Endocannabinoid signals in the control of emotion
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The appropriate control of emotional responses evoked by environmental stimuli is an important innate mechanism for ensuring quality of life and even for survival. Inappropriate responses and decreased abilities to adjust to changed environmental situations can lead to psychiatric disorders, such as posttraumatic stress disorders, phobia and depression. Endocannabinoid signalling has emerged as one of the regulatory systems of the brain supporting appropriate emotional responses. As various components of the endocannabinoid system have become therapeutic targets, understanding the endocannabinoids’ mechanism of action is an important research topic for a rationalized drug design and optimal therapeutic strategies.

Introduction
With the event of the synergistic use of numerous powerful techniques in neurosciences, mechanisms underlying emotion have been elucidated at cellular, synaptic and network levels. This can be illustrated with the detailed understanding of how the fear circuits are organized and which cellular mechanisms are involved in these circuits [1]. Several cortical and subcortical brain regions are engaged in these circuits, among which the amygdala, hippocampus and prefrontal cortex take central stages. In addition, several neurotransmitter systems and gene products are implicated in fear behaviours. Understanding the cellular mechanisms underlying emotion is also of clinical relevance, in order to define novel therapeutic targets for anxiety disorders, such as posttraumatic stress disorders (PTSD), general anxiety, phobia and depression [2].

A recently discovered signalling system modulating emotional responses to environmental impacts is constituted by the endocannabinoids (eCBs), which were identified as endogenous ligands of the cannabinoid receptors, which are G protein coupled receptors, initially characterized as receptors for the psychotropic Cannabis sativa constituent Δ⁹-tetrahydrocannabinol (THC) [3]. The most prominent eCBs are N-arachidonoyl ethanolamine (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG). They are engaged in a plethora of physiological functions in the nervous system, both in the adult (e.g. synaptic transmission, behaviours such as stress coping, anxiety, memory processing, neuroprotection, neuroinflammation, reward, feeding behaviour), but also play important roles during neural development (e.g. neuronal proliferation, neuronal migration, axonal growth). Furthermore, dysregulations of the eCB system have been implicated in various pathophysiological states (e.g. neurodegenerative disorders, epilepsy), thus emerging as a promising therapeutic target system [4]. As eCBs are amphipathic molecules and cannot be stored in vesicles, therefore, regulatory mechanisms of the biosynthesis and degradation pathways constitute central points in the appropriate execution of eCB signalling. Recent research has given detailed information on the enzymes involved in their synthesis from membrane lipids and their degradation [5*].

eCBs act via paracrine and autocrine mechanisms on membrane receptors. The most important receptor regarding synaptic plasticity and behaviour is the cannabinoid receptor type 1 (CB₁ receptor). However, promiscuity is present, in particular for anandamide, which is able to have several other targets. Most importantly, it activates the transient potential vanilloid receptor 1 (TRPV1, formerly called VR1), and peroxisome proliferator-activated receptor-α [6]. Furthermore, N-arachidonoyl dopamine (NADA) can act via CB₁ receptors and TRPV1 [7]. Other eCB-like compounds were also characterized, such as N-arachidonoyl glycinic acid, which signals via a G protein coupled receptor other than cannabinoid receptor [8], suggesting that this family of neuromodulatory lipids is still growing. An interaction between AEA and 2-AG was also reported. Mediated via TRPV1, AEA inhibits synthesis and physiological function of 2-AG in striatal neurons [9*]. Endogenous ligands that activate TRPV1 are called endovanilloids; the most important ones are AEA, NADA and 12-(S)-HPETE (12-hydroxyeicosatetraenoic acid, a 12-lipoxygenase product) [10]. Thus, these features illustrate the increasing complexity of the eCB system.

eCBs in the control of emotional responses
With the use of genetically modified mice lacking components of the eCB system and the pharmacological
In fear conditioning paradigms, CB1 receptors were and the serotonergic system [30]. Neurotransmitter systems, such as the opioid system [29], that TRPV1-deficient mice showed decreased anxiety signalling via TRPV1 is consistent with the observation mediated by TRPV1. This AEA-mediated anxiogenic effects, which is relevant to note that AEA mediates anxiogenic behaviours in mice with loss of CB1 receptors in specific neuronal population and brain regions [21], in order to understand the brain regions and neural circuits involved in anxiety. On the contrary, pharmacological or genetic blockade of the AEA-degrading enzyme fatty acid amide hydrolase (FAAH) can lead to anxiolytic effects, which are mediated via CB1 receptors [22–25]. When modulating FAAH activity, however, it must be considered that FAAH not only elevates AEA levels but also other bioactive lipids, such as N-acyl taurines [5*], and N-palmitoyl ethanolamine and N-oleoyl ethanolamine [23]. Secondly, it is relevant to note that AEA mediates anxiogenic behaviours, when AEA levels are above a certain threshold [26*,27], and that this effect appears to be mediated by TRPV1. This AEA-mediated anxiogenic signalling via TRPV1 is consistent with the observation that TRPV1-deficient mice showed decreased anxiety [28*]. A relevant aspect to be investigated regarding anxiolysis is the interaction of the eCB system with other neurotransmitter systems, such as the opioid system [29] and the serotonergic system [30].

In fear conditioning paradigms, CB1 receptors were shown to be involved in extinction of aversive memories, but not in the acquisition or consolidation of fear memory [16]. New insights into this CB1 receptor-dependent behaviour have been gained lately. (i) It was shown that CB1 receptors are involved in the non-associative component of fear memory extinction and that habituation-like processes are CB1 receptor-dependent [31*]. In this process, cortical glutamatergic neurons containing CB1 receptors are centrally involved, as shown by the impaired extinction in CB1 receptor-deficient mice lacking the receptor, specifically, in this neuronal population [32]. Furthermore, this recent work also showed that the eCB-dependent effects are not mediated via corticotropin-releasing hormone receptor type 1 and type 2, since pharmacological blockade of CB1 receptor in mice deficient in either of these receptors still leads to impaired extinction [32]. (ii) The initial hypothesis [33] that CB1 receptors are involved specifically in extinction of aversive memories but not of appetitive memories was further corroborated. In this work, the Barnes maze task was modified, in order to investigate both aversive and appetitive memory extinction [34]. (iii) Using CB1 receptor antagonists in the contextual fear conditioning paradigm, an essential function of CB1 receptors was attributed to the destabilization of previously consolidated memories following reactivation [35]. (iv) Apart from the amygdala, the dorsolateral periaqueductal grey, a midbrain structure, attracted more attention in the CB1 receptor-mediated reduction of fear after contextual fear conditioning [36] and also in the anxiolytic effects of eCBs [37].

The eCB system is also a stress-relieving system. After pharmacological and genetic blockade of CB1 receptors, serum corticosterone is increased as compared to control groups. Furthermore, after induction of stress (e.g. forced swim, social defeat, restraint stress), corticosterone responses are much stronger after CB1 receptor blockade than in control groups [14]. CB1 receptors were shown to be involved in the negative feedback of glucocorticoids in the activation of parvocellular neurons in the paraventricular nucleus of the hypothalamus [38]. Using conditional knockouts of CB1 receptors, it was indeed demonstrated that CB1 receptors in subcortical neurons are important and that CB1 receptor in cortical glutamatergic neurons and in GABAergic neurons are dispensable of the appropriate feedback regulation [39]. Of very high relevance is the concept that eCBs and CB1 receptors employ their functions in order to adjust the behaviour to repeated homotypic stress [13*]. In the context of mood disorders, such as depression, CB1 receptor agonists and FAAH inhibitors appear to act via similar mechanisms as other antidepressants. They are able to enhance serotonergic and noradrenergic transmission in the brain and promote neurogenesis in the hippocampus [15,40].

**eCB signalling in synaptic plasticity**

After observing that the eCB system is involved in these many aspects of emotional responses, the imminent question arises how to integrate and interpret these phenotypes in terms of synaptic processes and neuronal networks.

The past few years have provided detailed knowledge on the involvement of eCBs in the regulation of synaptic transmission [41]. On the basis of the widespread expression of CB1 receptors in the nervous system, it must be
concluded that a large portion of synapses contains eCBs as retrograde neurotransmitters, involved in the suppression of transmitter release from the presynaptic site. CB1 receptors are present not only on GABAergic and glutamatergic terminals, but increasing evidence supports the notion of their presence also on cholinergic, noradrenergic, dopaminergic and serotonergic synaptic terminals.

The mechanism of retrograde suppression induced by eCBs follows a general scheme. Postsynaptic activation by Ca\(^+\) influx (in vitro induced after postsynaptic depolarization steps or distinct stimulation protocols of afferents) and/or postsynaptic activation of phospholipase CB (induced by activation of Gq/11 coupled with metabotropic glutamate and acetylcholine receptors) lead to the synthesis of eCBs from membrane precursors, resulting in a release of eCBs, which pass to the presynaptic site in order to bind to and activate CB1 receptors. Thereupon, CB1 receptors inhibit the release of neurotransmitters. As CB1 receptors are present on presynaptic terminals of different neurotransmitter characteristics, it is conceivable to propose that such suppression can be observed in a multitude of synaptic connections of numerous neurotransmitter systems. However, the most detailed information is gained on the regulation of GABAergic and glutamatergic transmission. As concluded from electrophysiological experiments on slice preparations, the suppression of GABA release can last rather short (in the range of a minute or less; called DSI: depolarization-induced suppression of inhibition), but also for a long term (for 30 to 40 minutes; called LTDi, long-term depression of inhibitory current; or I-LTD, inhibitory long-term depression). Similarly, eCBs can not only modulate glutamatergic transmission for a short term (DSE, depolarization-induced suppression of excitation); but can also influence LTD of excitatory transmission. eCBs can also act in an autocrine mechanism, leading to a sustained hyperpolarization of the neuron, and thus, to long-lasting self-inhibition. This mechanism was observed on low-threshold-scaping cortical GABAergic interneurons. Finally, timing-dependent LTD in principal neurons of the visual cortex is also eCB-dependent.

Here, LTD on glutamatergic synapses is triggered by the pairing of presynaptic stimulations with postsynaptic depolarizations or action potentials. The time schedule of the pairing of the stimuli is important for LTD formation. This mechanism is very interesting with respect to behaviours, as the exact timing of neuronal activities induced by distinct behaviours may be highly relevant and may ultimately influence synaptic strength in a particular neuronal network. Very recently, the endovanilloid 12-(S)-HPETE was suggested to be a retrograde neurotransmitter, synthesized at the postsynaptic site of hippocampal GABAergic interneurons after activation of mGluR1/5, which stimulates phospholipase C and subsequently 12-lipoxygenase. TRPV1, located at the presynaptic site of glutamatergic inputs, is activated and glutamate release is enhanced. As a final result, TRPV1 activation can trigger LTD [42\(^+\)].

**It matters where and when**

Despite the detailed knowledge of the involvement of eCBs in the control of synaptic transmission, the link to behavioural phenotypes observed after interference with the activity of the eCB system has remained elusive. In particular, the very widespread occurrence of eCB-mediated suppression of neurotransmitter release contrasts the observation of rather specific phenotypes seen in mutant mice and/or pharmacologically treated rodents.

As discussed above, eCBs are able to suppress both GABAergic and glutamatergic transmission as a response of an activation of the postsynaptic site. This mechanism was deciphered on the basis of in vitro electrophysiological experiments. However, it is important to investigate which conditions are able to induce eCBs synthesis in vivo. In an acute model of seizure by systemic application of kainic acid, it was shown that CB1 receptors on glutamatergic neurons contain protective CB1 receptors and that eCBs are also increased after seizures [19\(^+\),43]. These results suggest that CB1 receptors on glutamatergic terminals convey an intrinsic break mechanism against excessive neuronal activity. As postsynaptic mGluR5 receptors and 2-AG synthesizing enzymes are located in the peri-synaptic machinery (PSM), the hypothesis was put forward that the induction of eCB synthesis after excessive glutamate release (e.g. after seizures) is mediated by an overflow of glutamate at the synapse, finally activating postsynaptic mGluR5, which in turn activates phospholipase CB, leading to the increased production of 2-AG and the subsequent retrograde suppression of glutamate release via presynaptic CB1 receptor activation [44]. This model can be applied to situations of excessive glutamate transmission, for example, during seizures. However, the question remains whether such a mechanism applies also to behaviours such as stress coping and alleviation of fear responses.

In fact, distinct behaviours are able to enhance the tissue content of eCBs, as shown for extinction of aversive memories, where both AEA and 2-AG are increased in the basolateral amygdala complex and in the hippocampus immediately after extinction trials [31\(^+\),45].

Pathological states were also investigated. Repeated (5-times) restrain stress was shown to lead to the decreased levels of AEA in the amygdala, but to increased levels of 2-AG in the amygdala and limbic forebrain [46]. CB1 receptor antagonist applied before the fifth restrain stress leads to increased c-fos protein expression (a correlate for neuronal activation) in prefrontal areas, nucleus accumbens and lateral septum. This study indicated that CB1 receptor activity is engaged in habituation processes after
homotypic stress situations [46]. In an animal model of depression (i.e. chronic unpredictable stress for 21 days), AEA, but not 2-AG, was downregulated in many regions, including prefrontal cortex, hippocampus, amygdala and ventral striatum, while CB1 receptor density was increased in the prefrontal cortex, but decreased in hippocampus and ventral striatum [47]. At this point, there is a lack of data that would allow to specifically allocating the altered eCB signalling to a particular neuronal subpopulation, thus, preventing clear conclusions on the underlying mechanisms.

Nevertheless, it is conceivable to propose that eCBs can convey a short-term and/or long-term break mechanism in network activities, aiming at, for example, sharpening and/or synchronizing network activities. To this end, the use of conditional CB1 receptor mutant mouse lines in the analysis of fear extinction/habituation processes [32] in conjunction with in vivo and in vitro electrophysiological investigations may give interesting new insights into network activities and the possible relation with behaviour.

An attempt to relate eCB-mediated synaptic processes with behaviour has been reached by computer simulation, suggesting that DSI in the prefrontal cortex is beneficial for working memory, while an exogenous CB1 agonist is detrimental [48]. Another study, recording hippocampal neural ensembles in the delayed-nonmatch-to-sample (DNMS) test, revealed a modulation of the encoding of short-term memory by eCBs and CB1 receptors [49].

In future experiments, it will be important to investigate the involvement of CB1 receptors and TRPV1 on particular neuronal subpopulations and brain regions, in order to be able to assign specific functions to a particular behaviour. It is speculated that for a distinct behaviour, a distinct neuronal network is engaged, where on-demand synthesized eCBs, acting via CB1 receptors and TRPV1, are able to modulate network activities, for example, by synchronizing them.

An emotional link to the human eCB system

As the eCB system is involved in the modulation of emotional responses, the question was raised whether or not dysregulations of the eCB system in humans lead to pathological states. To this end, in a first step towards addressing this issue, two recent studies investigated genetic variations of the human CB1 receptor gene (CNR1 gene) in emotion processing. Healthy human subjects were exposed either to happy or to disgusting facial expressions, and striatal responses were monitored by fMRI [50]. Four single nucleotide polymorphisms (SNPs) in the CNR1 gene locus correlated with increased striatal response to happy, but not to disgusting faces. SNP rs1049353 in the AA alleles gave higher striatal responses than the GG alleles. This polymorphism is located in the protein encoding sequence of the CB1 receptor, but does not lead to an amino acid change. However, this altered nucleotide sequence might have an impact, for example, on mRNA stability or translational efficiency. Thus, social reward responsiveness can be linked with a particular genotype, putting forward the notion that this genotype has implications for conditions involving hyporesponsiveness to emotional and social stimuli. Another study investigated, among other SNPs, also rs1049353 [51], further showing that the AA alleles relate with increased responsiveness in the amygdala, putamen and pallium. Remarkably, human subjects with major depression containing the GG alleles (which is characterized with a hyporesponsiveness) showed an increased risk of ineffective antidepressant treatment. This is an interesting result, as it suggests that high responsiveness to positive social cues is associated with a favourable outcome in the treatment of major depression, and rs1049353 AA might be a predictive marker. SNP rs1049353 was also suggested to be linked to PTSD [52]. However, this study must be replicated with another cohort.

Insights into the human eCB system can also be gained by CB1 receptor agonist treatments. Similar to the results in rodent models of anxiety, ingestion of THC in healthy subjects modulates emotional responses, where high dose of acute THC treatment is anxiogenic, as monitored by behavioural scores [53], while an acute low dose of THC is anxiolytic, as monitored by a decreased amygdala reactivity in fMRI after presenting angry and fearful faces [54].

The phase III clinical trials of the CB1 receptor antagonist rimonabant (Acomplia®; Sanofi-Aventis) in the treatment of obesity and associated metabolic dysregulations revealed that a chronic CB1 receptor antagonism leads to a higher incidence of psychiatric side effects as compared to placebo groups. The recently published clinical trial ‘The Strategy to Reduce Atherosclerosis Development Involving Administration of Rimona-bant—The Intravascular Ultrasound Study’ (STRADI-VARIUS) [55], which did not exclude patients with psychiatric disorders, thus, reflecting the real situation of the general practitioner, depression, anxiety and depressed mood altogether increased from 2.1% (placebo) to 7.6% (rimonabant) as an adverse event leading to discontinuation of the treatment. Similar side effects were observed in clinical trials with the CB1 receptor antagonist tarambant (Merck) [56]. Positron emission tomography (PET) imaging using the selective CB1 receptor tracer [18F]MK-9470 confirmed CB1 receptor occupancy levels of about 40–60% at doses of tarambant that were therapeutically effective [57]. Thus, beneficial metabolic and weight reduction effects were observed at doses that do block about 40–60% of CB1 receptors, but, on the contrary, unfortunately, such doses induce psychiatric side effects.
eCBs in shaping emotional networks
All constituents of the eCB system are highly abundant in the developing nervous system, from earliest stages of proliferation of neural progenitors during embryogenesis to the final events of the fine-tuning of the wiring of the neural networks during puberty and adolescence. Although identified several years ago, it was not until very recently when eCBs acting via CB1 receptors were recognized as signalling molecules controlling fundamental processes of neural development, such as neural progenitor proliferation, migration of cortical neurons, axonal fasciculation, and synapse positioning [58,59,60]. Furthermore, several investigations also showed roles of eCBs in the proliferation and differentiation of adult neural progenitors in hippocampus and subventricular zone [40,61].

These new insights will eventually help us understanding the deleterious effects of THC on the developing nervous system. It is now well established that THC consumption during crucial phases of neural development (ranging from gestation to puberty and adolescence) has long-lasting consequences on cognitive and emotional behaviours, both in rodents and humans [62,63]. Furthermore, there is evidence that smoking marijuana increases the prevalence of schizophrenia-like psychoses, in particular when smoking occurred during puberty and adolescence [64].

Altogether, the eCB system is employed in several crucial steps of neural development. Disturbances by either CB1 receptor agonists or CB1 receptor antagonists appear to have long-lasting and even irreversible consequences on behaviour, presumably by influencing the establishment of correct neural networks. Future research will aim at giving cellular and molecular annotations to these changes.

Conclusions
The eCB system offers numerous opportunities of pharmacological intervention in the context of mood disorders, such as anxiety disorders, PTSD, phobia and depression. FAAH inhibition appears to be the preferred strategy, as these drugs are basically without psychotropic side effects. However, as shown in animal model system, long-term FAAH inhibition leads to increased levels not only of AEA, but also of other lipids, which might cause side effects. Both CB1 receptor agonist treatment and FAAH inhibition may be applied in exposure therapies against phobia and PTSD, as in these instances the pharmacological treatments are very limited in duration. Inhibition of 2-AG degradation will be an alternative approach. In fact, highly potent compounds applicable in systemic treatments are emerging [65].

In order to employ rational strategies of treatments involving eCB modulations, the understanding of dysregulations of the eCB system in human mood disorders needs much more attention and new methodological approaches.

Conflict of interest
There is no conflict of interest.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:
- of special interest
- of outstanding interest

Exellent review discussing the involvement of the eCB system in the coping to stress; with relevant implications to mood disorders.


First report on the presence of functional CB1 receptor on forebrain glutamatergic neurons, showing the protective mechanism against excessive neuronal activity.


First relevant work in understanding eCB signalling via CB1 receptors and TRPV1 with opposing effects on anxiety.


First genetic evidence on TRPV1 function in anxiety, opposing CB1 receptor function.


Relevant work on the view that eCBs mediate habituation-like processes in fear extinction.


First report on a retrograde signalling mediated by and endovanilloid and TRPV1.


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64. Pavo´ n FJ, Serrano AM, Selley DE, Parsons LH: Extensive work on the function of CB1 receptor during neural development, showing importance of CB1 receptors on glutamatergic neurons.


68. Very good overview on the impact of THC during puberty on emotional responses at later age.

