Endocannabinoid Signaling in Neural Plasticity

Bradley E. Alger

Contents

1 Introduction ... 142
 1.1 Definitions, Scope, Limitations, and Caveats ... 142
 1.2 ECBs: Basic Principles ... 143
2 ECBs Regulate Synaptic Plasticity ... 144
 2.1 Short-Term Plasticity ... 144
 2.2 Short-Term Target-Dependent Plasticity ... 146
 2.3 Long-Term Plasticity ... 146
 2.4 Mechanisms of ECB-Dependent Long-Term Plasticity ... 151
 2.5 Sufficiency of CB_1R Activation for ECB-LTD (or -iLTD) Induction ... 151
 2.6 Molecular Mechanisms of ECB-LTD (or iLTD) Maintenance ... 153
 2.7 Spike-Timing Dependent Plasticity (STDP) ... 155
 2.8 Interneurons Mobilize ECBs ... 157
3 Plasticity of the ECB System ... 158
 3.1 Use-Dependence of CB_1R Efficacy ... 158
 3.2 Tonic CB_1R Activation and ECB Regulation ... 159
 3.3 Plasticity of ECB Mobilization ... 162
 3.4 ECB Transport as a Synaptically Modifiable Process ... 163
 3.5 The ECB System and Seizures ... 164
 3.6 Interactions Between AEA and 2-AG ... 165
4 Conclusion ... 166
References ... 167

Abstract Plasticity refers to a physiologically measured change that may last for short or long periods of time. Endocannabinoids (ECBs) are prevalent throughout most of the brain, and modulate synaptic transmission in many ways. This chapter will focus on the roles of ECBs in neural plasticity in the mammalian brain. The topics covered can be divided loosely into two themes: how ECBs regulate synaptic

B.E. Alger
Departments of Physiology and Psychiatry, University of Maryland School of Medicine, 655 W. Baltimore St./BRB 5-025, Baltimore, MD, 21201, USA
e-mail: balgerlab@gmail.com

D. Kendall and S. Alexander (eds.), *Behavioral Neurobiology of the Endocannabinoid System*, Current Topics in Behavioral Neurosciences 1,
DOI: 10.1007/978-3-540-88955-7_6, © Springer-Verlag Berlin Heidelberg 2009

plasticity, and how ECBs' actions themselves are regulated by neuronal activity. Because ECBs regulate synaptic plasticity, the modifiability of ECB mobilization constitutes a form of "metaplasticity" (as reported by Abraham and Bear (Trends Neurosci 19:126–130, 1996)), i.e., an upstream process that determines the nature and extent of synaptic plasticity. Many of their basic functions are still being discovered, and while there is consensus on large issues, many points of divergence exist as well. This chapter concentrates on developments in the roles of ECBs in synaptic plasticity that have come to light since the major review by Chevaleyre et al. (Annu Rev Neurosci 29:37–76, 2006).

Keywords DSI • DSE • LTD • iLTD • Inhibition • Seizure

1 Introduction

1.1 Definitions, Scope, Limitations, and Caveats

The ECB system consists of the principal brain cannabinoid receptor, CB_1R, its endogenous ligands – 2-arachidonylglycerol (2-AG) and anandamide (AEA) – as well as a transporter process, metabolic and catabolic enzymes. Neither the mainly peripheral CB_2R (Pertwee 2005) nor the newly discovered, putative cannabinoid receptor, GPR55 (Ryberg et al. 2007), have received much attention from CNS physiologists thus far, and will not be dealt with here. Present physiological techniques cannot unambiguously distinguish among ECB synthesis, release, and transport processes, and I use 'mobilization' to encompass all steps between initial stimulation of the ECB system and activation of CB_1R. Cannabinoids, including ECBs, can act via non-CB_1R mechanisms, but these will not be covered.

The terminology ECB-STD and ECB-LTD (or iLTD) (Chevaleyre et al. 2006) to identify short-term and long-term plasticities initiated by ECBs has been retained. ECBs can be mobilized by a rise in $[Ca^{2+}]_i$, activation of numerous G-protein coupled receptors (GPCRs), or the combined actions of the two. GPCRs that trigger ECBs mobilization include metabotropic receptors for glutamate (Varma et al. 2001; Maejima et al. 2001), acetylcholine (Kim et al. 2002), dopamine (Yin and Lovinger 2006; Kreitzer and Malenka 2005), cholecystokinin (Foldy et al. 2007), oxytocin (Oliet et al. 2007), and glucocorticoids (Di et al. 2005), to name a few. Various stimuli use different biochemical pathways for ECB mobilization, and when necessary they are distinguished by superscripts: ECB_{mGluR}, ECB_{mAChR}, ECB_{Ca}, etc.

The work reviewed here was done on in vitro preparations from the rat or mouse brain, mainly with whole-cell electrophysiological recording methods. Generally, acute slices (300–400 μm thick) were used, but in a few cases dissociated tissue culture or organotypic slices were studied. Lovinger and colleagues have developed a "isolated neuron/bouton" preparation that has provided novel insights (Zhu and Lovinger 2005). Slice preparation and maintenance techniques are fairly similar

across the various laboratories, yet substantial points of divergence can be found. Experimental temperatures range from 22 to 34°C; developmental ages range from neonatal to fully adult; intrapipette contents differ, sometimes widely; storage and recording chambers, flow rates, and drug application methods often change from laboratory to laboratory for sometimes unexplained reasons. Understandably, but unfortunately, there have been few systematic studies on whether or how such experimental variables affect results. On the one hand, the diversity of methods fosters confidence in the robustness of replicated observations. On the other hand, the occasional disagreements and assertions that some seemingly minor experimental factor is critically important emphasize the need for cautious interpretation. Despite the explosion of interest in the cellular physiology of the endocannabinoid system, these are still relatively "early days" and consensus is a work in progress.

1.2 ECBs: Basic Principles

The biochemistry and pharmacology of the ECB system are covered elsewhere in this volume (in the chapter "The life cycle of the endocannabinoids: formation and inactivation" by Alexander & Kendall; in the chapter "Endocannabinoid Receptor Pharmacology" by Mackie & Yao), and the reader is referred to those chapters for details. CB_1R is the principal brain ECB receptor, and is a heterotrimeric G-protein coupled receptor. Most CB_1Rs are located on presynaptic terminals (see the chapter "Endocannabinoid Receptors: CNS Localization of the CB_1 Cannabinoid Receptor" by Katona, this volume), and activation of CB_1R always inhibits transmitter release. In the mammalian brain, release of glutamate or GABA has received the most attention thus far, although glycine release is inhibited in the brain stem (Mukhtarov et al. 2005). A major mechanism by which CB_1R activation inhibits transmitter release is inhibition of presynaptic voltage-gated Ca channels (VGCCs), primarily N-type. Increases in presynaptic K channel activity occur at some synapses (Kreitzer et al. 2002). Cerebellar depolarization-induced suppression of inhibition (DSI) involves a suppression of tetrodotoxin (TTX)-insensitive miniature inhibitory post-synaptic currents (mIPSCs) (Llano et al. 1991; Diana and Marty 2003) and activation of CB_1Rs reduces TTX-insensitive mIPSC frequency (Takahashi and Linden 2000). Hence, in addition to presynaptic Ca channels, CB_1R activation could inhibit release by inhibiting a vesicle release step downstream of Ca influx. Suppression of mIPSCs is sensitive to the $[Ca^{2+}]_i$ in the terminals (Yamasaki et al. 2006), so this may itself be a regulated step. Diana and Marty (2003) estimated that, at Purkinje cell–interneuron synapses, suppression of the release machinery accounted for 13.4%, depression of interneuron firing, 23.2%, and depression of the probability of release given an action potential, 63.4% of the total synaptic depression caused by CB_1R activation. Long-term suppression of release (LTD or inhibitory LTD, iLTD) involves a variety of effectors (see Sect. 2.6).

Biochemical investigations have generated an enormous amount of well-validated information about synthesis of ECBs (in the chapter "The life cycle of the

endocannabinoids: formation and inactivation" by Alexander & Kendall, this volume). Stimuli for ECBs often trigger phospholipase C (PLC) activity, generating diacylglycerol for diacylglycerol lipase (DGL) to cleave, yielding 2-AG. Yet studies of ECB-mediated neural response plasticity imply that new levels of experimental resolution may be necessary to understand the ECB system at the cellular physiological level. For example, while much evidence implicates 2-AG as the ECB in many systems, it is now accepted that PLC is not required for ECB_{Ca} production. Neither PLC inhibitors, nor deletion of PLC in mutant mice, affect ECB_{Ca} despite abolishing ECB_{GPCR} (Hashimotodani et al. 2005). Whether DGL itself is required for ECB_{Ca} remains controversial, with positive and negative effects of DGL inhibition having been reported. Some inhibitors of ECB metabolism that are effective when applied extracellularly are ineffective when applied intracellularly (Edwards et al. 2006), throwing basic assumptions about how the system functions at the moment into question. Undoubtedly, data from multiple techniques will be required before a complete picture is available.

Cannabinoids cannot be collected or assayed at the single cell level, therefore key components in the toolkit of endocannabinoid researchers are the CB_1R antagonists, AM-251 and rimonabant, which are inverse agonists that can produce effects on their own and not true receptor antagonists (Pertwee 2005; see Sect. 2.9). Recent work highlights two additional caveats to using them: AM251 is a putative agonist at GPR55 (Ryberg et al. 2007), and rimonabant is an effective antagonist at the vanilloid receptor, TRPV1 (e.g. Gibson et al. 2008). Since their non-specific actions do not overlap, both antagonists should routinely be used to confirm results.

2 ECBs Regulate Synaptic Plasticity

2.1 Short-Term Plasticity

The first example of ECB-mediated short term plasticity was depolarization-induced suppression of inhibition (DSI) (Pitler and Alger 1992; Llano et al. 1991) and its major properties had been delineated (Alger and Pitler 1995) before Wilson and Nicoll (2001) in hippocampal slices, and Ohno-Shosaku et al. (2001) in dissociated hippocampal culture, found that DSI is mediated by ECBs. At the same time Kreitzer and Regehr (2001) reported the discovery of depolarization-induced suppression of excitation (DSE) in the cerebellum and showed that it was also mediated by ECBs. These phenomena involve a transient suppression of synaptic transmission that follows a substantial increase in $[Ca^{2+}]_i$ in the receiving neuron. The hallmarks of DSI and DSE are that they are retrograde signal processes, with ECBs originating in a postsynaptic target cell, crossing the synapse in the reverse direction from conventional neurotransmitter travel, and suppressing the release of neurotransmitters (Alger 2002; Freund et al. 2003 for reviews). Inhibition of presynaptic Ca influx by ECBs has been measured in cerebellar parallel fibers (Kreitzer and Regehr 2001; Brown et al. 2003, 2004), and is the likely cellular

mechanism for the short-term ECB phenomena. Presynaptic N-type Ca channels are affected by CB_1R activation in many instances (Wilson et al. 2001), but other Ca channels are inhibited by ECBs in cerebellum (Brown et al. 2004) as well. The magnitude and duration of DSI and DSE is dependent on temperature (Kreitzer et al. 2002) and rise in postsynaptic $[Ca^{2+}]_i$ (Pitler and Alger 1992; Wang and Zucker 2001; Brenowitz and Regehr 2003). A prolonged $[Ca^{2+}]_i$ rise can lower the peak $[Ca^{2+}]_i$ pulse required for ECB_{Ca} mobilization (Brenowitz et al. 2006), so the system is modifiable. DSI and DSE have now been reported to occur in numerous brain regions, and appear to have similar properties everywhere. Although widespread, the ability of cells to mobilize ECBs under conditions of transient, high $[Ca^{2+}]_i$ rises is not universal, even if the cells can mobilize ECBs with other stimuli. For instance, the medium spiny neurons of the dorsal lateral striatum do not produce DSE, although they can readily undergo ECB-LTD (Yin and Lovinger 2006; Kreitzer and Malenka 2005).

Typically, the relevant postsynaptic Ca for ECB mobilization comes through high voltage-activated VGCCs, probably postsynaptic N-type channels (Lenz et al. 1998), however calcium from intracellular stores may contribute in some cells (Robbe et al. 2002; Melis et al. 2004b), particularly in young or immature tissue (Isokawa and Alger 2006).

Activation of G protein-coupled and ionotropic glutamate receptors can trigger ECB mobilization (Brown et al. 2003; Ohno-Shosaku et al. 2007). Brief bursts of afferent stimulation induce ECB-dependent transient suppression of parallel fiber inputs onto Purkinje cells, a phenomenon that is similar to DSE and has been called SSE (Brown et al. 2003). If the concentration of agonist is high, then ECBs are mobilized in a relatively $[Ca^{2+}]_i$-independent way (Maejima et al. 2001; Kim et al. 2002). If the concentration of agonist is low, then direct mobilization may not occur, but the products of the G-protein receptor activation can synergize with Ca_i^{2+} to produce a very marked increase in ECB mobilization over what the rise in $[Ca^{2+}]_i$ alone could accomplish (Varma et al. 2001; Kim et al. 2002; Ohno-Shosaku et al. 2003). A molecular model that can account for this synergistic interaction proposes that PLCβ isoforms (β1 in hippocampus (Hashimotodani et al. 2005), β4 in cerebellum (Maejima et al. 2005)) act as coincidence detectors, i.e., they are activated by both $[Ca^{2+}]_i$ and G-protein products. They can therefore integrate the two kinds of signals, and the summed stimuli produce larger responses than either could alone. This appealing model may not explain all of the interactions between Ca and GPCR activation that lead to ECB mobilization, however: a Ca-dependent priming step is required to enable mGluRs to mobilize ECBs primary step that cannot be accounted for by coincidence detection is required (see Sect. 3.3). In many cases, the ECBs released by GPCRs affect synapses that were not responsible for triggering ECB mobilization, e.g., suppression of hippocampal GABAergic synapses by activation of mGluRs (Varma et al. 2002) or mAChRs (Kim et al. 2002). These are examples of "heterosynaptic" ECB actions (Chevaleyre et al. 2006). It has been suggested that normal intracellular action potential activity does not generate a large enough $[Ca^{2+}]_i$ rise to trigger ECB mobilization without the concurrence of glutamatergic synaptic activity (Hampson et al. 2003),

however postsynaptic action potential bursts are effective stimuli for DSI of carbachol-induced IPSP/Cs (Pitler and Alger 1992; Reich et al. 2005). Whatever the details of the molecular model, it seems very likely that coordination between increases in postsynaptic $[Ca^{2+}]_i$ and neurotransmitters, often GPCR activators, may be the most prevalent stimulus for ECB mobilization in the brain.

Brief presynaptic trains of stimuli induced very localized $[Ca^{2+}]_i$ signals and ECB release from cerebellar Purkinje cells (Brown et al. 2003). CB_1Rs are present on parallel fibers, and by directly monitoring presynaptic Ca influx into parallel fiber terminals as an assay of the ECB effect, it was found that ionotropic glutamate receptors as well as mGluR1 contributed to ECB mobilization (Brown et al. 2003). ECBs affected only activated synapses, implying this was largely a “homosynaptic” effect (Chevaleyre et al. 2006), and providing an example of the extremely localized nature of ECB signaling.

2.2 *Short-Term Target-Dependent Plasticity*

Target-dependent plasticity refers to cases in which the postsynaptic target cell influences the type or degree of plasticity expressed by the incoming presynaptic contacts it receives. This is especially clear when a given afferent fiber system contacts more than one target cell in a given region, and the synaptic plasticity differs at each target. ECBs participate in target-dependent plasticity in the cerebellum, where the parallel fibers contact Purkinje cells and golgi cells (Beierlein et al. 2007). At these synapses either post-tetanic potentiation or depression was produced only at parallel fiber-to-Purkinje cell synapses; the golgi cell synaptic input was essentially unchanged by the same stimuli. Whether the Purkinje cell inputs were enhanced or depressed depended on the locus of the stimulation and the resulting degree of mGluR activation produced. Parallel fiber stimulation in the molecular layer activated numerous proximate synapses, which enabled glutamate spill-over to summate, activate mGluRs and mobilize ECBs from the Purkinje cells (Marcaggi and Attwell 2005). This resulted in ECB-dependent, stimulus-induced suppression of inhibition (Beierlein et al. 2007), which accounted for post-tetanic depression. Blocking CB_1R with AM251 uncovered post-tetanic potentiation, showing that the potentiation is an intrinsic property of these synapses that is masked when ECBs prevent glutamate release from the terminals. The parallel fibers onto golgi cells did express CB_1R that could be activated by exogenous cannabinoids, but the golgi cells seemed to be incapable of generating ECBs. In summary, the target-dependent plasticity was largely attributable to differential mobilization of ECBs.

2.3 *Long-Term Plasticity*

Although DSI itself is short-lasting, it can markedly affect postsynaptic excitability (Wagner and Alger 1996) and enhance the inducibility of long-term plasticity, such

as LTP (Carlson et al. 2002). If a stimulus train that was too weak to initiate LTP was delivered during DSI, LTP was induced. The ECB-induced disinhibition enabled normally subthreshold excitation to become suprathreshold for LTP induction. The possibility that ECBs could themselves induce long-term plasticity was first established in the dorsal striatum by Gerdeman et al. (2002) and in nucleus accumbens (NAc) by Robbe et al. (2002). Inhibitory LTD of rat basolateral amygdala cells in vitro seemed to correlate with the resistance to extinction of fear conditioning in the behaving animal (Marsicano et al. 2002).

2.3.1 Striatum

Induction of LTD is caused by mGluR activation at excitatory glutamatergic synapses onto the striatal medium spiny neurons, and a retrograde messenger was known to be involved (Gubellini et al. 2004). LTD was initiated with brief-high frequency stimulus trains paired with postsynaptic depolarizations, and was induced in a postsynaptic, Ca-dependent way. LTD was expressed presynaptically as a decrease in the probability of glutamate release, and dopamine D2 receptor activation was mandatory. Exogenous CB_1R agonists inhibited glutamate release (Gerdeman and Lovinger 2001) and stimulation of dopaminergic afferents generated AEA in the striatum (Giuffrida et al. 1999), so ECBs were a good candidate messenger. Gerdeman et al. (2002) found that several brief high frequency stimulus trains induced LTD that was expressed presynaptically, absent in $CB_1R^{-/-}$ mice, and blocked by the CB_1R antagonist, rimonabant. Strongly buffering $[Ca^{2+}]_i$ prevented ECB-LTD induction, which besides showing that postsynaptic $[Ca^{2+}]_i$ was essential to it, argued that ECBs generated by other nearby cells could not travel enough to affect the EGTA-loaded cell. Bath application of the putative CB transporter blocker, AM404, rescued LTD induction, supporting the proposal that ECBs were key players in LTD induction, and revealing that when ECB removal was prevented, ECBs from other cells could affect multiple cells. The transporter is a major factor in defining the extremely local sphere of ECB actions, which as argued previously (Alger 2002) is a key feature of the ECB system. Interestingly, *intracellular* application of ECB transporter blockers did not facilitate, but suppressed ECB-iLTD (Ronesi et al. 2004) (see Sect. 3.4). Restrictions in the spread of ECBs permit the single cells originating them to undergo major long-term plasticities while neighboring cells remain unaffected. In this way information coding may be selectively addressed to cells that happen to mobilize ECBs at the same time.

ECB-LTD in the dorsal lateral striatum depends on activation of L-type VGCCs, and D2 receptors as well as group I mGluRs (Yin and Lovinger 2006; Kreitzer and Malenka 2005) (perhaps specifically mGluR1), internal stores of calcium, and postsynaptic PLC activation (Yin and Lovinger 2006). The bistable resting potential of the medium spiny cells may critically regulate ECB-LTD (Kreitzer and Malenka 2005). In the "up" state, a resting potential near −50 mV, L-type VGCCs are activated, and mGluR-induced ECB-LTD was easily induced; in the "down" state, near −70 mV, mGluR-LTD ECB-LTD was not readily induced, probably

because the L channels are not activated at the negative membrane potential. Moreover, D2 activation markedly enhanced the state-dependent-LTD induction. While several of these features have been replicated, there are some controversial aspects of ECB-LTD in the dorsal striatum. A disagreement about the adequacy of CB_1R activation to induce LTD is covered in detail below.

2.3.2 Nucleus Accumbens (NAc)

Initiation of ECB-LTD of glutamatergic synapses in the NAc requires considerably greater synaptic stimulation (13 Hz for 10 min) than is necessary in other brain regions (Robbe et al. 2002). Pharmacological and genetic tests confirmed the involvement of CB_1R in the process, and showed that induction required postsynaptic $[Ca^{2+}]_i$ increases and maintenance involved presynaptic suppression of release. In Nac, mGluR5 is the predominant mGluR receptor, and ECB-LTD was abolished by the specific mGluR5 antagonist, MPEP, as well as a broad spectrum mGluR antagonist. The group I mGluR agonist DHPG mimicked and occluded ECB-LTD; DHPG effects were prevented by rimonabant. Unlike other systems in which Ca entry via VGCCs is required (see e.g. Gerdeman et al. 2002), in NAc, calcium from ryanodine-sensitive calcium stores mediates ECB-LTD initiation. And, unlike the dorsal striatum, D2 receptors play no role in the NAc. Hence, ECB-LTD in NAc has a number of distinctive features.

2.3.3 Hippocampus

Chevaleyre and Castillo (2003) showed that ECB-iLTD could be induced at inhibitory synapses in the hippocampus with brief high frequency stimulus trains, although longer-lasting theta-burst trains (Chevaleyre and Castillo 2004), or a 5-min bout of low-frequency (1 Hz) stimulation (Zhu and Lovinger 2005), will also induce ECB-iLTD. A 10-min application of the mGluR agonist DHPG induces ECB-iLTD, and mGluR antagonists block both synaptic and DHPG-induced iLTD (Chevaleyre and Castillo 2003; Edwards et al. 2006). ECB-iLTD could be inhibited by AM251, or extracellular pretreatment of slices with PLC or DGL inhibitors, although intracellular application was ineffective (Edwards et al. 2006). Intracellular application of DGL inhibitors do block other ECB_{GPCR} actions in hippocampus (Edwards et al. 2006) or cerebellum (Safo and Regehr 2005), so inefficiencies of intracellular delivery are not obviously at work. By applying AM251 at various times after brief field stimulation, it was found that ECB-iLTD induction required many minutes of CB_1R activation (Chevaleyre and Castillo 2003). Once established, ECB-iLTD cannot be blocked by either CB_1R or mGluR antagonists, showing that it is independent of continued receptor activation.

A key physiological feature of ECB-iLTD is that it can account for the EPSP-spike (E-S) potentiation that had been noted by Bliss and Lomo (1973) as a distinct dimension of LTP. E-S potentiation means that a given field EPSP is

capable of triggering a larger population spike after LTP induction than before. By definition this was a different mechanism from the increase in the EPSP itself, since the hallmark of E-S coupling is that the EPSP size is held constant when making the comparison. Decreases in inhibition can account for E-S coupling (Abraham et al. 1987), but the mechanism of the persistent decrease in inhibition remained elusive. ECB-iLTD was recognized as being ideally suited for this role, and AM251 prevented induction of E-S potentiation (Chevaleyre and Castillo 2003). The facilitatory effects of ECB-iLTD could be localized to small regions of the dendrites. Fine focal theta-burst stimulation of glutamatergic fibers enabled Chevaleyre and Castillo (2004) to define affected dendritic areas as limited to 10 μm in length in which mGluR-dependent ECB release would induce iLTD, and concomitantly, LTP of excitatory synapses. A two-pathway experiment revealed that even though LTP was directly induced in only a small region very close to the theta-burst stimulating electrode, iLTD affected a broader dendritic penumbra. In this region, the decrease in inhibition lowered the threshold for LTP induction.

There is a relatively low level of CB_1R expression on hippocampal glutamatergic terminals (Kawamura et al. 2006), which accounts for the relatively small degree of ECB-mediated DSE in the hippocampus (Ohno-Shosaku et al. 2002). However, early in development from PN2-10, a heterosynaptic, glutamatergic ECB-LTD is associated with a homosynaptic cyclic AMP-dependent protein kinase (PKA) -dependent LTP (Yasuda et al. 2008). This developmentally transient form of plasticity declines with age until it is absent in mature hippocampus. It is unusual in having a very slow onset (many tens of minutes), and being associated with a decrease in the fiber volley – a measure of action potentials in presynaptic axons – that is prevented by the K channel blockers Ba, 4-AP and dendrotoxin. ECB-LTD was also prevented by these blockers.

2.3.4 Cerebellum

At parallel fiber–Purkinje cell synapses, ECBs released from the Purkinje cells regulate transmitter release presynaptically by DSE (Kreitzer and Regehr 2001). Pairing of brief bursts of parallel fiber stimuli and climbing fiber stimuli for 30 trials leads to ECB-LTD of the parallel fiber synapses (Safo and Regehr 2005). Bath application or intracellular infusion of DGL inhibitors abolished both short-term and long-term ECB effects induced by synaptic stimulation without, however, altering DSE. A rise in Purkinje cell $[Ca^{2+}]_i$ was required for ECB mobilization. The similarities with ECB-(i)LTD in striatum or hippocampus end at this point, because ultimate expression of cerebellar ECB-LTD is expressed postsynaptically, whereas the other expression mechanisms are presynaptic (see Sect. 2.6).

Besides participating in parallel fiber LTD induction, ECBs regulate parallel fiber plasticity in another, quite different way, by preventing parallel fiber LTP expression (van Beugen et al. 2006). When stimulated by themselves, parallel fibers undergo a presynaptic, PKA -dependent, form of LTP. Coactivation of climbing

fibers during parallel fiber tetanization prevented LTP induction. CB_1R inhibition rescued LTP, and WIN55212-2 mimicked climbing fiber stimulation in blocking LTP. The dual roles of climbing fiber ECB effects, promoting LTD of the parallel fiber synapse while at the same time suppressing the presynaptic induction of LTP at parallel fiber terminals, are seen as complementary actions: by preventing LTP at a synapse destined for LTD, the climbing fiber LTP inhibition acts as a "safety lock" that ensures that synaptic weakening occurs.

2.3.5 Amygdala

In the basolateral amygdala, ECBs mobilized by low-frequency stimulus trains that activate mGluR1s induce ECB-iLTD (Azad et al. 2004). Neither PLC nor DGL inhibition affected iLTD, but it was enhanced in fatty acid amide hydrolase (FAAH) knock-out mice, implicating AEA, and not 2-AG, in the process. Postsynaptic inhibitors of adenylyl cylase or PKA inhibited iLTD induction, suggesting that the triggering of this cascade by mGluR1 mobilized AEA. Interestingly, in the lateral amygdala exogenous cannabinoids activating CB_1Rs on inhibitory interneurons can abolish LTD of excitatory synapses (Azad et al. 2008). The mechanism of this effect, and a possible role for ECBs, are not worked out.

2.3.6 Ventral Tegmental Area (VTA)

ECBs mobilized from dopamine cells in the VTA can induce DSE of glutamatergic synapses (Melis et al. 2004b). In addition, brief train stimulation of afferents from prefrontal cortex to the VTA lead to an ECB- and CB_1R-dependent decrease in the excitatory post-synaptic currents (EPSCs) onto dopaminergic neurons (Melis et al. 2004a). ECB mobilization was triggered by mGluR1 activation, and was blocked by postsynaptic infusion of an ECB transporter blocker. A rise in postsynaptic $[Ca^{2+}]_i$ was essential; Ca from ryanodine-sensitive internal stores was involved. The ECB appeared to be 2-AG, as EPSC depression was prevented when the recording electrode contained a DGL inhibitor, but PLC inhibition had no effect. Activation of D2 receptors enhanced ECB mobilization, but was not required for it. Initial reports of ECB actions in VTA did not include long-term effects. However, repeated cocaine treatment facilitates LTP induction in the VTA brain slice by persistently suppressing GABAergic synapses. Recent evidence (Pan et al. 2008) shows that cocaine stimulates mGluR1- and D2-dependent ECB-iLTD of VTA GABAergic eIPSCs.

2.3.7 Cortex

DSI (Trettel and Levine 2002; Fortin et al. 2004) and DSE (Fortin and Levine 2007) affect GABAergic and glutamatergic synapses in neocortex, with evidence of

regional selectivity, and ECB-spike-timing dependent plasticity (STDP) occurs in somatosensory cortex (Nevian and Sakmann 2006). ECB-LTD of glutamatergic synapses was induced by low-frequency stimulation in layer 2/3 of visual cortex (Crozier et al. 2007). This LTD was initiated by activation of NMDA receptors (NMDARs), since AP5 or intracellular dizolcipine blocked it. Prior monocular deprivation occluded layer 2/3 ECB-LTD, and blocking CB_1Rs prevents the ocular dominance shift (Liu et al. 2008) suggesting that ECB-LTD contributes to the loss of visual responsiveness in the monocular deprivation model.

2.4 Mechanisms of ECB-Dependent Long-Term Plasticity

Although ECB-LTD occurs at numerous excitatory and inhibitory synapses, relatively little is known about its induction or maintenance mechanisms. ECB-LTD induction requires persistent activation of CB_1R; in experimental studies an mGluR agonist must be applied for between 5 and 10 min (10 is typical) to guarantee that LTD will occur. Field stimulation of glutamatergic afferents, which can induce LTD after bursts of stimuli lasting only 1 or 2 s (Chevaleyre and Castillo 2003), seems to be much more efficient. To some extent the very large time difference between exogenous application of mGluR agonist and synaptic stimulation is misleading, however. Since the *maintenance* of ECB-LTD does not depend on persistent activation of CB_1R, the time interval during which AM251 is effective in blocking ECB-LTD represents the duration of the *induction* phase; i.e., CB_1R must remain activated for at least that long for ECB-LTD induction to occur. It is possible to measure the duration of CB_1R activation by applying AM251 at various time intervals after field stimulation. This revealed that even a few seconds of field stimulation caused activation of CB_1R lasting for many minutes (Chevaleyre and Castillo 2003; Ronesi et al. 2004). In other words, there was no big temporal disparity between the duration of time that direct mGluR agonist application, or field stimulation, actually activates CB_1R.

2.5 Sufficiency of CB_1R Activation for ECB-LTD (or -iLTD) Induction

Given that long-duration CB_1R activation is a requirement for ECB-LTD induction, one can inquire why this is necessary, and whether long-duration CB_1R binding per se is sufficient for induction. A straightforward approach is to apply a CB_1R agonist for many minutes and ask if ECB-LTD is induced. The experiment has been done in several ways, and the results have been inconsistent. In cerebellum, parallel fiber LTD is ECB-dependent, but CB_1R activation alone by the synthetic agonist, WIN55212-2, is insufficient for induction (Safo and Regehr 2005); the response

returns to baseline once WIN55212-2 is removed. Similarly, loading hippocampal CA1 pyramidal cells with the G-protein activator, GTPγS, caused persistent ECB mobilization and activation of CB_1R, yet application of AM251 returned the eIPSCs to expected control amplitudes (Kim et al. 2002). Additionally, eliciting persistent, ECB-dependent eIPSC suppression by repetition of overlapping DSI trials for 10 min had no lasting effects (Edwards et al. 2006). Soon after the last DSI trial, the eIPSCs returned to control amplitudes with no evidence of ECB-iLTD. Finally, prolonged stimulation of ECB_{mAChR} with carbachol suppressed eIPSCs continuously for up to 20 min in CA1. Again, full recovery of eIPSCs to control levels occurred shortly after the mAChR agonist was removed and atropine applied. Stimulation with an mGluR agonist reliably produced ECB-iLTD. It appeared that persistent CB_1R activation was insufficient for the long-term effects, and that some other consequence of mGluR activation led to ECB-iLTD initiation (Edwards et al. 2006). In contrast, in NAc (Robbe et al. 2002) and hippocampus (Chevaleyre and Castillo 2003; Chevaleyre et al. 2007), minutes-long application of WIN55212-2 reportedly can cause a significant, and apparently irreversible suppression of IPSCs in CA1. However, lipophilic compounds such as WIN55212-2 are difficult to remove completely from slices, and the possibility of lingering WIN55212-2 was difficult to eliminate.

Within the striatum, contradictory results have also been obtained. Continuous eEPSC suppression caused by 20 min of WIN55212-2 application or by loading postsynaptic medium spiny cells with AEA (Ronesi et al. 2004) (which escapes and persistently suppresses incoming glutamate release) reportedly could be fully reversed by addition of AM251. However, in the same preparation, Kreitzer and Malenka (2005) found that WIN55212-2 alone did induce LTD.

Recent reports in cerebellum (Safo and Regehr 2005), striatum (Singla et al. 2007) and hippocampus (Yin et al. 2006) offer a possible resolution to some of the conflicting findings: it turns out that establishment of ECB-LTD requires CB_1R activation plus concomitant presynaptic activity. WIN55212-2 application in the absence of presynaptic stimulation caused reversible eEPSC depression, whereas synaptic stimulation delivered throughout the WIN55212-2 application caused long-term, AM251-resistant depression after the WIN55212-2 was removed. Somewhat surprisingly, a very low frequency of stimulation (0.05 Hz) was sufficient for this form of ECB-LTD induction (Singla et al. 2007). The long intervals between stimuli would seem to preclude build-up of an intracellular chemical factor, and the explanation for this efficacy is unknown. In any case, some of the negative results reported by earlier work may have reflected the absence of adequate presynaptic co-stimulation to provide whatever condition is needed in concert with CB_1R activation to induce ECB-LTD. In the experiments of Singla et al. (2007) the unknown element seemed to involve an increase in presynaptic $[Ca^{2+}]_i$, because if WIN55212-2 were applied when extracellular calcium ion concentration ($[Ca^{2+}]_e$) was reduced, concurrent stimulation did not cause LTD (assessed after restoration of normal $[Ca^{2+}]_e$). Filling the postsynaptic cell with the calcium chelator, BAPTA, did not affect LTD induction via ECB and stimulation, supporting the inference that LTD induction took place exclusively via presynaptic $[Ca^{2+}]_i$-dependent mechanisms.

Indeed, combined activation of L-type Ca channels, modest postsynaptic activation, and synaptic transmission is sufficient to induce striatal ECB-LTD (Adermark and Lovinger 2007a). An L-channel activator, FPL64176, could induce persistent, CB_1R-dependent LTD if the cells were depolarized to −50 mV and simultaneously stimulated at a low frequency. In general, the requirement for simultaneous stimulation confers synapse specificity of LTD targeting: only those synapses releasing neurotransmitter during CB_1R would be susceptible to LTD (Singla et al. 2007). Still unresolved are what exactly the co-stimulation does, and why some neurotransmitters that release ECBs do not induce LTD even when co-stimulation is given (Edwards et al. 2006).

2.6 Molecular Mechanisms of ECB-LTD (or iLTD) Maintenance

Maintenance of striatal ECB-LTD has been investigated in a reduced striatal slice preparation in which the cell bodies of the cortical afferent fibers had been removed (Yin et al. 2006). This ruled out the participation of gene transcription in cortical cell somata. ECB-LTD maintenance was prevented by bath application of protein translation inhibitors, but loading them into the postsynaptic cell had no effect. Neither cycloheximide nor anisomycin affected basal transmission, the activation of CB_1R, or of mGluR. Similarly, postsynaptic loading of transcription inhibitors also failed to affect ECB-LTD. The data suggested that local axonal protein translation was essential for ECB-LTD. The target(s) of these proteins were not clear.

Chevaleyre et al. (2007) investigated the presynaptic mechanisms of ECB-iLTD induction in the hippocampus. Pharmacological interference with cAMP-PKA system prevented ECB-iLTD induction. CB_1R activation often inhibits adenylyl cyclase, and indeed forskolin opposed the effect of WIN55212-2, and PKA inhibitors occluded it. Neither manipulation affected DSI, suggesting that postsynaptic ECB mobilization was not affected and that the mechanism by which CB_1R activation induces ECB-iLTD was different from the presynaptic DSI mechanism, i.e., primarily blockade of N-type Ca channels (Wilson and Nicoll 2001). Internal postsynaptic application of a PKA inhibitor failed to affect ECB-LTD, lending support to the conclusion that the PKA effects were presynaptic. To investigate the ability of CB_1Rs to target the GABA release machinery, TTX- and Cd-insensitive mIPSCs were studied in elevated $[Ca^{2+}]_e$. Both WIN55212-2 and PKA inhibitors reduced mIPSC frequency but not amplitude, and forskolin prevented WIN55212-2's actions. RIM1α is an active zone protein that is required for presynaptic LTP induction (Castillo et al. 2002) and is a substrate for PKA. In RIM1$\alpha^{-/-}$ mice, ECB-iLTD could not be induced (Chevaleyre et al. 2007), but DSI and short-term ECB_{mGluR} were normal, showing that ECB mobilization and CB_1Rs were unaffected by the knockout. Apparently, decreased phosphorylation of RIM1α by PKA is at

least partly responsible for ECB-iLTD induction. The RIM1$\alpha^{-/-}$ mice also had attenuated sensitivity to PKA inhibitors, and WIN55212-2, as if the iLTD process were fully saturated. After extending their conclusions to the basolateral amygdala, Chevaleyre et al. proposed that the PKA-RIM1α mechanism of ECB-iLTD may be a very general one.

Nevertheless other possibilities exist. In cerebellum, parallel fiber LTD is induced by a process involving nitric oxide (NO) and is expressed by a postsynaptic downregulation of AMPA receptors (AMPARs) (Ito 2001). This LTD is dependent on the activation of presynaptic CB_1R, and postsynaptic DGL (Safo and Regehr 2005), implying that 2-AG generated in the Purkinje cell is a key element. Blocking NO synthase with L-NAME confirmed that ECB-LTD is also NO-dependent. NO acted at a step downstream from the ECBs, since bypassing ECB synthesis and activating CB_1R directly with WIN55212-2 did not allow for LTD induction if NO synthesis was blocked. The apparent connection between CB_1R and NO is unknown. In fact, this issue has been further complicated by the finding that the NO synthase cascade is not localized to parallel fiber terminals, but rather to interneurons (Shin and Linden 2005). The study by van Beugen et al. (2006) (Sect. 2.3) offers a resolution: presynaptic CB1R activation simply suppresses presynaptic parallel fiber LTP induction, and thereby enhances the appearance of postsynaptic, NO-dependent LTD by preventing its occlusion by LTP. Inasmuch as parallel fiber LTP is dependent on presynaptic cAMP, PKA, and RIM1α (Castillo et al. 2002), the simplifying hypothesis would be that the major biochemical target of presynaptic CB_1R is the cAMP system, rather than NO. This is an appealing idea that can unify our understanding of the long-term ECB-dependent regulation of transmission at inhibitory and excitatory synapses. One gap remains to be filled: inhibition of PKA simply prevents LTP at excitatory synapses without causing LTD (van Beugen et al. 2006), whereas it causes LTD at inhibitory synapses (Chevaleyre et al. 2007).

In any event, the proposed downstream involvement of NO in cerebellar ECB-LTD (Safo and Regehr 2005) would be very different from the case of DSI in the hippocampus, where NO has been proposed to be upstream of ECB synthesis under conditions when mAChRs are also activated (Makara et al. 2007). In these experiments, blocking NO blocked DSI, and activating the NO pathway mimicked and occluded DSI. Neuronal NO synthase was found localized immediately postsynaptic to presynaptic terminals expressing NO-sensitive guanylate cyclase (Szabadits et al. 2007). NO released from the pyramidal cell would activate presynaptic guanylate cyclase. Indeed, guanylate cyclase activation caused cGMP accumulation in these terminals (Makara et al. 2007). Elements of the ECB system must be downstream of this step, but at present this connection remains mysterious. Unanswered questions include how and why mAChR activation could trigger the switch between an NO-independent DSI mechanism to an NO-dependent one. There is apparently no evidence that NO is involved in establishment of hippocampal ECB-iLTD, although strong, persistent activation of mAChRs, which copiously generates ECBs, does not cause iLTD (Edwards et al. 2006), perhaps arguing against this possibility.

Thus CB_1R activation is potentially coupled to at least four very different effectors, involving multiple biochemical pathways in the presynaptic cells: (1) direct G-protein-dependent suppression of Ca channels that mediates short-term DSI or DSE; (2) inhibition of cAMP production, leading to decreases in PKA, and an effect on the vesicle release machinery mediated by RIM1α; (3) activation of some component(s) of the NO signaling cascade; (4) opening of voltage-gated, presynaptic K channels through unknown biochemical pathways (exogenous cannabinoids do increase K currents via a PKA-dependent pathway in tissue culture (Mu et al. 2000)). A $[Ca^{2+}]_i$-dependent K conductance is turned on by CB1R autoreceptors in neocortical interneurons suggesting that CB_1R could be coupled to more than one K conductance (Bacci et al. 2004) (see Sect. 2.7).

2.7 Spike-Timing Dependent Plasticity (STDP)

Induction of Hebbian forms of plasticity depends on the occurrence of both pre- and postsynaptic activity. For the class of STDPs, the timing between pre- and postsynaptic events is critical (Dan and Poo 2004). The concept of STDP not only suggests that events must occur within a particular temporal window, but also that the order of events, whether the pre- or the postsynaptic cell activity occurs first, determines the type of plasticity (potentiation or depression) that is induced.

Timing-dependent LTD (tLTD) takes place in neocortical layer 5 principal neurons when the postsynaptic cell firing precedes presynaptic cell firing by 20–200 ms. Sjostrom et al. (2003) discovered that ECBs set the width of this temporal window. NMDAR activation was also required for tLTD, however tLTD only occurred if the ECBs, released by activity in the postsynaptic cell, were bound to CB_1Rs during the presynaptic activity. Interestingly, the released glutamate activated presynaptic NMDA autoreceptors, and hence the coincidence of presynaptic CB_1R and NMDAR activation was critical for tLTD. In fact, actual postsynaptic action potential firing is not required for this form of STDP – correctly timed subthreshold depolarizations are sufficient (Sjostrom et al. 2004) – making “spike-timing dependent plasticity” a misnomer.

In layer 2/3 pyramidal cells of somatosensory cortex, ECBs are also involved in STDP, although the mechanism is quite different from that in the layer 5 cells (Nevian and Sakmann 2006). In a study of the relationship between synaptic spine $[Ca^{2+}]_i$ and the long-term STDP produced at the synapse, Nevian and Sakmann found there was no simple correlation between them. STDP was produced by 60 pairings (at 0.1 Hz) in which a burst of three dc-triggered action potentials would precede or follow an eEPSP. When the burst occurred 50 ms before the eEPSP, LTD was induced; when it followed the eEPSP by 10 ms, LTP was induced. Two-photon excitation fluorescence microscopy revealed that spine $[Ca^{2+}]_i$ changes associated with the same stimuli that induced synaptic plasticity (though measured in different experiments) produced different effects. Calcium influx via NMDARs was essential for LTP induction, and not LTD. Conversely, calcium

influx via VGCCs mediated LTD not LTP. In neither case did the peak $[Ca^{2+}]_i$ amplitudes predict LTP or LTD; either change could be associated with a given $[Ca^{2+}]_i$, so the timing dependence was not conferred by differences in $[Ca^{2+}]_i$. mGluRs were necessary for LTD, but not LTP induction, and the mGluR antagonist did not affect $[Ca^{2+}]_i$. LTD was blocked by AM251 and by the PLC inhibitor, U73122, also without changes in spine $[Ca^{2+}]_i$. The conclusion was that STDP induction of LTD was caused by ECBs mobilized by VGCC-mediated Ca influx in combination with mGluR activation. The combined effects summated during the long induction protocol. IP_3-sensitive Ca stores contribute (Bender et al. 2008) to ECB-LTD in somatosensory cortex, and presynaptic NMDARs are probably also involved.

ECB-dependent STDP also occurs in the dorsal cochlear nucleus (Tzounopoulos et al. 2007), but only in the cartwheel interneurons, not in the principal cells for which they provide feedforward inhibition. The difference was not in the general source of the synaptic inputs, which are the same for both target cells, i.e., this is another example of target-dependent plasticity (see Sect. 2.2). The same excitatory afferent parallel fiber system induced opposing kinds of plasticity in the two cell types: a Hebbian LTP in the principal (fusiform) cells, and an "anti-Hebbian" LTD in the interneurons. The Hebbian LTP was a conventional, NMDAR and calmodulin-dependent protein kinase (CaMKII)-dependent phenomenon, requiring glutamate from the presynaptic cell and sufficient postsynaptic depolarization to permit postsynaptic NMDAR activation. Anti-Hebbian LTD at the parallel fiber–cartwheel interneuron synapse was caused when a postsynaptic spike reliably followed the EPSP. A crucial aspect of anti-Hebbian LTD induction was the very precise nature of the timing, which demands occurrence of the postsynaptic spike within a 10 ms window centered on the EPSP. This narrow window is set by the co-occurrence and mutual cancelation of LTP and ECB-dependent LTD, except at the shortest intervals where LTD dominates. Blocking CB_1R led to the production of only LTP; whereas preventing LTP increased the timing window for LTD induction. A puzzle was why the ECBs only affected parallel fiber inputs to the interneurons. Physiological release of ECBs (DSE), or application of a synthetic CB_1R agonist affected the parallel fiber inputs to both cartwheel and principal cells, albeit to a significantly larger extent at the synapses onto the interneurons. Electron microscopic analysis of labeled CB_1R revealed many fewer receptors on the parallel fiber terminals onto principal cells, thus accounting for the difference. An important open question is why the timing window is so narrow. The kinetics of ECB mobilization, even if faster than sometimes thought (cf. Wilson and Nicoll 2001; Heinbockel et al. 2005), are an order of magnitude too long. It will be interesting to learn what aspect of ECB mobilization conveys the temporal sensitivity to this STDP.

Exogenous cannabinoids disrupt the temporal coordination of cell assemblies, assessed as changes in the local EEG in the hippocampus without markedly altering the absolute firing rates of either pyramidal cells or interneurons (Robbe et al. 2006). This effect was explained by the applied agonists' ability to access the CB_1Rs on glutamatergic terminals that do not seem to be the main target of endocannabinoids in hippocampus. Somewhat surprisingly, application of the

CB_1R antagonist, rimonabant, applied on its own did not alter the rhythms, although it did prevent the actions of the exogenous cannabinoids. The implication that ECBs normally play no role in rhythm generation would have profound significance for the understanding of this system, and will no doubt be followed up in future work.

2.8 Interneurons Mobilize ECBs

The interneuron is a key player in many forms of neuronal plasticity and CB_1R-expressing interneurons are regulated by ECBs mobilized by other cells. Whether interneurons can exercise the same sort of autoregulation has been uncertain until relatively recently. An investigation of hippocampal CA1 stratum radiatum interneurons (Hoffman et al. 2003) concluded against the idea. Because the interneuron inputs were sensitive to exogenous CB_1R agonists, it appeared that these cells could not generate ECBs. Bacci et al. (2004), recording from low threshold-spiking, cholecystokinin (CCK)-expressing, neocortical interneurons, obtained a strikingly different result. Those interneurons do mobilize ECBs following stimuli that induced a large increase in $[Ca^{2+}]_i$, but the CB_1Rs that were activated were present on the interneurons themselves, and functioned as autoreceptors. By activating Ca-dependent K channels apparently on or near the interneuronal somata, the ECBs hyperpolarized and inhibited the interneurons. A notable feature of this self-inhibition was its very long duration (>10 min at 32°C), which seemed to be partly maintained by persistent action of ECBs as it remained sensitive to AM251 for many minutes.

While the results just described confirmed that interneurons were capable of mobilizing ECBs, it was not clear if the ECBs were also used for regulating interneuron synaptic inputs. Golgi cells in the cerebellum cannot mobilize ECBs (Beierlein et al. 2007), but two other local cerebellar interneurons, the basket and stellate cells, can regulate the strength of their excitatory parallel fiber inputs by mobilizing ECBs (Beierlein and Regehr 2006). Direct depolarization of the interneurons or brief synaptic stimulation induced DSE or SSE. Prevention of the effects either by CB_1R antagonists, or inhibition of DGL, implicated ECBs and particularly 2-AG in mediating the presynaptic inhibition. Interestingly, the synaptic release not only of mGluRs but also of NMDARs were fully capable of mobilizing ECBs, and it was necessary to block both receptors to prevent synaptic stimulation from initiating the ECB effects. Inasmuch as both interneurons are activated by the parallel fibers, this system provides for a feedforward inhibition of Purkinje cells, and therefore ECB actions would decrease this feedforward inhibition.

Ali (2007) carried out a paired-recording study of Schaffer-collateral-associated, CCK-expressing, interneurons in hippocampal CA1. She found the cells were interconnected with facilitating inhibitory synapses: brief stimulation of one cell led to increasingly large IPSCs in the target cell. Since the output of these interneurons is also directed towards the pyramidal cell dendrites, the facilitating IPSCs

would, by depressing the interneuron, disinhibit the pyramidal cells. If however the receiving interneuron were strongly stimulated independently, it would release ECBs, thus depressing the incoming facilitating IPSCs, and maintaining or heightening the pyramidal cell inhibition. Hence the combination of facilitating output, innervation by CB_1R-expressing GABAergic nerve terminals, and the capability of mobilizing ECBs constitutes a rich repertoire of tools whereby this network of CCK cells can modulate pyramidal cell firing.

A major twist on the idea that interneurons could directly mobilize ECBs and regulate their incoming synaptic input is that CCK cells in the hippocampus can regulate their own synaptic output, by triggering ECB mobilization from their target pyramidal cells (Foldy et al. 2006). In paired cell recordings, direct application of CCK reduced GABA release from CCK cells, but not from the parvalbumin (PV)-expressing basket cell interneurons. Most importantly, AM251 abolished the CCK-induced IPSC suppression. The ECBs appeared to originate from the pyramidal cells, because suppressive effect of CCK could be blocked by including the G-protein inhibitor, GDPβS, in the pyramidal cell recording pipette.

It is interesting that, although these other studies confirmed that CB_1R-expressing interneurons in other parts of the brain are competent to mobilize ECBs, thus far only the neocortical cells appear to respond with CB1R-mediated self-inhibition (Bacci et al. 2004) and the finding has not yet been replicated in the neocortex. This is surprising because the cortical interneurons are CCK-expressing and, like the CCK interneurons in other parts of the brain, express CB_1R, and as noted, some of them can release ECBs (Ali 2007). The ability of interneurons to mobilize ECBs allows them to regulate their involvement in circuit behaviors, particularly in the oscillations in which they play prominent roles.

3 Plasticity of the ECB System

3.1 Use-Dependence of CB_1R Efficacy

Even before ECBs were shown to be the retrograde messengers for DSI, it was clear that DSI could be regulated by presynaptic mechanisms. At a low concentration, 100 μM, 4-aminopyridine (4-AP) blocks only a few K channel subunits, yet at this concentration 4-AP abolished DSI (Alger et al. 1996). Other K channel blockers at much higher concentrations, e.g. 10 mM tetraethylammonium (TEA), were unable to do the same, implying that 4-AP's effect was fairly specific on an A- or perhaps D-type K current (Varma et al. 2002). One interpretation of this result was that the DSI messenger reduced synaptic transmitter release by activating presynaptic 4-AP sensitive channels. Another interpretation of 4-AP's effect was that it enhanced Ca influx into the terminal by blocking the K channels. In this scenario, the large influx of Ca would offset the suppression of Ca influx caused by DSI (cf., Klapstein and Colmers 1992). This hypothesis predicted that lowering extracellular $[Ca^{2+}]_e$ in the

presence of 4-AP would largely restore DSI, which was in fact observed (Varma et al. 2002), although full reversal was not obtained. The DSI-opposing effects of low concentrations of Na conductance inhibitors may have a similar explanation (Alger et al. 1996; Varma et al. 2002). Despite some residual uncertainty about the underlying mechanism of the K channel blockade on DSI, these data revealed a potential for use-dependence of DSI. The discovery that DSI is mediated by ECBs implied that CB_1R-mediated actions generally would similarly be use-dependent. Indeed, the CB_1R ligand THC increased A-type K currents (Deadwyler et al. 1995, some of which are 4-AP-sensitive, supporting this possibility. Moreover, Ba and 4-AP opposed the IPSC suppression caused by WIN55212-2 (Hoffman and Lupica 2000).

Confirmation of the use-dependence of DSI came during paired recordings from identified CCK-interneurons and pyramidal cells (Foldy et al. 2006). The experiments showed directly that unitary (u) IPSC suppression caused either by WIN55212-2 or DSI could largely be lifted by increasing the firing frequency of the interneuron. Brief (200 ms long) trains of directly induced action potentials at frequencies $\geq$20 Hz were necessary. With this protocol, the uIPSC amplitudes and failure rates approached normal rates even in the presence of WIN55212-2. The uIPSC suppression caused by the N-type Ca channel blocker, ω-conotoxin-GVIA, could not be overcome, consistent with the data (Wilson et al. 2001) that transmitter release by CB_1R-expressing (i.e., CCK) interneurons (Freund et al. 2003) takes place exclusively via the N-type Ca channel. DSI could be abbreviated by 40 Hz stimulation, although not fully abolished. When the protocol was changed to 15 pulse trains of 100 Hz stimulation, even complete (100% uIPSC suppression) DSI could be largely, though not completely, erased (Foldy et al. 2006). The data clearly demonstrated that DSI could be modulated by activity in the presynaptic interneuron. A question remains concerning the mechanism by which high frequency stimulation restores transmission in the face of CB_1R activation. While increasing the preterminal $[Ca^{2+}]_i$ seems certain to play a role, it might not be the only factor. G-protein-dependent blockade of N-type Ca channels is voltage-dependent, and can be relieved by strong depolarizations (Bean 1989; Ikeda 1991). This may also be important for use-dependence of CB_1R suppression. In principle, these factors could be distinguished by manipulations of preterminal $[Ca^{2+}]_i$, which would not affect the voltage-dependent relief while dramatically altering the CB_1R effects on $[Ca^{2+}]_i$.

3.2 *Tonic CB_1R Activation and ECB Regulation*

Participation of ECBs in neuronal plasticity is shaped by the mechanisms of ECB mobilization. Thus far the emphasis has been on instances of ECB mobilization triggered by a sudden, strong increase in $[Ca^{2+}]_i$, by certain GPCRs, or by the coincidence of both stimuli. All of these mechanisms have a definable point of onset. However, ECBs may also be tonically mobilized by an as yet incompletely

characterized, but persistent, Ca-dependent process with no clear point of onset. Interruption of this ongoing mechanism, in effect a negative regulation of ECB actions, represents another form of ECB-dependent neuronal plasticity.

Tonic actions of neurotransmitters or neuromodulators are inferred when a receptor antagonist alone produces effects that are opposite to the effect caused by the receptor agonist. Initial reports of ECB actions in the brain seemed to discount the possibility of tonic ECB actions: application of CB_1R antagonists did little or nothing on their own (e.g. Wilson and Nicoll 2001; Kim et al. 2002). The question of the tonic actions of ECBs is potentially tricky, because the CB_1R antagonists are inverse agonists (Pertwee 2005). In principle, CB_1R inverse agonists could induce effects that are the inverse of the agonists even in the absence of the agonist. They would do this by locking CB_1R, a G-protein binding receptor, into the GDP-bound state (Bouaboula et al. 1997; Vasquez and Lewis 1999). If CB_1R is constantly shuttling between GTP (active) and GDP (inactive) binding even in the agonist-unbound state, there could be a CB_1R "tone" that would be removed by the inverse agonist as it gradually trapped the receptor in the inactive state. Inhibition of CB_1R activated by tonically released ECB would have the same physiological effects as inverse agonism of intrinsically activated CB_1R. Nevertheless, the two mechanisms would be subject to strikingly different kinds of regulation. Despite this theoretical possibility, there seems to be no physiological evidence that CB_1R tone is set by intrinsic receptor activity, rather it is set by tonic mobilization of ECBs. In hippocampal CA1 (Wilson and Nicoll 2001) and NAc (Robbe et al. 2002), tonic ECB actions were only detected after the ECB transporter was blocked, implying that while ECBs may be tonically released, the transporter normally prevents CB_1R from being activated. In hippocampus, constitutive activation of the presynaptic, 2-AG degradative enzyme, monoacylglycerol lipase (Dinh et al. 2002), also plays a major role in preventing tonic activation of CB_1R, which is revealed when the enzyme is inhibited (Hashimotodani et al. 2007). Tonic release might be discovered in other areas when ECB transport or degradation are blocked.

In studying transmission between synaptically coupled GABA interneuron and CA3 pyramidal cell pairs, Losonczy et al. (2004) noted instances in which induction of a single action potential, or even a modest train of interneuronal action potentials, failed to initiate any synaptic response in the pyramidal cell. At first it appeared that the cells were simply not synaptically connected. However, a high frequency stimulus train lasting hundreds of milliseconds produced a gradually intensifying, erratic barrage of IPSCs in the pyramidal cell. Clearly the cells were synaptically coupled, but at low stimulus frequencies this was undetectable. Application of a CB_1R antagonist revealed strong coupling even at low stimulus frequencies, implying that the connection was actively "muted" by ECBs. The ability of high stimulus frequencies to reveal the synaptic connections by overcoming the CB_1R-induced suppression of GABA release demonstrated the use-dependence of ECB actions (see Sect. 3.1). In addition, since no overt stimulus of ECB mobilization had been applied, the experiments showed that the ECBs were tonically released.

These conclusions were confirmed and extended in the CA1 region (Neu et al. 2007). Paired CCK interneuron–pyramidal cell recordings revealed substantial variability in the probability of release from these interneurons. In many cases the connection was effectively silent until a CB_1R antagonist was applied. Although, in principle, tonically released ECBs might have come from any cell in the neighborhood of the recorded interneuron, they seemed to come only from the coupled pyramidal cell, because loading the postsynaptic cell with BAPTA abolished the tonic effects. Had ECBs spilled over from nearby cells to the target interneuron, then high BAPTA in a single pyramidal cell should have been ineffective. Tonic ECB release was not secondary to tonic activation of mAChR, mGluR, or NMDAR, all of which can induce ECB release in the hippocampus. Thus these experiments confirmed not only the reality of the tonic release phenomenon, but also supported the concept that ECB signaling is a local phenomenon; ECBs do not spill over from one cell to another under normal circumstances. Previous failures to observe tonic ECB release can probably be explained by the heterogeneity and relatively small numbers of interneurons susceptible to tonic release.

In identified pro-opiomelanocortin (POMC)-expressing neurons in the arcuate nucleus of the hypothalamus, Hentges et al. (2005) discovered that AM251 increased the baseline amplitudes of eIPSCs but not of eEPSCs. AM251 had no effect if the POMC cell had been loaded with 10 mM BAPTA, thus ruling out constitutive intrinsic activation of CB_1R as a mechanism, and showing that $[Ca^{2+}]_i$-dependent ECB mobilization was responsible, and that ECBs did not spill over from other cells. Another emerging theme highlighted in the POMC experiments was that, although eEPSCs were not suppressed by tonic ECBs, they were suppressed by WIN55212-2. When the ECB transport blocker VDM-11 was present, ECB-mediated suppression of eEPSCs did occur and this was suppressed by intracellular BAPTA in the recorded cell. Apparently CB_1Rs on excitatory terminals are located far enough from other sources of ECBs that, even with uptake blocked, ECBs cannot travel to them. As in neocortex and hippocampus (Marsicano et al. 2003; Monory et al. 2006), it is possible that hypothalamic CB_1Rs on glutamatergic terminals serve mainly as a back-up neuroprotective system that limits further glutamate release under conditions, such as seizures, when massive release of ECB-stimulating factors occurs.

Tonic release of ECBs has also been detected in the hypothamic paraventricular and supraoptic nuclei, specifically on the oxytocin (OT)-producing magnocellular neurosecretory cells (Oliet et al. 2007). GABAergic inputs onto these cells (but not onto the vasopressin-producing cells) typically have a low probability of release. Previous work had suggested that oxytocin action in these regions suppressed the inputs via a retrograde signaling process (Kombian et al. 1997). This seemed paradoxical, since the oxytocin receptors are exclusively localized on the postsynaptic OT cells, and not on incoming nerve terminals. A CB_1R agonist mimicked and occluded the ability of OT to suppress glutamate release in the supraoptic nucleus, while a CB_1R antagonist blocked them (Hirasawa et al. 2004), demonstrating that OT is another endogenous agent that causes retrograde effects indirectly by releasing ECBs. In the magnocellular cells of both the supraoptic and

paraventricular nuclei, ECBs tonically suppress GABAergic transmission (Oliet et al. 2007), but tonic ECB mobilization was secondary to tonic release of OT. Either an OTR or a CB_1R antagonist significantly increased the probability of GABA release from the interneurons, whereas agonists decreased it. Effects of other CB_1R antagonists were mutually occlusive. Interestingly, the OT-dependent ECB mobilization was also $[Ca^{2+}]_i$-dependent, and could be blocked by chelating postsynaptic calcium. From the perspective of synaptic plasticity, the ECBs caused the OT cells to act as low pass filters: GABAergic synapses, initially having a low neurotransmitter release probability, demonstrated marked facilitation when stimulated at high frequencies (> 20 Hz). Thus, because of tonic ECB release, GABAergic inhibition would normally be blocked, and the OT cells would readily fire. However, high frequency stimulation of the interneuron would break through the ECB-suppression of GABA release and inhibit OT cell firing. By tonically releasing ECBs, the OT cells participated in a feedback loop that regulated their own firing pattern.

Having been observed by at least five different laboratories in five different brain regions, it must be accepted that tonic ECB release is a genuine experimental phenomenon. Whether or not it is a physiological phenomenon, that is, to what extent it occurs under physiological circumstances when principal cells do not have electrodes stuck in them, is not yet clear. In view of the repeated demonstrations that the tonic ECB mobilization originates in the recorded principal cell, and is sensitive to its state of $[Ca^{2+}]_i$ buffering, G-protein activation, etc., this must be a concern. Assuming it is physiologically relevant, the concept of persistent ECB suppression of certain synapses, and with it the capabilities for use-dependent frequency filtering of inputs, make it possible for ECBs to play a wider variety of regulatory roles than previously realized.

3.3 Plasticity of ECB Mobilization

Recognition that ECBs were not stored in membrane-bound vesicles and yet could be increased by various forms of stimuli in biochemical experiments led to the idea that they are produced “on demand” to meet immediate physiological needs. The on-demand hypothesis makes some predictions that have not always been met when tested in cellular physiological systems: that application of an appropriate stimulus should directly lead to ECB synthesis and that synthesis and release are essentially coupled, with ECBs being released as soon as they are produced. The on-demand hypothesis does not obviously predict plasticity of ECB mobilization.

Repetitive synaptic stimulation (Zhu and Lovinger 2007) or transient application of a group I mGluR agonist DHPG (Edwards et al. 2008) persistently enhanced submaximal hippocampal DSI in CA1. Zhu and Lovinger also showed that low frequency (1 Hz) synaptic stimulation in stratum radiatum for 5 min also induced iLTD at these GABAergic synapses. Both iLTD and enhancement of DSI were prevented by pretreatment with mGluR antagonists. Short-term DHPG application

had a similar effect, and if mGluR antagonists were applied as DHPG was washed from the chamber, the DSI increase persisted (Edwards et al. 2008). Strong activation of mAChRs for several minutes increased DSI but did not have a lasting effect, implying that activation of ECB_{GPCR} alone was not sufficient to upregulate the ECB_{Ca} system. Since DSI can facilitate LTP induction (Carlson et al. 2002), upregulation of DSI can have lasting consequences. DSI and other forms of ECB_{Ca} are transient in nature, and may therefore offer greater flexibility in certain forms of neuronal network modification than long-lasting plasticities.

In many cells, application of an mGluR agonist may not mobilize ECBs even at high concentrations and even though the cells are otherwise capable of mobilizing them. Edwards et al. (2008) found that when the cells are first "primed" with a brief intense influx of Ca, then the same mGluR stimulus leads to robust ECB mobilization. At first glance the priming process has a lot in common with the molecular coincidence detector mechanism (Hashimotodani et al. 2005). Some critical features distinguish coincidence detection from priming, however. First, the coincidence detector model demands a strict temporal overlap in the elevation of $[Ca^{2+}]_i$ and the activation of the G-protein coupled receptor. Priming does not require such overlap and the two stimuli can be temporally separated by many minutes and facilitation of ECB mobilization will still occur. Indeed, if a cell is stimulated to produce a large Ca influx, and then allowed to fill with a high concentration of Ca chelator for tens of minutes, subsequent application of an mGluR agonist will evoke a robust ECB response. The induction of ECB-iLTD could be primed as well. Priming was not induced by an mAChR agonist, and it was suggested that the Ca-dependent step was closely linked to intracellular pathways accessed by group I mGluRs, although these pathways have not been identified. In summary, priming represents an upstream regulatory process that adjusts the responsiveness of the ECB_{mGluR} system, i.e., it is a form of "metaplasticity" (Abraham and Bear, 1996).

3.4 ECB Transport as a Synaptically Modifiable Process

The mechanism by which ECBs traffic between cells is not certain. The first direct evidence that ECBs could actually travel from a postsynaptic cell to presynaptic terminals seems to have been provided by Gerdeman et al. (2002), who directly loaded AEA into striatal cells, and observed CB_1R-dependent depression of glutamatergic synaptic input. However, AEA or 2-AG loaded into the postsynaptic cell could not escape and activate presynaptic CB_1R receptors if a ECB transporter blocker was also loaded (Ronesi et al. 2004). Since inhibiting the transporter from within prevents ECBs from reaching the CB_1R on the adjacent synaptic terminals, it appears that ECB extrusion from postsynaptic cells may depend on transporter-mediated facilitated diffusion. Extracellular and intracellular application of the ECB transporter blocker have diametrically opposite effects on ECB-LTD initiation; the former potentiates (Gerdeman et al. 2002) and the latter inhibits induction (Ronesi et al. 2004). The difference is attributable to differences in the direction of

ECB movements in the two cases: when applied extracellularly, transporter blockers will inhibit the uptake of ECBs into surrounding cells, thus retarding their clearance and prolonging their activation of CB_1R. The ECB transporter could be a substrate for regulation of ECB actions.

This has received support in a study of medium spiny neurons loaded with either AEA or 2-AG via the whole-cell pipette (Adermark and Lovinger 2007b) to investigate ECB release. Inhibition of afferent glutamatergic or $GABA_A$ergic inputs provided the bioassay for ECBs. Neither ECB diffused out of the cell and inhibited synaptic input significantly if the synapses were stimulated only infrequently with single pulses. Remarkably, synaptic responses evoked with double pulses delivered at the same rate were quickly depressed in a CB_1R-sensitive way. Direct activation of presynaptic CB_1R by WIN55212-2 was not stimulus-dependent, and conversely manipulations of postsynaptic cell properties – membrane potential, or $[Ca^{2+}]_i$ – did not alter ECB-mediated synaptic inhibition. The effects of loaded ECBs were prevented by co-loading the cell with ECB transport inhibitors, VDM-11 or AM404. Evidently, the rate of transporter-dependent postsynaptic release of ECBs was a function of afferent stimulation rate. The situation was similar at both excitatory and inhibitory synapses, except that inhibition of GABA release was much more sensitive to ECB release and, while stimulation did facilitate it, the release was not sensitive to the pattern of afferent activation. It is not clear if release or endogenous generation of cannabinoids is regulated by the same process, and the connection between the frequency of afferent stimulation and transport is mysterious.

3.5 The ECB System and Seizures

Seizures represent hyperexcitable brain states in which massive neuronal activity releases large quantities of neurotransmitters and neuromodulators into the extracellular space. Glutamate in particular can have numerous deleterious effects that lead to neurotoxicity. By acting on several receptor subtypes, glutamate and other neurotransmitters can also release ECBs, as can cellular depolarization, rise in intracellular $[Ca^{2+}]_i$, and other concomitants of seizures. As reviewed elsewhere in this volume "Genetic Models of the Endocannabinoid System" (Lutz), seizure-induced ECB release, by acting principally on CB_1Rs on glutamatergic terminals, can blunt the release of glutamate, and thereby retard and restrict the extent of neurotoxic damage. This work was based on the use of novel genetic mouse models involving targeted CB_1R deletions in various cell populations.

Seizures have other effects on the ECB system, and some have been assessed in physiological studies of the ECB system. In a developmental model, a single febrile seizure persistently upregulated ECB-mediated DSI in the hippocampal CA1 region (Chen et al. 2003). The strength of DSE was not affected, suggesting that the increase in DSI might not represent an increase in ECB mobilization. Responses to the exogenous cannabinoid, WIN55212-2, were also enhanced, implying that increased DSI might reflect an increase in CB_1R number, and Western blot analysis

revealed that in fact CB_1R number was increased by the seizure. As the numbers of CB_1R-expressing nerve terminals were not altered, the conclusion was that the density of CB_1Rs per terminal must have increased. Although the data were clear, the result was somewhat puzzling in view of the extremely high density of CB_1Rs that are normally found on the interneuron terminals (Freund et al. 2003). The relationship between CB_1R number and its response is not worked out, so a twofold increase in receptor number could perhaps explain the data. It will be interesting to learn if other aspects of CB_1R functioning may also be affected by seizure activity. A follow-up study, using tetanic stimulation of in vitro slices to model the seizure, confirmed the increase in CB_1Rs, although quantitatively the effect was smaller than the febrile seizure model (Chen et al. 2007). A prior in vivo seizure prevented subsequent in vitro tetanus-induced enhancement of DSI. The in vitro study added the novel information that CB_1R activation itself was necessary for the elevation of CB_1R number. Treatment with AM251 during the in vivo seizure stimulation prevented the in vitro increase in DSI.

These studies revealed complex regulation of the ECB system by seizures. The proposal was that seizure-induced upregulation of CB_1Rs on inhibitory terminals would, by suppressing inhibition, contribute to the development of the postseizure, hyperexcitable state. Prevention of CB_1R activation with a CB_1R antagonist during the seizure might increase excitability at that time, but be helpful in the long run by preventing the development of persistent hyperexcitability that is a deleterious sequel to febrile seizures.

Clearly the potential scenarios emerging from the genetic and physiological studies outlined above present very different pictures of the roles of CB_1Rs in epilepsy. Recently a comparative study of hippocampal tissue from human epileptic and control brains has reported results that appear to be in general agreement with the studies of genetically engineered mice (Ludanyi et al. 2008). Quantitative PCR and electron microscopy revealed a significant down-regulation of CB_1R, DGLα, and a CB_1R-interacting protein (CRIP-1a). There were no changes in other relevant enzymes. In a cellular comparison, it was found in the hippocampal dentate gyrus that there was a robust reduction in CB_1R levels associated with glutamatergic terminals with no changes in the receptors on GABAergic terminals. In accordance with the studies on modified mice, these data point to the loss of an ECB-mediated neuroprotective function resulting from repeated seizures. The physiological and therapeutic implications are complex. Administering CB_1R agonists might not be very effective if the CB_1Rs most important for excitability control are simply missing.

3.6 Interactions Between AEA and 2-AG

Although consensus is developing that 2-AG is the major ECB in the brain, AEA is an agonist of CB_1R and is produced by various stimuli, including $[Ca^{2+}]_i$ and neurotransmitters. It could be that in different brain regions one or the other

predominates. Yet the relationship between AEA and 2-AG has never been clarified, and it is possible that the two interact in some way. Maccarrone et al. (2008) have reported that mGluR5 (not mGluR1) activation in the striatum mobilizes ECBs and also increases CB_1R binding, perhaps by affecting receptor recycling. Contrary to a previous report (Giuffrida et al. 1999), stimulation increased 2-AG, but not AEA levels. The mGluR agonist also increased the activity of DGL and reduced MGL activity, both effects being associated with an increase in 2-AG levels. Most interestingly, increases in AEA, induced either by exogenous AEA or by downregulation of FAAH, decreased 2-AG and 2-AG-mediated actions. The common understanding that both AEA and 2-AG are primarily endocannabinoids might suggest competitive or other interactions among ECB-regulatory pathways. On the contrary, Maccarrone et al. (2008), showed that all of AEA's effects on 2-AG were mediated by TRPV1 channels, being abolished by pharmacological antagonism or genetic deletion of TRPV1 channels. AEA acting in its capacity as an endovanilloid (Starowicz et al. 2007) was responsible for the downregulation of 2-AG. It appears that TRPV1 inhibits glutathione-stimulated DGL, and hence the increases in 2-AG stimulated by DHPG. This critically important study demands follow-up, which, if confirmed, will certainly galvanize a major reevaluation of previous results pertaining to AEA and FAAH throughout the brain. In the context of ECB-mediated plasticity, it is easy to imagine how various mechanisms of AEA up- or downregulation would modify 2-AG-mediated synaptic plasticity.

AEA may be involved in a different form of regulation in the striatum. Ade and Lovinger (2007) showed that high-frequency stimulation (HFS) of glutamatergic afferents in dorsolateral induced LTP in young animals (PN12-14), but LTD in PN16-34 animals. This developmental shift in plasticity was correlated with changes in AEA levels. In young animals, stimulation increased only AEA levels without affecting 2-AG, and applied AEA permitted LTD induction. In the older animals, blocking CB_1R during HFS-induced LTD, and inhibiting the synthetic enzyme, DGL, had no effect on LTD. The authors suggest that a developmental increase in AEA may be the key factor in the shift from LTP to LTD induction with age.

4 Conclusion

This chapter has attempted an overview of the rapidly expanding field of ECBs and synaptic plasticity. The area is growing in both depth and breadth: firmly established phenomena, such as ECB-LTD and -iLTD are being investigated in greater cellular and molecular detail, and new phenomena, such as tonic ECB actions, the role of glia, and the interactions between AEA and 2-AG are coming to light. It appears that the field is in the phase of exuberant growth that characterizes developing systems; an eventual pruning back of some of the more extravagant claims, recognition of hidden connections between apparently disparate data, and a

further opening up of new vistas of regulation, both of and by ECBs, are all likely to occur.

References

Abraham WC, Bear MF (1996) Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci 19:126–130

Abraham WC, Gustafsson B, Wigstrom H (1987) Long-term potentiation involves enhanced synaptic excitation relative to synaptic inhibition in guinea-pig hippocampus. J Physiol (Lond) 394:367–380

Adermark L, Lovinger DM (2007a) Combined activation of L-type Ca2+ channels and synaptic transmission is sufficient to induce striatal long-term depression. J Neurosci 27:6781–6787

Ade KK, Lovinger DM (2007) Anandamide regulates postnatal development of long-term synaptic plasticity in the rat dorsolateral striatum. J Neurosci 27(9):2403–2409.

Adermark L, Lovinger DM (2007b) Retrograde endocannabinoid signaling at striatal synapses requires a regulated postsynaptic release step. Proc Natl Acad Sci USA 104:20564–20569

Alger BE (2002) Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. Prog Neurobiol 68:247–286

Alger BE, Pitler TA (1995) Retrograde signaling at GABAA-receptor synapses in the mammalian CNS. Trends Neurosci 18:333–340

Alger BE, Pitler TA, Wagner JJ et al. (1996) Retrograde signalling in depolarization-induced suppression of inhibition in rat hippocampal CA1 cells. J Physiol (Lond) 496:197–209

Ali AB (2007) Presynaptic inhibition of GABAA receptor mediated unitary IPSPs by cannabinoid receptors at synapses between CCK-positive interneurons in rat hippocampus. J Neurophysiol 98:861–869

Azad SC, Monory K, Marsicano G et al. (2004) Circuitry for associative plasticity in the amygdala involves endocannabinoid signaling. J Neurosci 24:9953–9961

Azad SC, Kurz J, Marsicano G et al. (2008) Activation of CB1 specifically located on GABAergic interneurons inhibits LTD in the lateral amygdala. Learn Memory 15:143–152

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

Bean BP (1989) Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. Nature 340:153–156

Beierlein M, Regehr WG (2006) Local interneurons regulate synaptic strength by retrograde release of endocannabinoids. J Neurosci 26:9935–9943

Beierlein M, Fioravante D, Regehr WG (2007) Differential expression of posttetanic potentiation and retrograde signaling mediate target-dependent short-term synaptic plasticity. Neuron 54:949–959

Bender VA, Bender KJ, Brasier DJ et al. (2008) Two coincidence detectors for spike timing-dependent plasticity in somatosensory cortex. J Neurosci 26:4166–4177

Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol (Lond) 232:331–356

Bouaboula M, Perrachon S, Milligan L et al. (1997) A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. J Biol Chem 272:22330–22339

Brenowitz SD, Regehr WG (2003) Calcium dependence of retrograde inhibition by endocannabinoids at synapses onto Purkinje cells. J Neurosci 23:6373–6384

Brenowitz SD, Best AR, Regehr WG (2006) Sustained elevation of dendritic calcium evokes widespread endocannabinoid release and suppression of synapses onto cerebellar Purkinje cells. J Neurosci 26:6841–6850

Brown SP, Brenowitz SD, Regehr WG (2003) Brief presynaptic bursts evoke synapse-specific retrograde inhibition mediated by endogenous cannabinoids. Nat Neurosci 6:1048–1057

Brown SP, Safo PK, Regehr WG (2004) Endocannabinoids inhibit transmission at granule cell to Purkinje cell synapses by modulating three types of presynaptic calcium channels. J Neurosci 24:5623–5631

Brown SP, Brenowitz SD, Regehr W (2003) Brief presynaptic bursts evoke synapse-specific retrograde inhibition mediated by endogenous cannabinoids. Nat Neurosci 6:1048–1057

Carlson G, Wang Y, Alger BE (2002) Endocannabinoids facilitate the induction of LTP in the hippocampus. Nat Neurosci 5:723–724

Castillo PE, Schoch S, Schmitz F et al. (2002) RIM1α is required for presynaptic long-term potentiation. Nature 415:327–330

Chen K, Ratzliff A, Hilgenberg L et al. (2003) Long-term plasticity of endocannabinoid signaling induced by developmental febrile seizures. Neuron 39:599–611

Chen K, Neu A, Howard AL et al. (2007) Prevention of plasticity of endocannabinoid signalling inhibits persistent limbic hyperexcitability caused by developmental seizures. J Neurosci 27:46–58

Chevaleyre V, Castillo PE (2003) Heterosynaptic LTD of hippocampal GABAergic synapses. A novel role of endocannabinoids in regulating excitability. Neuron 38:461–472

Chevaleyre V, Castillo PE (2004) Endocannabinoid-mediated metaplasticity in the hippocampus. Neuron 43:871–881

Chevaleyre V, Takahashi KA, Castillo PE (2006) Endocannabinoid-mediated synaptic plasticity in the CNS. Annu Rev Neurosci 29:37–76

Chevaleyre V, Heifets BD, Kaeser PS et al. (2007) Endocannabinoid-mediated long-term plasticity requires cAMP/PKA signaling and RIM1α. Neuron 54:801–812

Crozier RA, Wang Y, Liu C-H et al. (2007) Deprivation-induced synaptic depression by distinct mechanisms in different layers of mouse visual cortex. Proc Natl Acad Sci USA 104:1383–1388

Dan Y, Poo M (2004) Spike timing-dependent plasticity of neural circuits. Neuron 44:23–30

Deadwyler SA, Hampson RE, Mu J et al. (1995) Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-dependent process. J Pharmacol Exp Ther 273:734–743

Di S, Malcher-Lopes R, Marcheselli VL et al. (2005) Rapid glucocorticoid-mediated endocannabinoid release and opposing regulation of glutamate and gamma-aminobutyric acid inputs to hypothalamic magnocellular neurons. Endocrinology 145:4292–4301

Diana MA, Marty A (2003) Characterization of depolarization-induced suppression of inhibition using paired interneuron – Purkinje cell recordings. J Neurosci 23:5906–5918

Dinh TP, Freund TF, Piomelli D (2002) A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. Chem Phys Lipids 121:149–158

Edwards DA, Kim J, Alger BE (2006) Multiple mechanisms of endocannabinoid response initiation in hippocampus. J Neurophysiol 95:67–75

Edwards DA, Zhang L, Alger BE (2008) Metaplastic control of the endocannabinoid system at inhibitory synapses in hippocampus. Proc Natl Acad Sci USA 105:8142–8147

Foldy C, Neu A, Jones MV et al. (2006) Presynaptic, activity-dependent modulation of cannabinoid type 1 receptor-mediated inhibition of GABA release. J Neurosci 26:1465–1469

Foldy C, Lee SY, Szabadics J et al. (2007) Cell type-specific gating of perisomatic inhibition by cholecystokinin. Nature Neurosci 10:1128–1130

Fortin DA, Levine ES (2007) Differential effects of endocannabinoids on glutamatergic and GABAergic inputs to layer 5 pyramidal neurons. Cereb Cortex 17:163–174

Fortin DA, Trettel J, Levine ES (2004) Brief trains of action potentials enhance pyramidal neuron excitability via endocannabinoid-mediated suppression of inhibition. J Neurophysiol 92:2105–2112

Freund TF, Katona I, Piomelli D (2003) Role of endogenous cannabinoids in synaptic signaling. Physiol Rev 83:1017–1066
Gerdeman G, Lovinger DM (2001) CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. J Neurophysiol 85:468–471
Gerdeman GL, Ronesi J, Lovinger DM (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nature Neurosci 5:446–451
Gibson HE, Edwards JG, Page RS et al. (2008) TRPV1 channels mediate long-term depression at synapses on hippocampal interneurons. Neuron 57:746–759
Giuffrida A, Parsons LH, Kerr TM et al. (1999) Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nature Neurosci 2:358–363
Gubellini P, Pisani A, Centonze D et al. (2004) Metabotropic glutamate receptors and striatal synaptic plasticity: implications for neurological diseases. Prog Neurobiol 74:271–300
Hampson RE, Zhuang SY, Weiner JL et al. (2003) Functional significance of cannabinoid-mediated, depolarization induced suppression of inhibition (DSI) in the hippocampus. J Neurophysiol 90:55–64
Hashimotodani Y, Ohno-Shosaku T, Tsubokawa H et al. (2005) Phospholipase Cβ serves as a coincidence detector through its Ca2+ dependency for triggering retrograde endocannabinoid signal. Neuron 45:257–268
Hashimotodani Y, Ohno-Shosaku T, Kano M (2007) Presynaptic monoacylglycerol lipase activity determines basal endocannabinoid tone and terminates retrograde endocannabinoid signaling in the hippocampus. J Neurosci 27:1211–1219
Heinbockel T, Brager DH, Reich CG et al. (2005) Endocannabinoid signaling dynamics probed with optical tools. J Neurosci 25:9449–9459
Hentges ST, Low MJ, Williams JT (2005) Differential regulation of synaptic inputs by constitutively released endocannabinoids and exogenous cannabinoids. J Neurosci 25:9746–9751
Hirasawa M, Schwab Y, Natah S et al. (2004) Dendritically released transmitters cooperate via autocrine and retrograde actions to inhibit afferent excitation in rat brain. J Physiol (Lond) 559:611–624
Hoffman AF, Lupica CR (2000) Mechanisms of cannabinoid inhibition of $GABA_A$ synaptic transmission in the hippocampus. J Neurosci 20:2470–2479
Hoffman AF, Riegel AC, Lupica CR (2003) Functional localization of cannabinoid receptors and endogenous cannabinoid production in distinct neuron populations of the hippocampus. Eur J Neurosci 18:524–534
Ikeda SR (1991) Double-pulse calcium channel current facilitation in adult rat sympathetic neurones. J Physiol (Lond) 439:181–214
Isokawa M, Alger BE (2006) Ryanodine receptor regulates endogenous cannabinoid mobilization in the hippocampus. J Neurophysiol 95:3001–3011
Ito M (2001) Cerebellar long-term depression: characterization, signal transduction, and functional roles. Physiol Rev 81:1143–1195
Kawamura Y, Fukaya M, Maejima T et al. (2006) The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. J Neurosci 26:2991–3001
Kim J, Isokawa M, Ledent C et al. (2002) Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. J Neurosci 22:10182–10191
Klapstein GJ, Colmers WF (1992) 4-Aminopyridine and low Ca2+ differentiate presynaptic inhibition mediated by neuropeptide Y, baclofen and 2-chloroadenosine in rat hippocampal CA1 in vitro. Br J Pharmacol 105:470–474
Kombian SB, Mouginot D, Pittman QJ (1997) Dendritically released peptides act as retrograde modulators of afferent excitation in the supraoptic nucleus in vitro. Neuron 19:903–912
Kreitzer AC, Malenka RC (2005) Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. J Neurosci 25:10537–10545
Kreitzer AC, Regehr WG (2001) Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. Neuron 29:717–727

Kreitzer AC, Carter AG, Regehr WG (2002) Inhibition of interneuron firing extends the spread of endocannabinoid signaling in the cerebellum. Neuron 34:787–796

Lenz RA, Wagner JJ, Alger BE (1998) N- and L-type calcium channel involvement in depolarization-induced suppression of inhibition in rat hippocampal CA1 cells. J Physiol (Lond) 512:61–73

Liu C-H, Heynen AJ, Schuler MG et al. (2008) Cannabinoid receptor blockade reveals parallel plasticity mechanisms in different layers of mouse visual cortex. Neuron 58:340–345

Llano I, Leresche N, Marty A (1991) Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. Neuron 6:565–574

Losonczy A, Biro AA, Nusser Z (2004) Persistently active cannabinoid receptors mute a subpopulation of hippocampal interneurons. Proc Natl Acad Sci USA 101:1362–1367

Ludányi A, Eross L, Czirják S, Vajda J, Halász P, Watanabe M, Palkovits M, Maglóczky Z, Freund TF, Katona I (2008) Downregulation of the CB1 cannabinoid receptor and related molecular elements of the endocannabinoid system in epileptic human hippocampus. J Neurosci 28:2976–2990

Maccarrone M, Rossi S, Bari M et al. (2008) Anandamide inhibits metabolism and physiological actions of 2-arachidonoyglycerol in the striatum. Nature Neurosci 11:152–159

Maejima T, Hasimoto K, Yoshida T et al. (2001) Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. Neuron 31:463–475

Maejima T, Oka S, Hashimotodani Y et al. (2005) Synaptically driven endocannabinoid release requires Ca2+-assisted metabotropic glutamate receptor subtype 1 to phospholipase C β4 signaling cascade in the cerebellum. J Neurosci 25:6826–6835

Makara JK, Katona I, Nyiri G et al. (2007) Involvement of nitric oxide in depolarization-induced suppression of inhibition in hippocampal pyramidal cells during activation of cholinergic receptors. J Neurosci 27:10211–10222

Marcaggi P, Attwell D (2005) Endocannabinoid signaling depends on the spatial pattern of synapse activation. Nat Neurosci 8:776–781

Marsicano G, Wotjak CT, Azad SC et al. (2002) The endogenous cannabinoid system controls extinction of aversive memories. Nature 418:530–534

Marsicano G, Goodenough S, Monory K et al. (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science 302:84–88

Melis M, Perra S, Muntoni AL et al. (2004a) Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. J Neurosci 24:10707–10715

Melis M, Pistis M, Perra S et al. (2004b) Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. J Neurosci 24:53–62

Monory K, Massa F, Egertova M et al. (2006) The endocannabinoid system controls key epileptogenic circuits in the hippocampus. Neuron 51:455–466

Mu J, Zhuang S-Y, Hampson RE et al. (2000) Protein kinase-dependent phosphorylation and cannabinoid receptor modulation of potassium A current (IA) in cultured rat hippocampal neurons. Pflugers Arch 439:541–546

Mukhtarov M, Ragozzino D, Bregestovski P (2005) Dual Ca2+ modulation of glycinergic synaptic currents in rodent hypoglossal motoneurones. J Physiol (Lond) 569:817–831

Neu A, Foldy C, Soltesz I (2007) Postsynaptic origin of CB1-dependent tonic inhibition of GABA release at CCK-positive basket cell to pyramidal cell synapses in the CA1 region of the rat hippocampus. J Physiol (Lond) 578:233–247

Nevian T, Sakmann B (2006) Spine Ca2+ signalling in spike-timing-dependent plasticity. J Neurosci 26:11001–11013

Ohno-Shosaku T, Maejima T, Kano M (2001) Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. Neuron 29:729–738

Ohno-Shosaku T, Tsubokawa H, Mizushima I et al. (2002) Presynaptic cannabinoid sensitivity is a major determinant of depolarization-induced retrograde suppression at hippocampal synapses. J Neurosci 22:3864–3872

Ohno-Shosaku T, Matsui M, Fukudome Y et al. (2003) Postsynaptic M1 and M3 receptors are responsible for the muscarinic enhancement of retrograde endocannabinoid signalling in the hippocampus. Eur J Neurosci 18:109–116

Ohno-Shosaku T, Hashimotodani Y, Ano M et al. (2007) Endocannabinoid signaling triggered by NMDA receptor-mediated calcium entry into rat hippocampal neurons. J Physiol (Lond) 584:407–418

Oliet SHR, Baimoukhametova DV, Piet R et al. (2007) Retrograde regulation of GABA transmission by the tonic release of oxytocin and endocannabinoids governs postsynaptic firing. J Neurosci 27:1325–1333

Pan B, Hillard CJ, Liu QS (2008) Endocannabinoid signaling mediates cocaine-induced inhibitory synaptic plasticity in midbrain dopamine neurons. J Neurosci 28:1385–1397

Pertwee RG (2005) Inverse agonism and neutral antagonism at cannabinoid CB1 receptors. Life Sci 76:1307–1324

Pitler TA, Alger BE (1992) Postsynaptic spike firing reduces synaptic GABAA responses in hippocampal pyramidal cells. J Neurosci 12:4122–4132

Reich CG, Karson MA, Karnup SV et al. (2005) Regulation of IPSP theta rhythms by muscarinic receptors and endocannabinoids in hippocampus. J Neurophysiol 94:4290–4299

Robbe D, Kopf M, Remaury A et al. (2002) Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. Proc Natl Acad Sci USA 99:8384–8388

Robbe D, Montgomery SM, Thome A et al. (2006) Cannabinoids reveal importance of spike timing coordination in hippocampal function. Nat Neurosci 9:1526–1533

Ronesi J, Gerdeman GL, Lovinger DM (2004) Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. J Neurosci 24:1673–1679

Ryberg E, Larsson N, Sjogren S et al. (2007) The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol 152:1092–1101

Safo PK, Regehr WG (2005) Endocannabinoids control the induction of cerebellar LTD. Neuron 48:647–659

Shin JH, Linden DJ (2005) An NMDA receptor/nitric oxide cascade is involved in cerebellar LTD but is not localized to the parallel fiber terminal. J Neurophysiol 94:4281–4289

Singla S, Kreitzer AC, Malenka RC (2007) Mechanisms for synapse specificity during striatal long-term depression. J Neurosci 27:5260–5264

Sjostrom PJ, Turrigiano GG, Nelson SB (2003) Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors. Neuron 39:641–654

Sjostrom PJ, Turrigiano GG, Nelson SB (2004) Endocannabinoid-dependent neocortical layer-5 LTD in the absence of postsynaptic spiking. J Neurophysiol 92:3338–3343

Starowicz K, Nigam S, Di Marzo V (2007) Biochemistry and pharmacology of endovanilloids. Pharmacol Ther 114:13–33

Szabadits E, Cserep C, Ludanyi A et al. (2007) Hippocampal GABAergic synapses possess the molecular machinery for retrograde nitric oxide signaling. J Neurosci 27:8101–8111

Takahashi KA, Linden DJ (2000) Cannabinoid receptor modulation of synapses received by cerebellar Purkinje cells. J Neurophysiol 83:1167–1180

Trettel J, Levine ES (2002) Cannabinoids depress inhibitory synaptic inputs received by layer 2/3 pyramidal neurons of the neocortex. J Neurophysiol 88:534–539

Tzounopoulos T, Rubio ME, Keen JE et al. (2007) Coactivation of pre- and postsynaptic signaling mechanisms determines cell-specific spike-timing-dependent plasticity. Neuron 54:291–301

van Beugen BJ, Nagaraja RY, Hansel C (2006) Climbing fiber-evoked endocannabinoid signaling heterosynaptically suppresses presynaptic cerebellar long-term potentiation. J Neurosci 26:8289–8294

Varma N, Carlson GC, Ledent C et al. (2001) Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. J Neurosci 21(RC188):1–5

Varma N, Brager DH, Morishita W et al. (2002) Presynaptic factors in the regulation of DSI expression in hippocampus. Neuropharmacology 43:550–562

Vasquez C, Lewis DL (1999) The CB1 cannabinoid receptor can sequester G-proteins, making them unavailable to couple to other receptors. J Neurosci 19:9271–9280

Wagner JJ, Alger BE (1996) Increased neuronal excitability during depolarization-induced suppression of inhibition in rat hippocampus. J Physiol (Lond) 495:107–112

Wang J, Zucker RS (2001) Photolysis-induced suppression of inhibition in rat hippocampal CA1 pyramidal neurons. J Physiol (Lond) 533:757–763

Wilson RI, Nicoll RA (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 410:588–592

Wilson RI, Kunos G, Nicoll RA (2001) Presynaptic specificity of endocannabinoid signaling in the hippocampus. Neuron 31:453–462

Yamasaki M, Hashimoto K, Kano M (2006) Miniature synaptic events elicited by presynaptic Ca2+ rise are selectively suppressed by cannabinoid receptor activation in cerebellar Purkinje cells. J Neurosci 26:86–95

Yasuda H, Huang Y, Tsumoto T (2008) Regulation of excitability and plasticity by endocannabinoids and PKA in developing hippocampus. Proc Natl Acad Sci USA 105:3106–3111

Yin HH, Lovinger DM (2006) Frequency-specific and D2 receptor-mediated inhibition of glutamate release by retrograde endocannabinoid signaling. Proc Natl Acad Sci USA 103:8251–8256

Yin HH, Davis MI, Ronesi JA et al. (2006) The role of protein synthesis in striatal long-term depression. J Neurosci 26:11811–11820

Zhu PJ, Lovinger DM (2005) Retrograde endocannabinoid signaling in a postsynaptic neuron/synaptic bouton preparation from basolateral amygdala. J Neurosci 25:6199–6207

Zhu PJ, Lovinger DM (2007) Persistent synaptic activity produces long-lasting enhancement of endocannabinoid modulation and alters long-term synaptic plasticity. J Neurophysiol 97:4386–4389