Chapter 6

Neurochemical Communication: The Case of Endocannabinoids

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Additional information is available at the end of the chapter

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1. Introduction

The brain is equipped with a magnificent diversity of molecules that allow neurons to communicate with each other. Some of these molecules have been known to function as neurotransmitters for several decades such as GABA and glutamate while for others their involvement in brain signaling has been demonstrated more recently. Cannabinoids fall into the latter group. Even though the effects of cannabinoids as active ingredients in marijuana on human psyche and behavior have been experienced by humans for centuries or possibly millennia, their existence and production in the brain was described only some thirty years ago. Even more recently, their functional role in neural circuits of the brain has been discerned. This review focuses on these endogenously produced signaling molecules, endogenous cannabinoids or endocannabinoids (eCBs). Their functional role in the nervous system and interaction with other neurotransmitter systems will be described. One hallmark feature of endocannabinoid signaling is their ability to act as retrograde messengers in neural circuits. Two examples, one from the hippocampus and one from the main olfactory bulb, illustrate in detail this intercellular communication pathway.

Several features underscore the importance to understand the endocannabinoid system. Increasing evidence demonstrates the relevance of endocannabinoids in normal behaviors, including pain reception [1] and feeding [2, 3]. The therapeutic potential of cannabinoids has received increasing attention over the past few years [4]. endocannabinoids play a role in neuroprotection against acute excitotoxicity [5] and functional recovery after brain injury [6]. Endocannabinoids regulate human airway function and provide a means to treat respiratory pathologies [1]. Cannabinoids are in widespread use recreationally as psychoactive drugs and interact with other drugs of abuse. This fact emphasizes even more the need to understand the endocannabinoid system and the neurobiological substrate of their mood-altering capacity [7,

8]. Furthermore, the endocannabinoid system is crucially involved in processes of learning and memory, e.g., in the extinction of aversive memories [9].

2. The endocannabinoid system

Endocannabinoids are small lipids that regulate various aspects of brain function such as learning and memory including synaptic transmission and different forms of short-and long-term plasticity [10]. They also influence growth and development such as synapse formation and neurogenesis. Other biological functions modulated by endocannabinoids include eating and anxiety. Principally, two endocannabinoids, N-arachidonoylethanol-amide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are the natural agonists/ligands of the most widely expressed cannabinoid receptor in the brain, CB1R [11] (Figure 1).

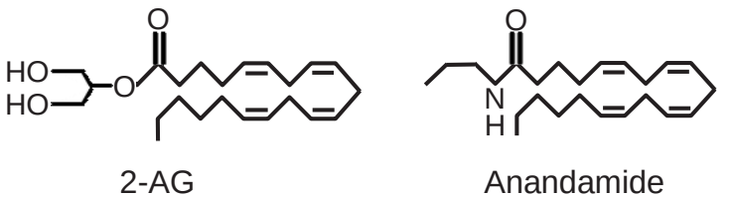

Figure 1. Two endocannabinoids, 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanol-amide (anandamide, AEA), are the natural agonists/ligands of cannabinoid receptors, CB1Rs, in the brain.

Endocannabinoids, as fatty-acid derived endogenous ligands, together with their G-protein coupled cannabinoid receptors form the endocannabinoid system. This system also includes associated biochemical machinery with endocannabinoid precursors, synthetic and degradative enzymes for these lipidic neurotransmitters, and transporters [12-15]. Two different cannabinoid receptors have been cloned, CB1 and CB2 receptors. They share 44% amino acid sequence homology [16, 17]. The expression pattern of the two cannabinoid receptors in various body parts is distinctly different. In the brain, CB1R is the most abundant G-protein coupled receptor [18]. CB2R is primarily expressed in immune cells and peripheral tissues [17]. Some level of CB2R expression has also been detected in the brainstem, cortex and cerebellar neurons and microglia [19, 20].

Cannabinoid receptors are found at high levels in the brain [21, 22], specifically at presynaptic nerve terminals [23, 24]. They can be activated by cannabis-derived drugs. Δ9-Tetrahydrocannabinol, THC, is the bioactive ingredient of the drugs marijuana and hashish [25] and can artificially activate cannabinoid receptors as exogenous cannabinoids. Cannabinoid receptors exist in all normal brains [18, 21, 22] where they subserve many essential brain functions when activated by their natural ligands. Cannabinoid receptors in the nervous system are predominantly $G_{i/o}$-protein-coupled type 1 cannabinoid receptors (CB1 receptors, CB1Rs). Their ligands, endocannabinoids are synthesized from membrane lipids [26]. Endocannabinoids can diffuse through membranes and are thus able to activate receptors in the same manner as

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exogenously applied cannabinoids such as cannabis. Anandamide and 2-AG were discovered in the early 1990s [27-29, reviewed in 30] while their functional role in neuronal communication remained obscure for years. Since their discovery, the role of endocannabinoids as retrograde messengers that suppress both excitatory and inhibitory transmission has been well-established. Endocannabinoids mediate retrograde signals in the hippocampus [31-35], cerebellum [36-38], neocortex [39, 40], amygdala [41, 42], and olfactory bulb [43]. Termination of endocannabinoidsignalling is accomplished by reuptake into both neurons and glia. Subsequently, anandamide and 2-AG are hydrolyzed intracellularly by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively [44].

3. Unusual and novel neurotransmitters

Endocannabinoids are different from conventional neurotransmitters because they are lipids that are not stored but rather are rapidly synthesized on demand at the site of need from components of the cell membrane. Upon cellular activation, they are released from places all over the cell. They are arachidonic acid-containing messengers generated by phospholipase action [45]. Stimuli that trigger release of endocannabinoids include rise of intracellular calcium levels inside the neuron or activation of certain G-protein-coupled receptors such as metabotropic glutamate receptors (mGluR5). Subsequent to their non-synaptic, non-vesicular release, endocannabinoids bind to cannabinoid receptors on nearby neurons such as presynaptic interneurons where they regulate presynaptic neurotransmitter release, e.g., through closure of specific ion channels.

Endocannabinoids are members of a loose family of unusual and novel neurotransmitters. Similar to endocannabinoids, other novel neurotransmitters such as nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H2S) do not adhere to the classic definition of neurotransmitters and challenge the notion of what constitutes a neurotransmitter [46, 47]. These synaptic molecules have changed markedly the definition of a neurotransmitter. They satisfy key neurotransmitter criteria but differ radically from classical transmitters. For example, endocannabinoids, nitric oxide and carbon monoxide are neither stored in synaptic vesicles nor released by exocytosis. Nitric oxide does not act via traditional receptors on postsynaptic membranes.

Like endocannabinoids, nitric oxide can serve as an intercellular messenger in the brain [48]. It acts as a retrograde factor at synapses and presynaptically regulates both glutamatergic and GABAergic synapses to alter release-probability in synaptic plasticity. Nitric oxide influences the synaptic machinery involved in transmitter release and, in a coordinated fashion, also the vesicular recycling mechanisms. Nitric oxide has a role in the coordination of local pre-and post-synaptic function during plasticity at individual synapses. It is involved in experience-dependent plasticity in the cerebral cortex. Likewise, cannabinoids mediate a variety of forms of short-and long-term synaptic plasticity that have been reviewed in detail elsewhere [49-51].

4. Depolarization-induced suppression of inhibition

The relevance of the endocannabinoid system for neural signaling and brain function in general has been explored only recently [13]. Endocannabinoids mediate a new type of neuronal communication, called DSI, **D**epolarization-induced **S**uppression of **I**nhibition (Fig. 2) (reviewed in [10, 12, 30]. A short rise in intracellular calcium concentration in a principal neuron, e.g., a pyramidal cell of the hippocampus, results in a transient decline of incoming inhibitory signals in the form of GABA arriving from other neurons. This observation led to the hypothesis that during DSI, some unknown messenger must travel from the postsynaptic cell to the presynaptic GABA-releasing one and somehow turns off neurotransmitter release. Conventional chemical synaptic signaling between two neurons involves activation of a presynaptic neuron resulting in transmitter release and subsequent activation of the postsynaptic neuron, e.g., a GABAergic inhibitory interneuron makes synaptic contacts with a glutamatergic pyramidal cell in the hippocampus. When the interneuron is activated it releases the inhibitory neurotransmitter GABA and inhibits the pyramidal cell. In contrast, during DSI, when a pyramidal cell is activated, e.g., through direct current injection, the inhibitory input onto that pyramidal cell is reduced. As a major breakthrough in our understanding of endocannabinoid signaling, endocannabinoids were found to act as retrograde signaling molecules that mediate communication between postsynaptic pyramidal cells and presynaptic inhibitory interneurons and evoke the reduction in GABA release. Since endocannabinoids are lipids, they do not diffuse over great distances in the watery extracellular environment of the brain. Rather, DSI acts as a short-lived local effect that enables individual neurons to disconnect briefly from their neighbors and encode information [12].

The announcement of this breakthrough has been given the Latin term 'Dies mirabilis' (wonderful day) by Alger [10]. In March of 2001 four independent labs described in three different journals their studies culminating in the conclusion that endocannabinoids function mainly as retrograde messengers. Elphick and Egertova [52] analyzed prior pharmacological and anatomical studies of the actions of cannabinoid receptor agonists and combined this with their knowledge of the localization of cannabinoid receptors and degradative enzymes for anandamide, fatty acid amide hydrolase (FAAH) to reason that endocannabinoids act as retrograde messengers. Pivotal work by Wilson and Nicoll [34] and Ohno-Shosaku et al. [32] established that DSI was mimicked by activating cannabinoid receptors whereas blockade of cannabinoid receptors prevented DSI. A corresponding phenomenon, DSE, **D**epolarization-induced **S**upression of **E**xcitation, mediated by retrograde action of endocannabinoids, was identified by Kreitzer and Regehr [36] at cerebellar excitatory synapses. DSI and DSE are based on a presynaptic effect as shown by an increase in calcium in the postsynaptic cells and corresponding changes in paired pulse ratio of neurotransmitter release.

G-protein coupled receptors (GPCRs) are involved in mediating the transduction of extracellular stimuli, such as neurotransmitters, into intracellular signaling cascades. Activation of specific G-protein coupled receptors triggers the release of endocananbinoids for many minutes, e.g., dopamine [53], metabotropic glutamate [33, 37, 54] or muscarinic M1/M3 acetylcholine receptors [55, 56]. Even though endocannabinoids are typically released in a

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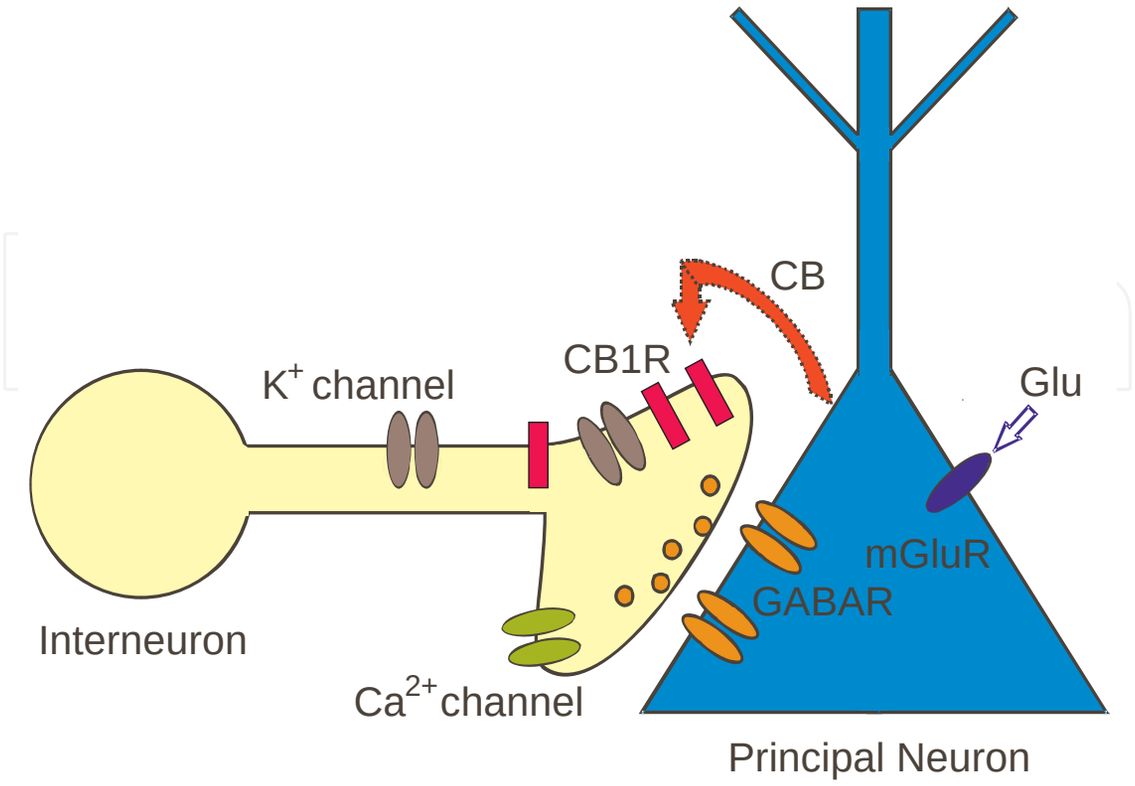

Figure 2. Depolarization-induced **S**uppression of **I**nhibition (DSI) is a model for retrograde signaling in the brain and allows assaying real time release of endocannabinoids from principal neurons as a brief cessation of GABA ouput. Activation of metabotropic glutamate receptors (mGluRs) on principal neurons or depolarization of postsynaptic principal cells evokes synthesis and release of cannabinoids (CBs). Cannabinoids bind to presynaptic cannabinoid receptors (CB1R) on GABAergic interneurons and transiently reduce GABA release from synaptic terminals. As a consequence, $GABA_A$ receptor-mediated synaptic currents and GABAergic inhibition are temporarily suppressed in postsynaptic principal neurons.

calcium-dependent manner [57, 58], in the mGluR-and mAChR-dependent pathways, no clear rise in intracellular calcium $[Ca^{2+}]_i$ [37, 55] is necessary. The release of endocananbinoids can be initiated even in the presence of high intracellular concentrations of calcium chelators, although endocannabinoids may nevertheless be sensitive to the ambient intracellular calcium concentration [59]. Studies by the Alger lab and others indicate that G-protein coupled receptor activation of postsynaptic cells leads to enhancement of DSI, e.g., glutamate acting on group I metabotropic glutamate receptors (mGluRs) directly generates endocannabinoids and enhances DSI ([37, 33]. It is now established that activation of many G-protein coupled receptors is linked to the use of endocannabinoids to deliver or fine-tune their messages to target cells [11].

The discovery of DSI has been a major advance in our understanding of the endocannabinoid system for brain function [60]. DSI is a type of short-term synaptic plasticity originally observed in the cerebellum and hippocampus [12, 14]. Endocannabinoids are retrograde signaling molecules that are released from depolarized principal neurons and travel to presynaptic inhibitory interneurons to reduce GABA release. DSI is a novel, regulatory process that manifests itself as a transient suppression of synaptic $GABA_A$ responses mediated by retro-

grade signaling of endocannabinoids from principal neurons (Fig. 3). Through the retrograde signaling process neurons alter the strength of synapses made onto them and thereby control their own synaptic excitability in an activity-dependent manner, which is functionally important in information processing by neuronal networks [14]. In the cerebellum, a retrograde signaling process that is similar to DSI reduces synaptic excitation by suppressing presynaptic glutamate release and is called DSE [61], see above.

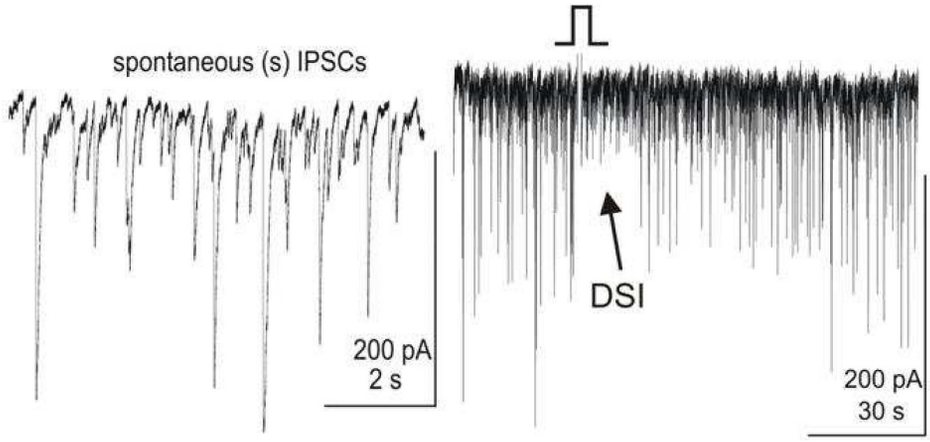

Figure 3. Right panel: Hippocampal pyramidal cells show spontaneous inhibitory postsynaptic currents (IPSCs). Left panel: In response to a 1-s voltage pulse the pyramidal cell reveals DSI, a transient reduction in IPSC activity as a result of endocannabinoids acting on CB1R on presynaptic GABAergic interneurons.

5. Hippocampal depolarization-induced suppression of inhibition

Endocannabinoids are lipids and, unlike classic neurotransmitters, are not stored but rather rapidly synthesized from components of the cell membrane. They are synthesized in, and released from, postsynaptic somatodendritic domains that are readily accessible to whole-cell patch electrodes. The effects of these lipid signals are detected electrophysiologically as CB1R-dependent alterations in conventional synaptic transmission, which, therefore, provide a sensitive means of bioassay in gendocannabinoid levels and actions. Endocannabinoid release can be triggered through Ca^{2+}-dependent or relatively Ca^{2+}-independent pathways, with different down-stream effects. As discussed above, endocannabinoids are released non-synaptically, non-vesicular from places all over cells when levels of calcium rise inside the neuron or when certain G-protein-coupled receptors are activated. After cellular release, endocannabinoids travel to cannabinoid receptors on nearby neurons and evoke a reversible, short-term depression of synaptic transmission, DSI. In activated hippocampal pyramidal cells, DSI leads to a transient reductionof GABA release from presynaptic terminals of inhibitory interneurons. Direct insights into the actions of endocannabinoids have been based primarily on pharmacological experiments. The hydrophobicity of endocannabinoids severely limits their penetration into brain tissue, and endocannabinoids are rapidly degraded by abundant endogenous lipases. These intrinsic properties of endocannabinoids make it difficult

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to directly study physiological effects of endocannabinoids. The development of a highly water-soluble caged anandamide that is inert to lipases circumvents these problems [62]. When perfused into hippocampal slice preparations, the caged anandamide serves as a latent endocannabinoid pool, and focal photolysis rapidly liberates highly hydrophobic anandamide *in situ* to activate CB1R. Photolysis is an alternative experimental approach to chemically stimulate synapses, cells, or circuits by directly applying neurotransmitter or neuromodulators. Often pharmacological approaches yield little control of the stimulation in terms of timing, space and specificity. However, photo-uncaging of caged neurotransmitters has made the pharmacological approach more sophisticated. Photo-uncaging uses localized, patterned light and yields higher spatial and temporal resolution. One application of photostimulation, the flash photolysis technique, can be used to determine signaling kinetics of the endocannabinoid system [60, 62]. The endocannabinoid system can be used as a tool for bioassaying the temporal dynamics or kinetics of lipid signaling. Combining whole-cell voltage patch-clamp recording, intracellular calcium measurements, and photorelease of caged glutamate and a novel, caged cannabinoid, anandamide (AEA) allows determining endocannabinoid signaling kinetics. Flash photolysis of caged compounds (photolysis using so-called molecular optical probes or photoprobes) is an important tool in this endeavor. Caged compounds are inert, biologically inactive (e.g., a caged cannabinoid or caged glutamate) until a flash of laser light breaks open the molecular cage, releases the caged molecule and generates a biologically active effector molecule in situ [63]. Chemically, the caged compound is a modified signal molecule. The modification of the molecule prevents its bioactivity until light absorption results in a photochemical change of the signal molecule such that its bioactivity is restored.

The lipid signaling pathway comprises several temporal components that can be determined to quantify the time that it takes from the DSI-inducing stimulus to the onset of DSI. These components contribute to the latency to onset of DSI (start of DSI-inducing stimulus to initial suppression of IPSCs). Among them is the rise of calcium to initiate endocannabinoid synthesis (t-Ca). The rise in intracellular calcium leads to endocannabinoid synthesis and release, followed by travel of these molecules to cannabinoid receptors on presynaptic interneurons, t-EC. The next step is the activation of CB1R and downstream effects, t-CB1R (t-DSI = t-Ca + t-EC + t-CB1R). Experiments carried out using the above-mentioned technological advances allowed determining the time for synthesis and release of eCB from the postsynaptic neuron, which was estimated to be around 150 ms at room temperature, comparable with the timescale of metabotropic signaling and at least an order of magnitude faster than previously thought. A major portion of the DSI onset time, t-DSI, reflects activation of presynaptic CB1Rs and downstream consequences. The data suggest that, far from simply serving long-term neuromodulatory functions, endocannabinoid signaling is sufficiently fast to exert moment-to-moment control of synaptic transmission. The DSI onset latency after a voltage step, t-DSI, is 350 to 400 ms. t-CB1R, the direct activation of CB1R by photoreleasing anandamide which results in suppression of sIPSCs, takes ~180ms (Fig. 4). A transient rise in intracellular calcium sufficient to obtain minimal DSI, t-Ca, is evoked by a 50-ms voltage step and takes ~60 ms. The time needed for endocannabinoid synthesis and release to occur, t-EC, is about 150 ms.

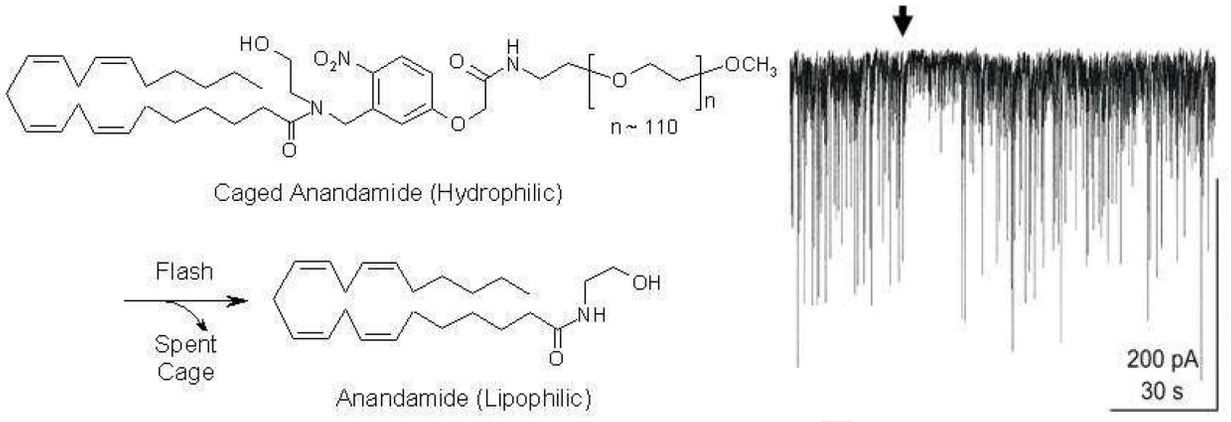

Figure 4. Left panel: Photolysis of caged anandamide yields bioactive anandamide. Right panel: Photorelease of anandamide suppresses sIPSCs after a delay of ~180 ms. Modified from [62] with permission of the Society for Neuroscience.

Voltage-activated DSI works through a rise in intracellular calcium concentration. However, release of endocannabinoids can be triggered even in the presence of high intracellular concentrations of calcium chelators, although they may nevertheless be sensitive to ambient intracellular calcium [59]. To test if these two pathways function on the same time scale, the dynamic components of the mGluR-induced endocannabionoid response on sIPSC frequency in pyramidal cells are compared (Fig. 5) [62]. The mean onset latency, duration and magnitude of the IPSC suppression evoked by uncaged glutamate are similar to that caused by uncaged AEA (Figs. 4, 5). No reduction in sIPSCs occurred for 221 ms (determined by extrapolation of the exponential fit to the control sIPSC level). The time-to-onset of IPSC suppression evoked by the mGluR-induced endocannabinoid process (time to mGluR-dependent suppression of inhibition, $t_{mGluRSI}$) is described by: t-mGluR-SI = 221 ms = t-eCB(mGluR) + t-CB1R, where t-eCB(mGluR) is the time for activation of the mGluR-dependent endocannabinoid synthesis and release, and t-CB1R is ~180 ms (see above). This leaves t-eCB(mGluR) to be < 50 ms, which is even faster than endocannabinoid synthesis and released evoked by a voltage step.

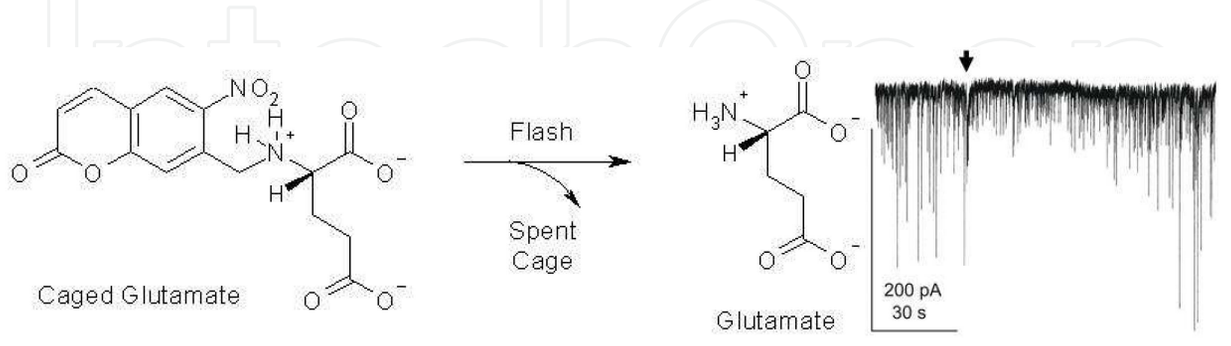

Figure 5. Dynamics of mGluR-dependent endocannabinoid suppression of sIPSCs in cultured hippocampal slices. Left panel: Photorelease of glutamate. Right panel: Recording from a pyramidal cell illustrates the transient reduction in spontaneous (s) IPSC frequency of CA1 pyramidal cells after flash photorelease of caged glutamate (photolysis induced suppression of inhibition, PSI). Arrow indicates laser flash. From [62] with permission of the Society for Neuroscience.

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Anandamide can be released from its caged form by a UV-laser flash and rapidly activates presynaptic CB1Rs to suppress the release of GABA [62]. A specific CB1R antagonist, AM 251, blocks the suppression of spontaneous IPSCs. This establishes that uncagedanandamide can be used as a CB1R agonist to study activation of CB1R in the brain. Similarly, uncaged glutamate acts at mGluRs on hippocampal pyramidal cells to evoke cannabinoid release and subsequent suppression of presynaptic GABA release [62]. The data provide the first detailed attempt to determine the minimal time required for activation of an intercellular neuronal lipid messenger system. This signaling system requires a major portion of DSI onset time, t-DSI, for activation of presynaptic CB1R and downstream consequences. Endocannabinoids, and by extension similar lipid messengers, can be mobilized and evoke responses as quickly as conventional metabotropic, G-protein receptor-coupled neurotransmitters. The speed with which neuromodulators such as endocannabinoids act places critical constraints on the physiological roles they can play. Endocannabinoids and other lipids function in brain signaling not simply in homeostatic processes or slowly-activating forms of regulation, but rather lipids can affect neuronal excitability in moment-to-moment information processing.

6. Depolarization-induced suppression of inhibition in glomerular circuits of the olfactory bulb

The olfactory bulb is the first relay station in the CNS for processing of sensory information that comes from olfactory receptor cells in the nasal epithelium. Cannabinoid receptors are expressed at high levels in the olfactory bulb, specifically in the input region, the glomerular layer [21, 64-66]. Neurons in the glomerular layer are immunoreactive for enzymes that synthesize endocannabinoids [67-69]. Our understanding of the physiological role of endocannabinoids and cannabinoid receptors for neural signaling in the olfactory system is just emerging. Recent electrophysiological evidence has established that the endocannabinoid system plays a functional role in regulating neuronal activity and signaling in olfactory bulb glomeruli [43].

Neurons in the glomerular fall into three subpopulations: periglomerular (PG), external tufted (eTC), and short-axon (SA) cells. Periglomerular cells are neurochemically and functionally heterogeneous [70-72]. Periglomerular cells are GABAergic, short-axon cells express both GABA and dopamine, and external tufted cells are glutamatergic [72, 73]. Periglomerular cells receive input from the olfactory nerve or dendrodendritic glutamatergic input from external tufted or mitral cells, e.g., as spontaneous bursts of EPSCs [70, 73-74]. Periglomerular cells presynaptically inhibit olfactory receptor neurons through GABAergic transmission [76, 77]. External tufted cells receive spontaneous bursts of inhibitory postsynaptic currents (sIPSCs) from periglomerular cells at inhibitory GABAergic synapses as well as spontaneous glutamatergic EPSCs [74; 78]. In the glomerular layer, external tufted cells can be a potential source of endocannabinoids.

Cannabinoid receptors directly regulate membrane properties of periglomerular cells as shown by the effects of CB1R antagonist AM251 and agonist WIN in the presence of ionotropic

glutamate and $GABA_A$ receptor blockers (synaptic blockers: CNQX, APV, gabazine) [43]. This indicates that the actions of cannabinoids on periglomerular cells are mediated through CB1R expressed by periglomerular cells. AM251 directly activates periglomerular cells and enhances their GABA release. Periglomerular cells are synaptically connected to external tufted cells. Therefore, any CB1R-mediated regulation of activity of periglomerular cells could affect GABA release and synaptic transmission to external tufted cells. CB1R is also expressed in external tufted cells and may participate in modulating external tufted cell activity.

In external tufted cells, neither AM251 nor WIN influences firing frequency or membrane potential [43]. However, in the presence of synaptic blockers cannabinoid drugs have a modest effect on external tufted cells. In this condition, AM251 slightly increases the firing rate of external tufted cells without membrane depolarization. In synaptic blockers, WIN slightly decreases firing of external tufted cells without a clear change in membrane potential. The effects of AM251 and WIN in the presence of synaptic blockers, i.e., during pharmacological isolation of external tufted cells, indicate that CB1R mediates a direct effect on external tufted cells. The direct excitatory effect of a CB1R antagonist on external tufted cells is opposed by increased GABAergic synaptic input from periglomerular cells onto external tufted cells, i.e., the enhanced GABA release from periglomerular cells triggered by a CB1R antagonist may dominate and mask the CB1R antagonist-evoked direct excitation of external tufted cells.

The CB1R effects on periglomerular and external tufted cell prompt the questions if DSI is present in the glomerular layer of the olfactory bulb. In external tufted cells, DSI can be induced with a 5-sec depolarizing voltage step from a holding potential of -60 mV to 0 mV (Fig. 5). In external tufted cells DSI is visible as a decrease in the amplitude and frequency of sIPSCs. The response to a single depolarizing step is a suppression of sIPSC area by ~40 % of control which then gradually recoveres. External tufted cells exhibit a distinct intrinsic bursting pattern [74]. In order to mimic spontaneous rhythmic bursting of an external tufted cell a train of depolarizing steps can be applied to the cell. This experimental paradigm allows determining a possible functional role of DSI in glomeruli. A train of depolarizing steps results in a transient 60% reduction in sIPSC area (20 steps, 0.75 Hz) (Fig. 4B, F). DSI can be completely eliminated in the presence of AM251, indicating that DSI is mediated by CB1R (Fig. 5C, F). The bursting frequency of external tufted cells ranges from 0.5 to 6.5 Hz with a mean frequency of 2.7 bursts/sec [74]. Depolarizing voltage pulses at 2 Hz (20 steps, pulse duration: 250 ms) evoke DSI as a reduction of sIPSCs in external tufted cells, similar to the results obtained with voltage steps at 0.75 Hz to 0 mV. In external tufted cells, single depolarizing voltage steps as well as a train of voltage steps evoke suppression of inhibition (DSI). This suggests that spontaneous rhythmic bursting of these cells triggers the release of endocannabinoids which function as retrograde messengers to reduce GABA release from periglomerular cells which in turn, regulates the activity of periglomerular cell synaptic targets such as external tufted cells.

Endocannabinoids regulate neuronal activity and signaling in olfactory bulb glomeruli. They function in the form of DSI through CB1R-mediated retrograde signaling among glomerular neurons. Endocannabinoids are released from external tufted cells and act as retrograde messengers to control the excitability of presynaptic neurons, i.e., periglomerular cells, and to regulate their transmitter release. Endocannabinoids are synthesized and

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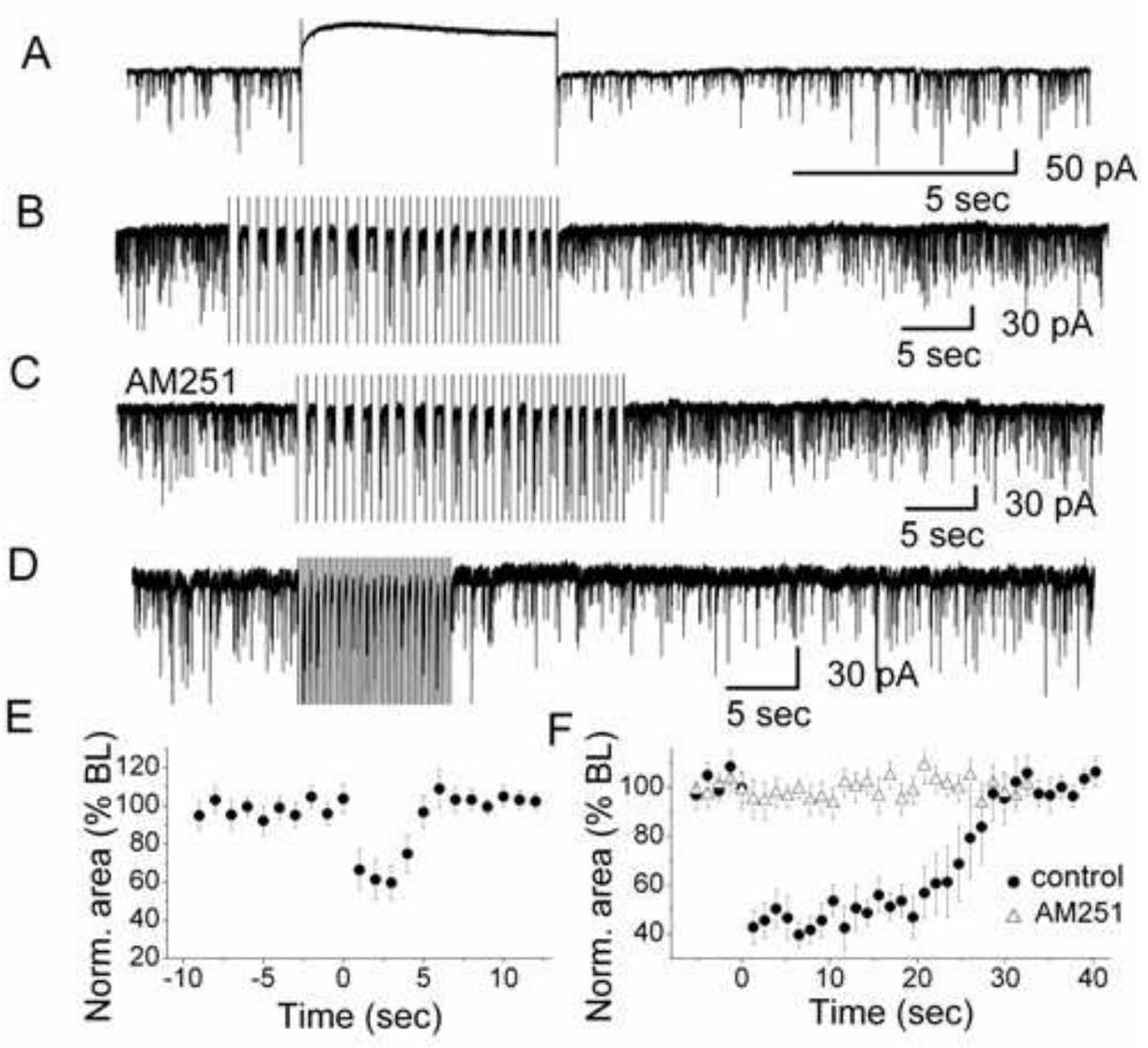

Figure 6. Depolarization-induced **S**uppression of **I**nhibition (DSI) in olfactory glomeruli. **A** A depolarizing voltage step evoked DSI in a representative external tufted cell. High Cl^--based pipette solution was used for recording sIPSCs. Depolarization was achieved by stepping from-60 mV holding potential to 0 mV for 5 sec. **B** In the presence of CNQX and 5-AP, a train of 20 voltage steps to 0 mV (0.75 Hz; step duration: 667 ms) transiently reduced sIPSCs in an external tufted cell. Holding potential was-60 mV. **C.** In the presence of AM251, no sIPSC suppression was observed. **D** A train of 20 voltage steps to-30 mV (2 Hz; step duration: 250 ms) transiently reduced sIPSCs in an external tufted cell (in CNQX and 5-AP). **E** Normalized sIPSCs area illustrating the magnitude and time course of DSI elicited by a 5-sec depolarizing pulse (n=7). The averaged values between 0 – 5 sec after the end of the voltage step were significantly different from the baseline (ANOVA and Bonferroni post-hoc analysis, p< 0.05). **F** Normalized sIPSC area illustrating the magnitude and time course of DSI elicited by a train of depolarizations to 0 mV (n=12) in control and in the presence of AM251 (n=10). In control conditions, the averaged values between zero to 2 5 seconds after the end of the train of voltage steps were significantly different from the baseline (ANOVA and Bonferroni post-hoc analysis, p< 0.05). From [43] with permission of the Society for Neuroscience.

released from neuronal cell bodies as a result of cellular excitation [11]. One potential source of endocannabinoids in the olfactory bulb is neurons that synapse onto presynaptic cells, i.e., periglomerular cells, and receive feedback synaptic inputs. This profile fits external

tufted cells and they could be a potential endocannabinoid source in the olfactory bulb which is supported by the fact that DSI is found in external tufted cells. DSI in external tufted cells is subject to the level of cellular activation, i.e., voltage step duration and step number. DSI cannot be evoked with step durations of 1 sec or less while a step duration closer to 5 seconds evokes transient DSI. A train of depolarizing voltage steps (>3) generates particularly prominent DSI and strengthens the inhibition of sIPSCs. This suggests that excitation of external tufted cells in the form of rhythmic bursting triggers the release of endocannabinoids and regulates glomerular activity. Bursting is intrinsic to external tufted cells and mediated by several cell intrinsic conductances [79]. Bursting of neurons may modulate endocannabinoid release not only in the olfactory bulb but also in other brain systems and constitute a general phenomenon of endocannabinoidsignaling.

Olfactory sensory neurons form direct synaptic contacts with external tufted cells. Sensory or synaptic input to external tufted cells can trigger the release of endocannabinoids which have an inhibitory effect on CB1Rs in presynaptic periglomerular cells. Endocannabinoids thus reduce inhibitory input to external tufted cells and enhance external tufted cell sensitivity to weak sensory inputs by depolarizing the membrane potential closer to spike threshold. This CB1R-mediated inhibition of periglomerular cells reduces their GABA release and, in turn, modifies the firing pattern of external tufted cells and, potentially, also reduces inhibition of mitral cells and presynaptic olfactory nerve terminals. The functional relevance of this signaling pathway lies in a potential increase of the overall sensitivity of the glomerulus to sensory inputs resulting from activation of CB1R on periglomerular cells.

7. Endocannabinoid-evoked physiological responses and crosstalk with other neurotransmitters

Endocannabinoids can evoke physiological responses that are not mediated by presynaptic CB1Rs but rather by postsynaptic CB1Rs [14], e.g., via regulation of K^+ conductances present on the extrasynaptic dendritic surface of neurons or modulation of postsynaptic NMDA receptors or even non-CB1R, e.g., [80]. Several conventional CB1R ligands have been reported to have CB1R unspecific effects or activate non-CB1 receptors [14]. Electrophysiological evidence suggests that the CB1R agonist WIN55,212-2 produces non-CB1R mediated effects on the excitability of principal neurons in the basolateral amygdala [81], thus providing evidence for a non-CB1R site of action of WIN55,212-2 [82, 83]. Cannabinoid drugs can activate other 'non-CB' receptors, such as GPR55, peroxisome proliferator-activated receptors (PPARs), and vanilloid type TRP channels [84, 85].

The accepted view of endocannabinoid action is based on hippocampal studies demonstrating that endocannabinoids reduce synaptic inhibition of the principal cell (DSI), see above. Endocannabinoids were found to possess other properties, namely, to mediate self-modulation of neocortical pyramidal neurons [86] or long-lasting self-inhibition in neocortical GABA-containing interneurons [87]. This self-inhibition is mediated by autocrine release of endocannabinoids and does not depend on glutamatergic and/or GABAergic neurotransmis-

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sion but rather on activity-dependent long-lasting hyperpolarization due to the activation of a K^+-conductance. Endocannabinoids released by these interneurons target the same cells and mediate a lasting hyperpolarization that is blocked by a CB1R antagonist. Self-inhibited cells can become hyperpolarized below spike threshold and are effectively removed from the neural circuit in which they reside.

The endocannabinoid system reciprocally modulates other neurotransmitter systems [88]. Examples include interactive cross-talk with the endogenous opioid system [89, 90]. Incidentally, like the endogenous opiate system, the endocannabinoid system was first discovered because it can be activated by a plant-derived compound – in the case of the endocannabinoids, this is Δ9-tetrahydrocannabinol, the bioactive ingredient of the drugs marijuana and hashish [25]. Other studies detected an interaction of the endocannabinoid system at the molecular and functional levels with other neurotransmitters such as the dopaminergic and adenosinergic systems [91-93]. Recent evidence has suggested cross-modulation between the endocannabinoid and hypocretinergic system [88]. This idea is based on the overlap observed in the neuroanatomical distribution of both systems as well as their putative functions. Functionally, both endocannabinoids and hypocretins can contribute to the regulation of appetite, reward and analgesia. Furthermore, biochemical and functional studies have demonstrated heterodimers between CB1 cannabinoid receptor and hypocretin receptor-1. Activation of hypocretin receptor-1 stimulates the synthesis of 2-arachidonoyl glycerol which through retrograde endocannabinoid signaling results in inhibition of neighboring cells. This interaction would allow endocannabinoids to contribute to hypocretin effects and provide potential therapeutic applications to currently existing drugs targeting these systems [88]. However, these two neuromodulatory systems exert antagonistic effects in the regulation of the sleep/wake cycle and anxiety-like responses which contributes even more to the excitement of performing research targeting the endocannabinoid system.

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