AEA-degrading enzyme FAAH\textsuperscript{38} and the pharmacological blockade of the 2-AG-degrading enzyme MAGL\textsuperscript{117} led to increased NSC proliferation. Synthetic cannabinoid receptor agonist–stimulated neurogenesis appears to require both CB\textsubscript{1} and CB\textsubscript{2} receptors.\textsuperscript{118} Furthermore, the phytocannabinoid THC can also stimulate neurogenesis.\textsuperscript{119} Interestingly, the nonpsychotropic phytocannabinoid cannabidiol (CBD) was also reported to enhance neurogenesis,\textsuperscript{120} possibly through facilitating eCB signaling.\textsuperscript{121} Interestingly, CBD treatment was shown to increase AEA levels.\textsuperscript{122}

Exercise is an efficient intervention for increasing adult neurogenesis. Pharmacological blockade of the CB\textsubscript{1} receptor has been shown to blunt exercise-induced increase in proliferation in the SGZ.\textsuperscript{123} However, in another study, using CB\textsubscript{1} receptor–deficient mice, no genotype differences were observed in neurogenesis over a 6-week running period, but the CB\textsubscript{1} receptor–deficient mice showed reduced motivation to run.\textsuperscript{124} These divergent results might be explained by the differences in the pharmacological versus genetic blockade of CB\textsubscript{1} receptor. Results from an investigation in a mouse model of Down syndrome are very different. Here, interestingly, pharmacological blockade of CB\textsubscript{1} receptor led to the alleviation of impaired cognitive performance, synaptic plasticity, and neurogenesis,\textsuperscript{125} an effect possibly explained by the pathology of enhanced hippocampal CB\textsubscript{1} receptor expression and concomitant increased receptor function at excitatory terminals.

NSCs contain a functional ECS, including the expression of CB\textsubscript{1} and CB\textsubscript{2} receptors, and eCB-synthesizing and -degrading enzymes.\textsuperscript{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.\textsuperscript{110,112,116} For the CB\textsubscript{1} 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\textsubscript{2} receptor activation.\textsuperscript{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\textsubscript{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.\textsuperscript{38} The question arises whether CB\textsubscript{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\textsubscript{1} receptor in NSCs revealed decreased proliferation.\textsuperscript{39} Furthermore, CB\textsubscript{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\textsubscript{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\textsubscript{1} receptor–deficient mice would also suggest a postsynaptic function of the CB\textsubscript{1} receptor that is required for maturation, but eventually, CB\textsubscript{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).\textsuperscript{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.\textsuperscript{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\textsubscript{1}/CB\textsubscript{2} receptors seem to induce cell-cycle arrest.\textsuperscript{37} The ECS is also present in mature oligodendrocytes.\textsuperscript{24,35,36} CB\textsubscript{1}/CB\textsubscript{2}-agonist stimulation leads to increased myelin basic protein (MBP) expression.\textsuperscript{128} Furthermore, in animal models of demyelination, stimulation of the ECS can alleviate various pathologies associated with demyelination.\textsuperscript{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,\textsuperscript{131} two important cellular events for maintaining homeostasis in the body.
State of the art
Neurobiology of cannabinoid receptor signaling - Lutz

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, 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), which leads to decreased protein stability of FAAH in the A allele, leading to increased AEA levels. This point mutation was also introduced into the mouse genome, allowing for comparative studies in human and mouse. These investigations indicate that the A allele promotes fear extinction, reduces anxiety, and provides protection against stress-induced decreases in AEA. On the other hand, the A allele of FAAH constitutes a risk factor for developing anxiety and depression from repetitive childhood trauma, 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. Several polymorphisms in the CB₂ receptor gene (CNR1) have been described. None of them lead to overt biochemical changes in CB₂ receptor protein functions. However, these polymorphisms might contribute to the susceptibility for development of certain neuropsychiatric disorders, although the data are not as clear as for the FAAH C385A polymorphism; further studies are needed to substantiate previous observations with independent cohorts. Investigations on a CB₂ receptor polymorphism (CNR2) recently summarized in a meta-analysis suggested an association of a particular SNP (rs2501432) with depression. 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.

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. Also, the course of therapy can be followed up by monitoring eCB levels, such as antidepressant treatment with electroconvulsive intervention. 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, 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 CB₁ receptor binding. Data on aberrant CB₁ 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-
State of the art
Neurobiology of cannabinoid receptor signaling - Lutz

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 (e.g., 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); CB1/CB2, cannabinoid type 1/type 2 receptor; DAGL, diacylglycerol lipase; eCB, endocannabinoid; ET, eCB transporter; FAAH, fatty acid amide hydrolase; MAGL, monoaoylglycerol lipase; NAM, negative allosteric receptor modulator; NAPE-PLD, N-acyl phosphatidylethanolamide-specific phospholipase D; PAM, positive allosteric receptor modulator; PTSD, posttraumatic stress disorder.
State of the art
Neurobiology of cannabinoid receptor signaling - Lutz

Components 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
Bioavailability 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.70 Furthermore, AEA and 2-AG have different pharmacological profiles with regard to interaction with their receptors, CB1 and CB2 receptors, but can also activate other receptors, such as TRPV1 and GABA receptors, respectively.5,21 Furthermore, endogenous peptides called pepcans or hemopressins were recently characterized151-152; these can act on CB1 and CB2 receptors, thereby modifying biological processes.

Cannabinoid receptors
For many years, the focus has been on CB1 receptor antagonism/inverse agonism. However, the failure of rimonabant (Acomplia) because of CNS side effects153 stopped clinical applications. Nevertheless, convincing alternative strategies, in particular peripherally acting CB1 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 CB2 receptor.13,159 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 CB1 receptor155 and a PAM for CB2 receptor.160 A further strategy to enhance specificity is to generate dual-target drugs.13 For example, a mainly peripherally acting hybrid CB1/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 CB1 receptor activity, although with slower progress.163 Particularly in the field of neuroinflammation and neurodegeneration, CB2 receptor agonism seems to be promising.44 CB2 receptor as a target is particularly attractive because of the lack of psychotropic activity, which is present in the case of CB1 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,173 suggesting mechanisms via an indirect stimulation of the ECS, thereby possibly explaining the low level of side effects induced by CBD.
State of the art

Neurobiology of cannabinoid receptor signaling - Lutz

**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 (mCB\(_1\)). 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, TRPV\(_1\), peroxisome proliferator-activated receptor-\(\gamma\) [PPAR\(\gamma\)]). 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 transferable 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.

**Acknowledgments/Disclosures:** There is no conflict of interest. This work was supported by the German Research Foundation DFG (CRC 1193 “Neurobiology of Resilience”; TRR-CRC 58 “Fear, Anxiety, Anxiety Disorders”). The author would like to thank Michael Plenikowski for his excellent work in the design of the illustrations.
Cannabinoid receptors control stress-induced impairment of memory consolidation. Proc Natl Acad Sci U S A. 2016;113:9904-9909. 

18. Ruiz de Azua I, Mancini G, Srivastava RK, et al. Adipocyte cannabinoid receptor CB1 regulates energy homeostasis and alternatively activated macrophages. J Clin Invest. 2017;127:4148-4162. 

19. Bellochio L, Soria-Gomez E, Quarta C, et al. Activation of the sympathetic nervous system mediates hypoglycemic and anxiety-like effects of CB1 receptor blockade. Proc Natl Acad Sci U S A. 2013;110:4786-4791. 

20. Suarez J, Rivera P, Aparisi RA, et al. Adipocyte cannabinoid CB1 receptor deficiency alleviates high fat diet-induced memory deficit, depressive-like behavior, neuroinflammation and impairment in adult neurogenesis. Psychoneuroendocrinology. 2019;110:104418. 

21. Pertwee RG, Howlett AC, Abood ME, et al. International union of basic and clinical pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. Pharmacol Rev. 2010;62:588-631. 

22. Wootten D, Christophouls A, Marti-Solano M, Babu MM, Sexton PM. Mechanisms of signalling and biased agonism in G protein-coupled receptors. Nat Rev Mol Cell Biol. 2018;19:638-653. 

23. Han J, Kesner P, Meta-Laurant M, et al. Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. Cell. 2012;148:1039-1050. 

24. Ilyasov AA, Milligan CE, Pharr EP, Howlett AC. Endocannabinoids in the health and disease. Front. Neurosci. 2018;12:733. 

25. Araujo DJ, Tjoa K, Saijo K. The endocannabinoid system components as potential biomarkers in psychiatry. Front Psychiatry. 2020;11:315. 

26. Cota D, Steiner MA, Marsicano G, et al. Requirement of cannabinoid receptor type 1 for the basal modulation of hypothalamic-pituitary-adrenal axis function. Endocrinology. 2007;148:1574-1581. 

27. Marsicano G, Lutz B. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur J Neurosci. 1999;11:4213-4225. 

28. Katona I, Uchigashima M, Watanabe M. Endocannabinoid system components as the cornerstone of exostasis. Muzik O, Diwadkar V A. Hierarchical control systems for the regulation of physiological homeostasis and the expanded endocannabinoid system in neuroendocrine physiology. Nat Rev Neurosci. 2003;4:873-884. 

29. Tomaselli G, Vallee M. Stress and drug abuse-relevant actions of the endocannabinoid system and the brain. Annu Rev Psychol. 2013;64:21-47. 

30. Mony K, Massa F, Egertova M, et al. The endocannabinoid system controls key epi-leptogenic circuits in the hippocampus. Neuron. 2006;51:455-466. 

31. Lange MD, Daldrup T, Remmers F, et al. Cannabinoid CB1 receptors in distinct circuits of the extended amygdala determine fear responsiveness to unpredictable threat. Mol Psychiatry. 2017;22:1422-1430. 

32. Mony K, Massa F, Egertova M, et al. The endocannabinoid system controls key epi-leptogenic circuits in the hippocampus. Neuron. 2006;51:455-466. 

33. Katona I, Urban GM, Wallace M, et al. Molecular composition of the endocannabinoid system at glutamatergic synapses. J Neurosci. 2006;26:5628-5637. 

34. Gutierrez-Rodriguez A, Bonilla-Del RI, Puente N, et al. Localization of the cannabinoid type-1 receptor in subcellular astrocyte compartments of mutant mouse hippocampus. Glia. 2018;66:1417-1431. 

35. Molina-Holgado E, Vela JM, Arevalo-Martin A, et al. Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. J Neurosci. 2002;22:9742-9753. 

36. Gomez O, Sanchez-Rodríguez MA, Ortega-Gutierrez S, et al. A basal tone of 2-arachidonoylglycerol contributes to early oligodendrocyte progenitor proliferation by activating phosphatidylinositol 3-kinase (PI3K)/Akt and the mammalian target of rapamycin (MTOR) pathways. J Neuroinflammation. 2015;12:309-317. 

37. Aguado T, Palazuelos J, Monory K, et al. The endocannabinoid system promotes astroglial differentiation by acting on neural progenitor cells. J Neurosci. 2006;26:1551-1561. 

38. Zimmermann T, Maroso M, Beer A, et al. Neuronal stem cell lineage-specific cannabinoid type-1 receptor regulates neurogenesis and plasticity in the adult mouse hippocampus. Cereb Cortex. 2018;28:4454-4471. 

39. Lange MD, Daldrup T, Remmers F, et al. Cannabinoid CB1 receptors in distinct circuits of the extended amygdala determine fear responsiveness to unpredictable threat. Mol Psychiatry. 2017;22:1422-1430. 

40. Hebert-Chaletain E, Desprez T, Serrat A, et al. A cannabinoid link between mitochondria and memory. Nature. 2016;539:555-559. 

41. Robin LM, Oliveira da Cruz JF, Langlais VC, et al. Astroglial CB2 receptors determine synaptic D-serine availability to enable recognition memory. Neuron. 2018;98:935-944. 

42. Tanaka M, Sackett S, Zhang Y. Endocannabinoid modulation of microglial phenotypes in neuropsycho-phology. Front Neurol. 2020;11:87.
State of the art

Neurobiology of cannabinoid receptor signaling - Lutz

44. Jordan CJ, Xi ZX. Progress in brain cannabinoid CB1 receptor research: from genes to behavior. Neurosci Biobehav Rev. 2019;98:208-220.

45. Van Sickle MD, Duncan M, Kingsley PJ, et al. Identification and functional characterization of brainstem cannabinoid CB1 receptors. Science. 2005;310:329-332.

46. Stempel AV, Stumpf A, Zhang HY, et al. Cannabinoid type 2 receptors mediate a cell type-specific plasticity in the hippocampus. Neuron. 2016;90:795-809.

47. Zhang HY, Gao M, Liu QR, et al. Cannabinoid CB2 receptors modulate midbrain dopamine neuronal activity and dopamine-related behavior in mice. Proc Natl Acad Sci U S A. 2014;111(46):E5007-E5015.

48. Zhang HY, Gao M, Shen H, et al. Expression of functional cannabinoid CB2 receptor in VTA dopamine neurons in rats. Addict Biol. 2017;22:725-765.

49. Yu SJ, Reiner D, Shen H, Wu KJ, Liu QR, Wang Y. Time-dependent protection of CB2 receptor agonist in stroke. PLoS One. 2015;10:e0132487.

50. Katona I, Freund TF. Multiple functions of endocannabinoid signaling in the brain. Annu Rev Neurosci. 2012;35:529-559.

51. Nyulas R, Dudok B, Urban GM, et al. Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals. J Neurosci. 2008;28:10588-10598.

52. Egertova M, Cravatt BF, Elphick MR. Comparative analysis of fatty acid amide hydrolase and CB1 cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. Neuroscience. 2003;119:481-496.

53. Gibson HE, Edwards JG, Page RS, Van Hook MJ, Kauer JA. TRPV1 channels mediate long-term depression at synapses on hippocampal interneurons. Neuron. 2008;57:746-759.

54. Guggenhuber S, Romo-Parra H, Bindila L, et al. Impaired 2-AG signaling in hippocampal glutamatergic neurons: aggravation of anxiety-like behavior and unaltered seizure susceptibility. J Neurophysiopharmacology. 2015;19:pp.091.

55. Zimmermann T, Bartisch JC, Beer A, et al. Impaired anandamide/palmitoylethanolamide signaling in hippocampal glutamatergic neurons alters synaptic plasticity, learning, and emotional responses. Neurophysiopharmacology. 2019;44:1377-1388.

56. Bacci A, Huguenard JR, Prince DA. Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. Nature. 2004;431:312-316.

57. Marielli S, Pacioni S, Cannich A, Marsicano G, Bacci A. Self-modulation of neocortical pyramidal neurons by endocannabinoids. Nat Neurosci. 2009;12:1488-1490.

58. Maroso M, Szabo GG, Kim HK, et al. Cannabinoid control of learning and memory through HCN channels. Neuron. 2016;89:1059-1073.

59. Thibault K, Carrel D, Bonnard D, et al. Activation-dependent subcellular distribution patterns of CB1 cannabinoid receptors in the rat forebrain. Cereb Cortex. 2013;23:2581-2591.

60. Fletcher-Jones A, Hildick KL, Evans AJ, Nakamura Y, Wilkinson KA, Henley JM. The C-terminal helix 9 motif in rat cannabinoid receptor type 1 regulates axonal trafficking and surface expression. Elife. 2018;9:8.

61. Wickert M, Hildick KL, Baillie GL, et al. The F238L point mutation in the cannabinoid type 1 receptor enhances basal endocytosis via lipid rafts. Front Mol Neurosci. 2018;11:230.

62. Schneider M, Kasanetz F, Lynch DL, et al. Enhanced functional activity of the cannabinoid type-1 receptor mediates adolescent behavior. J Neurochem. 2015;135:799-813.

63. Dudok B, Barna L, Ledri M, et al. Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling. Nat Neurosci. 2015;18:75-86.

64. Imperatore R, Morell G, Luongo L, et al. Genetic deletion of monoacylglycerol lipase leads to impaired cannabinoid receptor CB1R signaling and anxiety-like behavior. J Neurochem. 2015;135:799-813.

65. Cravatt BF, Demarest K, Patricelli MP, et al. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc Natl Acad Sci U S A. 2001;98:9371-9376.

66. Benard G, Massa F, Puente N, et al. Mitochondrial CB1 receptors regulate neuronal energy metabolism. Nat Neurosci. 2012;15:558-564.

67. Jimenez-Blasco D, Busquets-Garcia A, Hebert-Chatelean E, et al. Glucose metabolism links astroglial mitochondria to cannabinoid effects. Nature. 2020;583(7817):603-608.

68. Alger BE, Kim J. Supply and demand for endocannabinoids. J Neurosci. 2015;35:304-315.

69. Kanaän T. Biased receptor signaling in drug discovery. Pharmaco Rev. 2019;71:267-315.

70. Zhao P, Furness SGB. The nature of efficacy at G protein-coupled receptors. Biochem Pharmacol. 2019;170:113647.

71. Laprairie RB, Bagher AM, Denovan-Wright EM. Cannabinoid receptor ligand bias: implications in the central nervous system. Curr Opin Pharmacol. 2017;32:22-43.

72. Al-Zoubi R, Morales P, Reggio PH. Structural Insights into CB1 receptor biased signaling. Int J Mol Sci. 2019;20(8):1837.

73. Borroto-Escuela DO, Fuxe K. Oligomeric receptor complexes and their allosteric receptor-receptor interactions in the plasma membrane represent a new biological principle for integration of signals in the CNS. Front Mol Neurosci. 2019;12:230.

74. Borroto-Escuela DO, Carlsson J, Ambrogini P, et al. Understanding the role of GPCR heteroreceptor complexes in modulating the brain networks in health and disease. Front Cell Neurosci. 2017;11:37.

75. Wager-Miller J, Westenbroek R, Mackie K. Dimerization of G protein-coupled receptors: CB1 cannabinoid receptors as an example. Chem Phys Lipids. 2002;121:83-89.

76. Callen L, Moreno E, Barroso-Chinea P, et al. Cannabinoid receptors CB2 and CB1, form functional heteromers in brain. J Biol Chem. 2012;287:20851-20865.

77. Navarro G, Varani K, Reyes-Rojo I, et al. Cannabigerol action at cannabinoid CB1 and CB2 receptors and at CB1-CB2 heteroreceptor complexes. Front Pharmacol. 2018;9:632.

78. Bagher AM, Laprairie RB, Kelly ME, Denovan-Wright EM. Antagonism of dopamine receptor 2 ligands affects cannabinoid receptor 1 signaling in a cell culture model of striatal medium spiny projection neurons. Mol Pharmacol. 2016;89:652-666.

79. Bagher AM, Laprairie RB, Toguri JT, Kelly MEM, Denovan-Wright EM. Bidirectional allosteric interactions between cannabinoid receptor 1 (CB1) and dopamine receptor 2 long (D2) heterotetramers. Eur J Pharmacol. 2017;813:66-83.

80. Vinals X, Moreno E, Lanfumey L, et al. Cognitively impaired induction by delta9-tetrahydrocannabinol occurs through heteromers between cannabinoid CB1 and serotonin 5-HT3 receptors. PLoS Biol. 2015;13:e002194.

81. Moreno E, Chiarlonare A, Medrano M, et al. Singular location and signaling profile of adenosine A1,-cannabinoid CB1 receptor heteromers in the dorsal striatum. Neurophysiopharmacology. 2018;45:964-977.

82. Navarro G, Varani K, Reyes-Rojo I, et al. Cannabigerol action at cannabinoid CB1 and CB2 receptors and at CB1-CB2 heteroreceptor complexes. Front Pharmacol. 2018;9:632.

83. Blasco-Benito S, Moreno E, Seijo-Vila M, et al. Therapeutic targeting of HER2-CB1R heteromers in HER2-positive breast cancer. Proc Natl Acad Sci U S A. 2019;116:3863-3872.

84. Glass M, Felder CC. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments aAMP accumulation in striatal neurons: evidence for a γ linkage to the CB1 receptor. J Neurosci. 1997;17:5327-5333.

85. Kearn CS, Blake-Palmer K, Daniel E, Mackie K, Glass M. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor crosstalk? Mol Pharmacol. 2005;67:1697-1704.

86. Caballero-Floran RN, Conde-Rojas I, Oviedo CA, et al. Cannabinoid-induced depression of synaptic transmission is switched to stimulation when dopaminergic tone is increased in the globus pallidus of the rodent. Neuropharmacology. 2016;110:407-418.

87. Navarrete M, Araque A. Endocannabinoids mediate neuron-astrocyte communication. Neuron. 2008;57:883-893.

88. Matthes T, Kukla K, Zimmermann T, Tenzer S, Lutz B. Cell type-specific tandem affinity purification of the mouse hippocampal CB1 receptor-associated proteome. J Proteome Res. 2016;15:3585-3601.
State of the art
Neurobiology of cannabinoid receptor signaling - Lutz

89. Diez-Alarcia R, Ibarra-Lecue I, Lopez-Cardona AP, et al. Biased agonism of three different cannabinoid receptor agonists in mouse brain cortex. Front Pharmacol. 2016;7:415.

90. Steindel F, Lerner R, Häring M, et al. Neuron-type specific cannabinoid-mediated G protein signalling in mouse hippocampus. J Neurochem. 2013;124:795-807.

91. O’Brien JB, Wilkinson JC, Roman DL. Regulator of G-protein signaling (RGS) proteins as drug targets: progress and future potentials. J Biol Chem. 2019;294:18571-18585.

92. Song C, Anderson GR, Sutton LP, Dao M, Martemyanov KA. Selective role of RGS9-2 in regulating retrograde synaptic signaling of indirect pathway medium spiny neurons in dorsal striatum. J Neurosci. 2018;38:7120-7131.

93. Raehal KM, Bohn LM. The role of beta-arrestin2 in the severity of antinociceptive tolerance and physical dependence induced by different opioid pain therapeutics. Neuropharmacology. 2011;60:58-65.

94. Gurevich VV, Gurevich EV. GPCR signaling regulation: the role of GRKs and arrestins. Front Pharmacol. 2019;10:125.

95. Delgado-Peraza F, Ahn KH, Noguera-Ortiz C, et al. Mechanisms of biased b-arrestin-mediated signaling downstream from the cannabinoid 1 receptor. Mol Pharmacol. 2016;89:619-629.

96. Morgan DJ, Davis BJ, Kearns CS, et al. Mutation of putative GRK phosphorylation sites in the cannabinoid receptor 1 (CB1R) confers resistance to cannabinoid tolerance and hyperresponsiveness to cannabinoids in mice. J Neurosci. 2014;34:5152-5163.

97. Younts TJ, Monday HR, Dubok D, et al. Presynaptic protein synthesis is required for long-term plasticity of GABA release. Neuron. 2016;92:479-492.

98. Puighermanal E, Marsicano G, Busquets-Garcia J, et al. Blockade of monoacylglycerol lipase inhibits CB1 receptor signaling in hippocampal neurons. J Neurosci. 2010;30:2017-2024.

99. Jiang X, Qu H, Xiao X, et al. Oligodendrocytes in development, myelin generation and repair. Int J Mol Sci. 2015;17:25-32.

100. Booth WT, Walker NB, Lowther WT, Howlett AC. Cannabinoid receptor interacting protein 1a CRIP1a: structure and function. Molecules. 2019;24:3672.

101. Ruehle S, Wager-Miller J, Straker A, et al. Discovery and characterization of two novel CB1 receptor splice variants with modified N-termini in mouse. J Neurochem. 2017;142:521-533.

102. Liu QK, Huang NS, Qu H, et al. Identification and characterization of novel mouse and rat CB1R isoforms and in silico modeling of human CB1R for peripheral cannabinoid therapeutics. Acta Pharmacol Sin. 2019;40:387-397.

103. Ryberg E, Vu HK, Larsson N, et al. Identification and characterization of a novel splice variant of the human CB1 receptor. FEBS Lett. 2005;579: 259-264.

104. Straker A, Wager-Miller J, Hutchens J, Mackie K. Differential signalling in human cannabinoid CB1 receptors and their splice variants in autaptic hippocampal neurones. Br J Pharmacol. 2012;165:2660-2671.

105. Bagher AM, Laprairie RB, Kelly ME, Denvan-Vight EM. Co-expression of the human cannabinoid receptor coding region splice variants (hCB1) affects the function of hCB1 receptor complexes. Eur J Pharmacol. 2013;721:341-354.

106. Rapino C, Castellucci A, Lizzi AR, et al. Modulation of endocannabinoid-binding receptors in human neuroblastoma cells by tunicamycin. Molecules. 2019;24:1432.

107. Bond AM, Ming GL, Song H. Adult mammalian neural stem cells and neurogenesis: five decades later. Cell Stem Cell. 2015;17:385-395.

108. Toda T, Parylak SL, Linker SB, Gage FH. The role of adult hippocampal neurogenesis in brain health and disease. Mol Psychiatry. 2019;24:67-87.

109. Miller SM, Sahay A. Functions of adult-born neurons in hippocampal memory interference and stress. Nat Rev Neurosci. 2014;15:786-801.

110. Prendergill JA, Kelly AM, Downer EJ. The role of cannabinoids in adult neurogenesis. Br J Pharmacol. 2015;172:3950-3963.

111. Oddi S, Scipioni L, Maccarrone M. Endocannabinoids and their receptors in neural progenitor cell proliferation in the hippocampus. Neuropharmacology. 2010;59:207-213.

112. Maccarrone M, Guzman M, Mackie K, Doherty P, Harkany T. Programming of neural cells by endocannabinoids: from physiological rules to emerging therapies. Nat Rev Neurosci. 2014;15:786-801.

113. Toda T, Parylak SL, Linker SB, Gage FH. The role of adult hippocampal neurogenesis in brain health and disease. Mol Psychiatry. 2019;24:67-87.

114. Miller SM, Sahay A. Functions of adult-born neurons in hippocampal memory interference and stress. Nat Rev Neurosci. 2014;15:786-801.

115. Miller SM, Sahay A. Functions of adult-born neurons in hippocampal memory interference and stress. Nat Rev Neurosci. 2014;15:786-801.

116. Oddi S, Scipioni L, Maccarrone M. Endocannabinoids and their receptors in neural progenitor cell proliferation in the hippocampus. Neuropharmacology. 2010;59:207-213.

117. Maccarrone M, Guzman M, Mackie K, Doherty P, Harkany T. Programming of neural cells by endocannabinoids: from physiological rules to emerging therapies. Nat Rev Neurosci. 2014;15:786-801.

118. Toda T, Parylak SL, Linker SB, Gage FH. The role of adult hippocampal neurogenesis in brain health and disease. Mol Psychiatry. 2019;24:67-87.

119. Miller SM, Sahay A. Functions of adult-born neurons in hippocampal memory interference and stress. Nat Rev Neurosci. 2014;15:786-801.

120. Wolf SA, Bick-Sander A, Fabel K, et al. Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. Cell Commun Signal. 2010;8:12.

121. Campos AC, Ortega Z, Palazuelos J, et al. The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: involvement of the endocannabinoid system. Int J Neuropsychopharmacol. 2013;16:1407-1419.

122. Leishman E, Manchanda M, Thelen R, Miller S, Mackie K, Bradshaw HB. Cannabidiol’s upregulation of N-acetyl ethanolamines in the central nervous system requires N-acetyl phosphatidyl ethanolamine-specific phospholipase D. Cannabis Cannabinoid Res. 2018;3:228-241.

123. Hill MN, Titterness AK, Morrish AC, et al. Endogenous cannabinoid signaling is required for voluntary exercise-induced enhancement of progenitor cell proliferation in the hippocampus. Hippocampus. 2010;20:513-523.

124. Dubrez S, Koehl M, Abrous DN, Marsicano G, Chaoulloff F. CB1 receptor deficiency decreases wheel-running activity: consequences on emotional behaviours and hippocampal neurogenesis. Exp Neurol. 2010;224:106-113.

125. Navarro-Romero A, Vazquez-Oliver A, Gomis-Gonzalez M, et al. Cannabinoid type-1 receptor blockade restores neurochemical phenotypes in two models for Down syndrome. Neuropsych Dis. 2019;125:92-106.

126. Kirdjajova D, Anderova M. NG2 cells and their neurogenic potential. Curr Opin Pharmacol. 2019;50:53-60.

127. Kuhn S, Gritt L, Crooks D, Dombrowski Y. Oligodendrocytes in development, myelin generation and beyond. Cells. 2019;8:1424.

128. Gomez O, Sanchez-Rodriguez A, Le M, Sanchez-Caro C, Molina-Holgado F, Molina-Holgado E. Cannabinoid receptor agonists modulate oligodendrocyte differentiation by activating PI3K/Akt and the mammalian target of rapamycin mTOR. pathways. Br J Pharmacol. 2011;163:1520-1532.

129. Bernal-Chico A, Canedo M, Manterola A, et al. Blockade of monoacylglycerol lipase inhibits oligodendrocyte excitotoxicity and prevents demyelination in vivo. Glia. 2015;63:163-176.

130. Tomas-Roig J, Agamenony HY, Celarain N, Quintana E, Ramiro-Torrenta L, Havemann-Reinecke U. Dose-dependent effect of cannabinoid WIN-55,212-2 on myelin repair following a demyelinating insult. Sci Rep. 2020;10:590.

131. Garcia-Arencibia M, Molina-Holgado E, Molina-Holgado F. Effect of endocannabinoid signalling on cell fate: life, death, differentiation and proliferation of brain cells. Br J Pharmacol. 2019;176:1361-1369.

132. Hurd YL, Manzoni OJ, Pletnikov MV, Lee FS, Bhattacharyya S, Melis M. Cannabidiol and the develop-
Neurobiology of cannabinoid receptor signaling - Lutz

State of the art

opiing brain: insights into its long-lasting effects. J Neurosci. 2019;39:8250-8258.
133. Frau R, Miczan V, Tracis F, et al. Prenatal THC exposure produces a hyperdopaminergic phenotype rescued by pregnenolone. Nat Neurosci. 2019;22:1975-1985.
134. de Salas-Quiroga A, Garcia-Rincon D, Gomez-Dominguez D, et al. Long-term hippocampal interneuronalopathy drives sex-dimorphic spatial memory impairment induced by prenatal THC exposure. Neuropsychopharmacology. 2020;45:877-886.
135. Rodrigues RS, Lourenco DM, Paulo SL, et al. Cannabinoid actions on neural stem cells: implications for pathophysiology. Molecules. 2019;24:1350.
136. Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc Natl Acad Sci U S A. 2002;99:8394-8399.
137. Dincheva I, Drysdale AT, Hartley CA, et al. FAAH genetic variation enhances fronto-amygdala function in mouse and human. Nat Commun. 2015;6:3935.
138. Mayo LM, Araztian A, Linde J, et al. Protective effects of elevated anandamide on stress and fear-related behaviors: translational evidence from humans and mice. Mol Psychiatry. 2020;25:993-1005.
139. Lee GG, Fetcho RN, Jing D, et al. Individual differences in frontolimbic circuitry and anxiety emerge with adolescent changes in endocannabinoid signaling across species. Proc Natl Acad Sci U S A. 2016;113:4500-4505.
140. Habib AM, Okorokov AL, Hill MN, et al. Microdeletion in a FAAH pseudogene identified in a patient with high anandamide concentrations and pain insensitivity. Br J Anaesth. 2019;123:e249-e253.
141. Kong X, Miao Q, Lu X, et al. The association of endocannabinoid receptor genes (CBR1 and CBR2) polymorphisms with depression: a meta-analysis. Medicine (Baltimore). 2019;98:e17403.
142. Lazary J, Eszleri N, Juhas G, Bagdy G. A functional variant of CB2 receptor gene interacts with childhood trauma and FAAH gene on anxious and depressive phenotypes. J Affect Disord. 2019;237:716-722.
143. Hillard CJ. Circulating endocannabinoids: from whence do they come and where are they going? Neuropharmacology. 2018;43:155-172.
144. Muller-Vahl KR, Bindila L, Berti B, et al. Cerebrospinal fluid endocannabinoid levels in Gilles de la Tourette syndrome. Neuropharmacology. 2020;152:1323-1329.
145. Kranaster L, Hoyer C, Mindt S, et al. The novel seizure quality index for the antidepressant outcome prediction in electroconvulsive therapy: association with biomarkers in the cerebrospinal fluid. Eur Arch Psychiatry Clin Neurosci. 2019 Nov 23. Epub ahead of print. doi:10.1007/s00406-019-01086-x.
146. Sloan ME, Grant CW, Gwion JL, Ramchandani VA, Le Foll B. Endocannabinoid signaling in psychiatric disorders: a review of postin emission tomography studies. Acta Pharmacol Sin. 2019;40:342-350.
147. Russo EB. Cannabis therapeutics and the future of neurology. Front Integr Neurosci. 2018;12:51.
148. Sarris J, Sinclair J, Karamacosa D, Davidson M, Firth J. Medicinal cannabis for psychiatric disorders: a clinically-focused systematic review. BMC Psychiatry. 2020;20:24.
149. Black N, Stockings E, Campbell G, et al. Cannabinoids for the treatment of mental disorders and symptoms of mental disorders: a systematic review and meta-analysis. Lancet Psychiatry. 2019;6:951-1010.
150. Nomura DK, Morrison BE, Blankman JL, et al. Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. Science. 2011;334:809-813.
151. Gomes I, Grushko JS, Golebiowska E, et al. Novel endogenous peptide agonists of cannabinoid receptors. FASEB J. 2009;23:3020-3029.
152. Bauer M, Chicca A, Tamborrini M, et al. Identification and quantification of a new family of peptide endocannabinoids (Pepecans) showing negative allosteric modulation at CB1 receptors. J Biol Chem. 2012;287:36944-36967.
153. Wei F, Zhao L, Jing Y. Signaling molecules targeting cannabinoid receptors: hemopressin and related peptides. Neuropeptides. 2020;79:101998.
154. Christensen R, Kristensen PK, Bartels EM, et al. Peptide endocannabinoids (Pepcans) showing negative allosteric modulation at CB1 receptors. Biochem Pharmacol. 2019;2011;334:809-813.
155. Amato G, Khan NS, Maitra R. A patent update on cannabinoid receptor 1 antagonists (2015-2018). Expert Opin Ther Pat. 2019;29:261-269.
156. Khurana L, Mackie K, Piomelli D, Kendall DA. Modulation of CB1 cannabinoid receptor by allosteric ligands: pharmacology and therapeutic opportunities. Neuropharmacology. 2017;124:3-12.
157. Slivicki RA, Xu Z, Kulkami PM, et al. Positive allosteric modulation of cannabinoid receptor type 1 suppresses pathological pain without producing tolerance or dependence. Biol Psychiatry. 2018;84:722-733.
158. Slivicki RA, Iyer V, Mali SS, et al. Positive allosteric modulation of CB1 cannabinoid receptor signaling enhances morphine antinociception and attenuates morphine tolerance without enhancing morphine-induced dependence or reward. Front Mol Neurosci. 2020;13:54.
159. Tomaselli G, Vallee M. Stress and drug abuse-related disorders: the promising therapeutic value of neurosteroids focus on pregnenolone-progesterone-allopregnanolone pathway. Front Neuroendocrinol. 2019;55:100789.
160. Petruci V, Chicca A, Glasmacher S, et al. Pepecan-12 (RVD-hemopressin) is a CB2 receptor positive allosteric modulator constitutively secreted by adrenals and in liver upon tissue damage. Sci Rep. 2017;7:9560.
161. Iyer MR, Cinar R, Katz A, et al. Design, synthesis, and biological evaluation of novel, non-brain-penetrant, hybrid cannabinoid CB1R inverse agonist/inducible nitric oxide synthase (iNOS) inhibitors for the treatment of liver fibrosis. J Med Chem. 2017;60:1126-1141.
162. Xu B, Xiao J, Xu K, et al. VF-13, a chimeric peptide of VH-hemopressin(u) and neuropeptide VF, produces potent antinociception with reduced cannabinoid-related side effects. Neuropsychopharmacology. 2020;108178.
163. Morales P, Goya P, Jagerovic N. Emerging strategies targeting CB1, cannabinoid receptor: biased agonism and allosterism. Biochem Pharmacol. 2018;157:8-17.
164. Fazio D, Criscuolo E, Piccoli A, Barboni B, Fezza F, Maccarrone M. Advances in the discovery of fatty acid amide hydrolase inhibitors: what does the future hold? Expert Opin Drug Discov. 2020;1-14.
165. Gil-Ordonez A, Martin-Fontecha M, Ortega-Gutierrez S, Lopez-Rodriguez ML. Monoacylglycerol lipase (MAGL) as a promising therapeutic target. Biochem Pharmacol. 2018;2019;157:18-32.
166. Bedse G, Hill MN, Patel S. 2-Arachidonoyl glycerol modulation of anxiety and stress adaptation: from grass roots to novel therapeutics. Biol Psychiatry. 2020;8006-3232(20)30049-4. doi:https://doi.org/10.1016/j.biopsych.2020.01.015.
167. Ren SY, Wang ZZ, Zhang Y, Chen NH. Potential application of endocannabinoid system agents in neuropsychiatric and neurodegenerative diseases-focusing on FAAH/MAGL inhibitors. Acta Pharmacol Sin. 2020 Mar 18. Epub ahead of print. doi:10.1038/s41401-020-0385-7.
168. Vozella V, Ahmed F, Choobchian P, et al. Pharmacokinetics, pharmacodynamics and safety studies on URB937, a peripherally restricted fatty acid amide hydrolase inhibitor, in rats. J Pharm Pharmacol. 2019;71:1762-1773.
169. D’Souza DC, Cortes-Briones J, Creatura G, et al. Efficacy and safety of a fatty acid amide hydrolase inhibitor (PF-04457845) in the treatment of cannabis withdrawal and dependence in men: a double-blind, placebo-controlled, parallel group, phase 2a single-site randomised controlled trial. Lancet Psychiatry. 2019;6:35-45.
170. Mayo LM, Araztian A, Linde J, et al. Elevated anandamide, enhanced recall of fear extinction, and attenuated stress responses following inhibition of fatty acid amide hydrolase: a randomized, controlled experimental medicine trial. Biol Psychiatry. 2020;87:538-547.
171. van Esbroeck ACM, Janssen APA, Cognetta AB III, et al. Activity-based protein profiling reveals off-target proteins of the FAAH inhibitor BIA 10-2474. Science. 2017;356:1084-1087.
172. Mock ED, Mustafa M, Gunduz-Cinar O, et al. Discovery of a NAPE-PLD inhibitor that modulates emotional behavior in mice. Nat Chem Biol. 2020;16:667-675.
173. Nicolussi S, Gertsch J. Endocannabinoid transport revisited. Vitam Horm. 2015;98:441-485.
State of the art

Neurobiology of cannabinoid receptor signaling - Lutz

174. Chicca A, Nicolussi S, Bartholomaus R, et al. Chemical probes to potently and selectively inhibit endocannabinoid cellular reuptake. *Proc Natl Acad Sci U S A*. 2017;114:E5006-E5015.
175. Reynoso-Moreno I, Chicca A, Flores-Soto ME, Viveros-Paredes JM, Gertsch J. The endocannabinoid reuptake inhibitor WOBE437 is orally bioavailable and exerts indirect polypharmacological effects via different endocannabinoid receptors. *Front Mol Neurosci*. 2018;11:180.
176. Shen CJ, Zheng D, Li KX, et al. Cannabinoid CB1 receptors in the amygdalar cholecystokinin glutamatergic afferents to nucleus accumbens modulate depressive-like behavior. *Nat Med*. 2019;25:337-349.
177. Marcus DJ, Bedse G, Gaulden AD, et al. Endocannabinoid signaling collapse mediates stress-induced amygdalo-cortical strengthening. *Neuron*. 2020;105:1062-1076.
178. Hermann M, Stillhard P, Wildner H, et al. Binary recombinase systems for high-resolution conditional mutagenesis. *Nucleic Acids Res*. 2014;42:3894-3907.
179. Madisen L, Garner AR, Shimaoka D, et al. Transgenic mice for intersectional targeting of neural sensors and effectors with high specificity and performance. *Neuron*. 2015;85:942-958.
180. Karimova M, Baker O, Camgoz A, Naumann R, Buchholz F, Anastassiadis K. A single reporter mouse line for Vika, Flp, Dre, and Cre-recombination. *Sci Rep*. 2018;8:14453.
181. Yao S, Yuan P, Ouellette B, et al. RecV recombinase system for in vivo targeted optogenomic modifications of single cells or cell populations. *Nat Methods*. 2020;17:422-429.
182. Shalem O, Sanjana NE, Zhang F. High-throughput functional genomics using CRISPR-Cas9. *Nat Rev Genet*. 2015;16:299-311.
183. Navabpour S, Kwapis JL, Jarome TJ. A neuroscientist’s guide to transgenic mice and other genetic tools. *Neurosci Biobehav Rev*. 2020;108:732-748.
184. Arlotta P, Pasca SP. Cell diversity in the human cerebral cortex: from the embryo to brain organoids. *Curr Opin Neurobiol*. 2019;56:194-198.
185. Sidhaye J, Knoblich JA. Brain organoids: an ensemble of bioassays to investigate human neuro-development and disease. *Cell Death Differ*. 2020 Jun 1. Epub ahead of print. doi:10.1038/s41418-020-0566-4.
186. Fischer J, Heide M, Huttner WB. Genetic modification of brain organoids. *Front Cell Neurosci*. 2019;13:558.
187. Ao Z, Cai H, Havert DJ, et al. One-stop microfluidic assembly of human brain organoids to model prenatal cannabis exposure. *Anal Chem*. 2020;92:4630-4638.
188. Velasco S, Kedaigle AJ, Simmons SK, et al. Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. *Nature*. 2019;570:523-527.