

Current Review

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Seizing an Opportunity for the Endocannabinoid System

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Exogenous cannabinoids can limit seizures and neurodegeneration, and their actions are largely mimicked by endogenous cannabinoids (endocannabinoids). Endocannabinoids are mobilized by epileptiform activity and in turn influence this activity by inhibiting synaptic transmission; both excitatory and some inhibitory synapses can be suppressed, leading to potentially complex outcomes. Moreover, the endocannabinoid system is not a fixed entity, and its strength can be enhanced or reduced. Endocannabinoids and their receptors are altered by epileptic seizures in ways that can reduce the efficacy of both exogenous and endogenous cannabinoids in sometimes unexpected ways.

Exogenous cannabinoids are receiving renewed attention for their anticonvulsant properties in the context of medical marijuana. Alternative or adjunctive approaches seek to exploit the endogenous cannabinoid (endocannabinoid, eCB) system to take advantage of the suppressive power of the brain's main cannabinoid receptor, CB1R, while avoiding some of the drawbacks associated with marijuana use. Collectively, the eCBs, their receptors, degradative and synthetic enzymes, uptake systems, and CB1Rs compose the endocannabinoid system (ECS). Though generally anticonvulsant at low doses, exogenous cannabinoids can cause seizures at high doses. While non-ECS-related reasons for this profile are conceivable, it is also possible that the inherent complexity of the ECS could also explain it. This brief review highlights a few of opportunities as well as the complexities that arise in the quest to use eCBs in the treatment of epilepsy involving hippocampal and neocortical systems. Space does not permit a discussion of cannabidiol (CBD) (1), a nonpsychotropic cannabinoid that does not directly affect CB1R but reportedly is anticonvulsant in certain forms of epilepsy (2).

Drugs derived from cannabis plants are "cannabinoids," and the psychoactive agent, tetrahydrocannabinol (THC) is an agonist of CB1R. The normal agonists of CB1R are eCBs, mainly the fatty acid derivatives, N-acetyethanolamide (anandamide) and 2-arachidonolyglycerol (2-AG), with 2-AG being predominant in most cases (3). eCBs are produced and released ("mobilized") from postsynaptic cells and travel across the synaptic cleft in the reverse ("retrograde") direction from conventional neurotransmission. Anandamide is metabolized by fatty acid amide hydrolase (FAAH), while 2-AG is degraded by monoglyceride lipase (MGL) (3) and α - β -hydrolase domain 6 (ABHD6) (4).

CB1Rs are primarily found on axonal terminals near presynaptic release sites. CB1R activation always reduces neurotransmitter release, regardless of transmitter identity. eCBs can be mobilized by strong postsynaptic activity that raises intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, in pyramidal cells; the resulting reduction of GABA release is called "depolarization-induced suppression of inhibition" (DSI), whereas reduction of glutamate release, which happens when eCBs released from pyramidal cells activate CB1Rs on glutamatergic cells, is called DSE. DSI and DSE are transient (tens of seconds) periods of depressed transmitter release. A separate, Ca^{2+} -independent mobilization of eCBs is caused by activation of some G-protein-coupled receptors (GPCRs), especially type I metabotropic glutamate receptors (mGluRs) (5, 6) and M1 or M3 types of muscarinic acetylcholine receptors (mAChRs) (7) (8); the Ca^{2+} -dependent and -independent pathways also interact synergistically (5), probably via PLC β 1 (9). Hence eCB mobilization can be driven exclusively by postsynaptic ($[Ca^{2+}]_i$ -dependent) or presynaptic (GPCR-dependent) processes or by their combination. Minutes-long stimulation of CB1Rs by mGluRs causes long-term forms of synaptic depression (LTD) lasting for ≥ 1 hours (10).

Epileptiform activity often arises when an imbalance of excitatory (E) and inhibitory (I) synaptic transmission (the E/I ratio) within a neuronal circuit causes increased E, decreased I, or both. The possibility that eCBs might participate in E/I shifts in the hippocampus and neocortex is implicit in the distribution of CB1Rs, which are found at high density on certain inhibitory interneuron synapses and at very low density at excitatory synapses (3). Given the universal inhibitory actions of eCBs on transmitter release, their net effect on neuronal circuits comprising both excitatory and inhibitory elements is not easy to predict.

Circuit Breaker or Dimmer Switch?

Cannabinoids have been viewed as "circuit breakers" because of their ability to halt seizures and limit degeneration (11).

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eCBs are mobilized by brain insults that cause widespread cellular depolarization accompanied by Ca^{2+} influx and the massive release of GPCR-coupled neurotransmitters. By inhibiting further glutamate release, eCBs help dampen seizures and reduce the neuronal cell death that occurs as a consequence of status epilepticus.

The effects of eCBs are more nuanced than the circuit breaker model suggests, however. While eCBs are sufficiently powerful to silence a synapse completely, silencing can be overcome, for example, by blocking axonal K^+ channels (12) or vigorously activating the presynaptic (CB1R-expressing) neuron (13, 14). Because CB1Rs are generally confined to the synaptic axonal terminal away from the neuronal cell body and dendrites, CB1R activation does not influence somatic action potential firing. As a result, excitation of dendrites can trigger repetitive somatic action potentials that readily travel to the synaptic terminals where they can reduce and ultimately abolish the CB1R-mediated inhibition of release. If overall excitability is not kept within certain bounds, cannabinoid actions that are effective in containing moderate levels of excitability may wane and lose the ability to limit glutamate release. The resulting increase will boost excitability, further decrease eCB efficacy, and so on; the ensuing positive feedback could contribute to the onset of seizures.

An example of modulation of eCB-mediated synaptic suppression occurs in the case of mAChRs in hippocampus and neocortex. The mAChR agonists stimulate CB1R+ interneurons, while eliciting eCB release from pyramidal cells (7). In brain slices, there is a continuous barrage of IPSPs that remains highly sensitive to eCB-mediated DSI (15–17). Clearly, the IPSPs originate from CB1R+ interneurons and are not fully blocked. The interaction between eCB inhibition and direct mAChR-dependent excitation of the interneurons achieves an intermediate, steady-state level of synaptic transmission that can either be further depressed by an increase in the eCBs (i.e., by DSI), or increased by a CB1R antagonist.

Such considerations are directly relevant to the study of epilepsy: Pilocarpine is a convulsant that is used experimentally to induce status epilepticus, leading to spontaneous seizures that develop over a period of days to weeks. After establishment of the epileptic state in the mouse pilocarpine model of TLE, application of anandamide or 2-AG suppresses the frequency of spontaneous excitatory postsynaptic currents and secondary population discharges in the dentate gyrus (18), that is, the eCBs are anticonvulsant in this model. Yet pilocarpine is also an mAChR agonist that mobilizes copious quantities of eCBs. Why do these eCBs not prevent the initial seizure induction? They are certainly produced because the severity of the seizures during the induction phase is markedly enhanced if activation of CB1R is prevented either pharmacologically or by genetic deletion (19). Similar to their effects in *in vitro* slices, mAChRs probably simultaneously excite cells and release eCBs that only partially counteract the glutamate release; they cannot fully break the circuit.

Epileptiform Activity Generates eCBs and Vice Versa

The artificial stimuli most often used to induce short-term eCB mobilization are 1- to 10-second-long depolarizations of the postsynaptic neuronal membrane by about 70 mV. Such

events crudely resemble epileptiform paroxysmal depolarizing shifts (PDSs), or burst potentials, and trigger Ca^{2+} influx that mobilizes eCBs. Spontaneous PDSs in an Mg^{2+} -free *in vitro* model (mean duration ~0.33 seconds) (20) and “theta-bursts” induce eCB-dependent IPSP depression (21, 22). Therefore, the hyperexcitable epileptiform events themselves mobilize eCBs. Bouts of theta-burst potential firing produces a pronounced eCB-mediated, long-term depression of inhibitory synapses (iLTD) in the hippocampus (22), which will increase excitability for prolonged periods. Most importantly, stimulation that effectively mobilizes eCBs and suppresses IPSPs (DSI) does not affect hippocampal EPSPs (22, 24). Whether this is because eCBs spread only very small distances from a somatic point of mobilization to nearby GABAergic terminals or is attributable to other factors is not known (23). In any case, the eCBs produced by burst potential firing will tilt the system toward enhanced excitation.

Another factor contributing to the bias toward eCB suppression of excitation is that CB1Rs are found on much lower density on glutamatergic than on GABAergic terminals (25), but there may be others: Febrile seizures in early development cause an upregulation of CB1Rs only on inhibitory interneurons (26). Either the eCBs mobilized by the seizures could not influence the CB1Rs on the excitatory terminals, or perhaps there are differences in cellular machinery downstream of CB1Rs on the excitatory and inhibitory neurons. Evidence that widespread eCB-generating activity selectively targets inhibitory synapses is puzzling—but not unique—and is seen with GPCR-dependent eCB stimulation as well (27, 28).

Perhaps only really massive seizure activity is capable of releasing sufficient glutamate onto dendritic spine synapses to activate the mGluRs, activate the 2-AG synthetic machinery sequestered there (3), and mobilize sufficient 2-AG to achieve at least partial protection. Kainic acid (a convulsant that does not directly stimulate eCB mobilization) causes seizures and mobilizes eCBs but does not do so directly, as does pilocarpine. Rather, the increase in activity that leads to neuronal degeneration also mobilizes eCBs and limits the extent of the damage, which again is inferred from the much greater damage seen in the global absence of functional CB1Rs (29). The neuroprotective effect in the kainic acid model is provided exclusively by the CB1Rs on glutamate cells, as their selective elimination exacerbates the damage as much as does global CB1R loss (30). Elimination of CB1Rs from GABAergic interneurons was ineffective.

Mixed Reception?

Levels of surface expression of CB1R on neuronal membranes are important determinants of cannabinoid efficacy and yet are not stable, rapidly becoming down-regulated in cultured cells with steady activation of CB1R (31, 32). The degree of downregulation is also dependent on the identity of the CB1R agonist, being more marked for WIN55212-2 than for THC. In organized tissue, persistent stimulation of the ECS suppresses neurotransmitter release steadily over a period of an hour— not declining as expected if functionally significant CB1Rs were quickly disappearing from the neuronal surface. Moreover, tonic (steady-state, not directly resulting from specific stimulation) eCB actions do occur (33–35) and, indeed, may



arise as a consequence of seizure activity (27, 36). Clearly such tonic effects are at odds with rapid, use-dependent downregulation of CB1Rs. It is not known whether the receptors that are removed in these cases are superfluous, ineffective, or quickly replaced.

Pilocarpine-induced status epilepticus up-regulates CB1Rs on excitatory fibers in the dentate gyrus (18), but febrile seizures in early development cause a selective, long-lasting (at least months) upregulation of CB1Rs on CB1R+ interneuron axon terminals (26, 27). The increase of CB1Rs on inhibitory terminals will, by decreasing inhibition, increase the E/I ratio, and move the system toward hyperexcitability. Intriguingly, the upregulation of CB1Rs is itself dependent on CB1R activation during the seizures and can be prevented by CB1R antagonists (27). The implication is that a treatment that could acutely exacerbate the severity of an infantile seizure could prevent the development of a very long-lasting, perhaps permanent, alteration (increase in the density of CB1Rs at GABAergic synapses) that could predispose the system to the establishment of epilepsy. The protective effect of the CB1R antagonist occurs even if it is given after the onset of seizures, opening a potential new treatment door, if the underlying biochemical mechanism can be understood and manipulated.

Cannabinoids—exoCB and endoCBs Are Not the Same

Although both exogenous and endogenous cannabinoids act on the same receptors and have qualitatively the same inhibitory effects on neurotransmitter release, they are not the same, and their differences have practical implications. Exogenous cannabinoids act globally—essentially all CB1Rs in the brain are activated. eCBs act very locally—only CB1Rs in the immediate vicinity of the eCB-releasing cells are activated and for only as long as the eCBs are mobilized (their longer lasting sequelae, potentiation or depression, are independent of continued CB1R activation). The therapeutic implications are fairly obvious: Exogenous cannabinoids have a greater ability to interrupt seizures quickly over broad expanses of the brain. The main drawback is their influence on brain regions having nothing to do with seizures and their unwanted psychoactive side effects. Controlled stimulation of the ECS might be accomplished by selective targeting of conventional neurotransmitter receptors known to be concentrated in specific brain regions, or by limited prevention of eCB degradation. Ideally, ECS effects would be relatively restricted to the neighborhood of the cells producing eCBs. This should help avoid the psychoactive actions—but possibly at the cost of insufficient coverage of the hyperactive areas.

Behavioral tolerance to cannabis caused by changes in CB1R numbers does develop over a period of days or weeks, but the time course and even direction of the changes (up or down) are markedly different across brain regions (31, 37–39). Hopes of manipulating the ECS pharmacologically to increase eCB levels by reducing their degradation have been tempered by observations that, following prolonged and profound inhibition of the degradative enzyme for 2-AG, MGL (40), cannabinoids lose efficacy, suggesting that CB1R desensitization takes place. Interestingly, similar effects are not seen with inhibition of anandamide degradation when FAAH is blocked. Confusingly, the FAAH^{-/-} mouse has a seizure phenotype, not a seizure-

resistant one as anticipated (41), suggesting that a persistent increase in anandamide promotes—rather than prevents—hyperexcitability, perhaps by suppressing inhibition via CB1R activation. Such complexities and others pose problems for potential therapeutic strategies based on the ECS (42).

Endocannabinoids Go Up and Down in Fragile X Syndrome

A dramatic example of the challenges to implementing an ECS-based anticonvulsant therapy is provided by fragile X syndrome (FXS), an autism spectrum disorder that is the most common cause of mental retardation attributable to a single gene defect (43). FXS patients suffer from anxiety, hyperactivity, and seizures. The *Fmr1* gene, which is disrupted by large numbers of trinucleotide repeats, codes for the fragile X mental retardation protein (FMRP), a protein translation inhibitor. Loss of FMRP is associated with overexpression of a number of proteins, especially those regulated by mGluR5. In the *Fmr1*^{-/-} mutant mouse, mGluR5-dependent eCBs are produced in excess at inhibitory synapses (44–46) and cause pronounced depression of synaptic inhibition and a concomitant increase in excitatory synaptic plasticity. Surprisingly, the situation at excitatory synapses is very different, with both a deficit (47) and an excess (48) of eCBs being reported (although in autaptic cultures [48], excess production of 2-AG leads to CB1R downregulation, and eventually a functional deficit in eCB signaling). Note that a deficit of eCB at excitatory synapses will contribute to a net increase in network excitability, as will an excess of eCBs at inhibitory synapses. The major therapeutic problem is that the implications for an ECS-based treatment seem diametrically opposed: Restoring balance would involve decreasing inhibition at inhibitory synapses (e.g., with a CB1R antagonist) and increasing inhibition at excitatory synapses (e.g., with a CB1R agonist). It is not yet known if it is necessary for both to occur to prevent seizures—perhaps one or the

Highlights

1. Endocannabinoids can affect, and be affected by, seizures.
2. Cannabinoid receptors are present at both glutamatergic and some GABAergic synapses; they always inhibit transmitter release.
3. The strength of the endocannabinoid system is not fixed but can vary up or down as a function of disease process, activity-including seizures, and drug treatment, including drug identity, amount, and duration of exposure.
4. Understanding the endocannabinoid system will help explain the multitude of exogenous cannabinoid actions, including both anticonvulsant and convulsant effects.
5. Taking advantage of the ubiquity and potency of the ECS for therapeutic uses should be possible but may not be easy.



other action is dominant, but determining if it is possible to provide therapy with a single drug, or whether a combination therapy could be devised, will be challenging.

Conclusions

The ECS remains a powerful and tempting target for development of anticonvulsant drugs. While acute activation of CB1Rs can suppress abnormal excitability, many factors can diminish their efficacy with long-term continuous use, including activity-dependent modulation and receptor downregulation. Attempts to avoid possible problems associated therapeutic, long-term use of exogenous cannabinoids by targeting the ECS and altering eCBs will no doubt have to confront some of the same problems. Increasing local eCB levels by preventing their degradation eventually leads to CB1R desensitization and internalization. Exploiting the ECS for therapeutic uses will require detailed information about the underlying condition, brain region involved, and developmental age, among other considerations.

References

1. Carlini EA, Mechoulam R, Lander N. Anticonvulsant activity of four oxygenated cannabidiol derivatives. *Res Commun Chem Pathol Pharmacol* 1975;12:1–15.
2. Devinsky O, Cilio MR, Cross H, Fernandez-Ruiz J, French J, Hill C, Katz R, Di Marzo V, Jutras-Aswad D, Notcutt WG, Martinez-Orgado J, Robson PJ, Rohrback BG, Thiele E, Whalley B, Friedman D. Cannabidiol: Pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia* 2014;55(6):791–802.
3. Kano M, Ohno-Shosaku T, Hashimoto Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev* 2009;89:309–380.
4. Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, Bodor AL, Muccioli GG, Hu SS, Woodruff G, Fung S, Lafourcade M, Alexander JP, Long JZ, Li W, Xu C, Möller T, Mackie K, Manzoni OJ, Cravatt BF, Stella N. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat Neurosci* 2010;13:951–957.
5. Varma N, Carlson GC, Ledent C, Alger BE. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J Neurosci* 2001;21:RC188.
6. Maejima T, Hashimoto K, Yoshida T, Aiba A, Kano M. Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. *Neuron* 2001;31:463–475.
7. Kim J, Isokawa M, Ledent C, Alger BE. Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. *J Neurosci* 2002; 22:10182–10191.
8. Ohno-Shosaku T, Matsui M, Fukudome Y, Shosaku J, Tsubokawa H, Taketo MM, Manabe T, Kano M. Postsynaptic M1 and M3 receptors are responsible for the muscarinic enhancement of retrograde endocannabinoid signalling in the hippocampus. *Eur J Neurosci* 2003;18:109–116.
9. Hashimoto Y, Ohno-Shosaku T, Tsubokawa H, Ogata H, Emoto K, Maejima T, Araishi K, Shin HS, Kano M. Phospholipase C β serves as a coincidence detector through its Ca $^{2+}$ dependency for triggering retrograde endocannabinoid signal. *Neuron* 2005;45:257–268.
10. Heifets BD, Castillo PE. Endocannabinoid signaling and long-term synaptic plasticity. *Annu Rev Physiol* 2009;71:283–306.
11. Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat Med* 2008;14:923–930.
12. Morishita W, Kirov SA, Alger BE. Evidence for metabotropic glutamate receptor activation in the induction of depolarization-induced suppression of inhibition in hippocampal CA1. *J Neurosci* 1998;18:4870–4882.
13. Losonczy A, Biró AA, Nusser Z. Persistently active cannabinoid receptors mute a subpopulation of hippocampal interneurons. *Proc Natl Acad Sci U S A* 2004;101:1362–1367.
14. Földy C, Neu A, Jones MV, Soltesz I. Presynaptic, activity-dependent modulation of cannabinoid type 1 receptor-mediated inhibition of GABA release. *J Neurosci* 2006;26:1465–1469.
15. Pitler TA, Alger BE. Postsynaptic spike firing reduces synaptic GABA responses in hippocampal pyramidal cells. *J Neurosci* 1992;12:4122–4132.
16. Wilson RI, Nicoll RA. Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. *Nature* 2001;410:588–592.
17. Trettel J, Levine ES. Endocannabinoids mediate rapid retrograde signaling at interneuron-pyramidal neuron synapses of the neocortex. *J Neurophysiol* 2003;89:2334–2338.
18. Bhaskaran MD, Smith BN. Cannabinoid-mediated inhibition of recurrent excitatory circuitry in the dentate gyrus in a mouse model of temporal lobe epilepsy. *PLoS One* 2010;5:e10683.
19. Kow RL, Jiang K, Naydenov AV, Le JH, Stella N, Nathanson NM. Modulation of pilocarpine-induced seizures by cannabinoid receptor 1. *PLoS One* 2014;9:e95922.
20. Beau FE, Alger BE. Transient suppression of GABA A -receptor-mediated IPSPs after epileptiform burst discharges in CA1 pyramidal cells. *J Neurophysiol* 1998;79:659–669.
21. Reich CG, Karson MA, Karnup SV, Jones LM, Alger BE. Regulation of IPSP theta rhythm by muscarinic receptors and endocannabinoids in hippocampus. *J Neurophysiol* 2005;94:4290–4299.
22. Younts TJ, Chevaleyre V, Castillo PE. CA1 pyramidal cell theta-burst firing triggers endocannabinoid-mediated long-term depression at both somatic and dendritic inhibitory synapses. *J Neurosci* 2013; 33:13743–13757.
23. Alger BE. Endocannabinoids at the synapse a decade after the dies mirabilis (29 March 2001): What we still do not know. *J Physiol* 2012;590:2203–2212.
24. Wagner JJ, Alger BE. Increased neuronal excitability during depolarization-induced suppression of inhibition in rat hippocampus. *J Physiol* 1996;495:107–112.
25. Ohno-Shosaku T, Tsubokawa H, Mizushima I, Yoneda N, Zimmer A, Kano M. Presynaptic cannabinoid sensitivity is a major determinant of depolarization-induced retrograde suppression at hippocampal synapses. *J Neurosci* 2002;22:3864–3872.
26. Chen K, Ratzliff A, Hilgenberg L, Gulyás A, Freund TF, Smith M, Dinh TP, Piomelli D, Mackie K, Soltesz I. Long-term plasticity of endocannabinoid signaling induced by developmental febrile seizures. *Neuron* 2003;39:599–611.
27. Chen K, Neu A, Howard AL, Földy C, Echegoyen J, Hilgenberg L, Smith M, Mackie K, Soltesz I. Prevention of plasticity of endocannabinoid signaling inhibits persistent limbic hyperexcitability caused by developmental seizures. *J Neurosci* 2007;27:46–58.
28. Gulyás AI, Szabó GG, Ulbert I, Holderith N, Monyer H, Erdélyi F, Szabó G, Freund TF, Hájos N. Parvalbumin-containing fast-spiking basket cells generate the field potential oscillations induced by cholinergic receptor activation in the hippocampus. *J Neurosci* 2010;30:15134–15145.



29. Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Canich A, Azad SC, Cascio MG, Gutiérrez SO, van der Stelt M, López-Rodríguez ML, Casanova E, Schütz G, Zieglgänsberger W, Di Marzo V, Behl C, Lutz B. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 2003;302:84–88.
30. Monory K, Massa F, Egertová M, Eder M, Blaudzun H, Westenbroek R, Kelsch W, Jacob W, Marsch R, Ekker M, Long J, Rubenstein JL, Goebels S, Nave KA, During M, Klugmann M, Wölfel B, Dodt HU, Zieglgänsberger W, Wotjak CT, Mackie K, Elphick MR, Marsicano G, Lutz B. The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron* 2006;51:455–66.
31. Sim-Selley LJ. Regulation of cannabinoid CB1 receptors in the central nervous system by chronic cannabinoids. *Crit Rev Neurobiol* 2003;15:91–119.
32. Blair RE, Deshpande LS, Sombati S, Elphick MR, Martin BR, DeLorenzo RJ. Prolonged exposure to WIN55,212-2 causes downregulation of the CB1 receptor and the development of tolerance to its anticonvulsant effects in the hippocampal neuronal culture model of acquired epilepsy. *Neuropharmacol* 2009;57:208–218.
33. Hentges ST, Low MJ, Williams JT. Differential regulation of synaptic inputs by constitutively released endocannabinoids and exogenous cannabinoids. *J Neurosci* 2005;25:9746–9751.
34. Kim J, Alger BE. Reduction in endocannabinoid tone is a homeostatic mechanism for specific inhibitory synapses. *Nat Neurosci* 2010;13:592–600.
35. Hashimoto-dani Y, Ohno-Shosaku T, Kano M. Presynaptic monoacylglycerol lipase activity determines basal endocannabinoid tone and terminates retrograde endocannabinoid signaling in the hippocampus. *J Neurosci* 2007;27:1211–1219.
36. Deshpande LS, Sombati S, Blair RE, Carter DS, Martin BR, DeLorenzo RJ. Cannabinoid CB1 receptor antagonists cause status epilepticus-like activity in the hippocampal neuronal culture model of acquired epilepsy. *Neurosci Lett* 2007;411:11–16.
37. Zhuang S, Kittler J, Grigorenko EV, Kirby MT, Sim LJ, Hampson RE, Childers SR, Deadwyler SA. Effects of long-term exposure to delta9-THC on expression of cannabinoid receptor (CB1) mRNA in different rat brain regions. *Brain Res Mol Brain Res* 1998;62:141–149.
38. Lundberg DJ, Daniel AR, Thayer SA. Delta(9)-Tetrahydrocannabinol-induced desensitization of cannabinoid-mediated inhibition of synaptic transmission between hippocampal neurons in culture. *Neuropharmacol* 2005;49:1170–1177.
39. Falenski KW, Blair RE, Sim-Selley LJ, Martin BR, DeLorenzo RJ. Status epilepticus causes a long-lasting redistribution of hippocampal cannabinoid type 1 receptor expression and function in the rat pilocarpine model of acquired epilepsy. *Neuroscience* 2007;146:1232–1244.
40. Schlosburg JE, Blankman JL, Long JZ, Nomura DK, Pan B, Kinsey SG, Nguyen PT, Ramesh D, Booker L, Burston JJ, Thomas EA, Selley DE, Sim-Selley LJ, Liu QS, Lichtman AH, Cravatt BF. Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. *Nat Neurosci* 2010;13:1113–1119.
41. Clement AB, Hawkins EG, Lichtman AH, Cravatt BF. Increased seizure susceptibility and proconvulsant activity of anandamide in mice lacking fatty acid amide hydrolase. *J Neurosci* 2003;23:3916–3923.
42. Pacher P, Kunos G. Modulating the endocannabinoid system in human health and disease—Successes and failures. *FEBS J* 2013;280:1918–1943.
43. Bhakar AL, Dölen G, Bear MF. The pathophysiology of fragile X (and what it teaches us about synapses). *Annu Rev Neurosci* 2012;35:417–443.
44. Zhang L, Alger BE. Enhanced endocannabinoid signaling elevates neuronal excitability in fragile X syndrome. *J Neurosci* 2010;30:5724–5729.
45. Maccarrone M, Rossi S, Bari M, De Chiara V, Rapino C, Musella A, Bernardi G, Bagni C, D Centonze. Abnormal mGlu 5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. *Neuropsychopharmacol* 2010;35:1500–1509.
46. Busquets-García A1, Gomis-González M, Guegan T, Agustín-Pavón C, Pastor A, Mato S, Pérez-Samartín A, Matute C, de la Torre R, Dierssen M, Maldonado R, Ozaita A. Targeting the endocannabinoid system in the treatment of fragile X syndrome. *Nat Med* 2013;19:603–607.
47. Jung KM, Sepers M, Henstridge CM, Lassalle O, Neuhofer D, Martin H, Ginger M, Frick A, DiPatrizio NV, Mackie K, Katona I, Piomelli D, Manzoni OJ. Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. *Nat Commun* 2012;3:1080.
48. Straiker A, Min KT, Mackie K. *Fmr1* deletion enhances and ultimately desensitizes CB(1) signaling in autaptic hippocampal neurons. *Neurobiol Dis* 2013;56:1–5.