



The Double-Edged Sword: Are Endocannabinoids Doing More Harm than Good in Epilepsy?

Epilepsy Currents
2022, Vol. 22(2) 139–140
© The Author(s) 2021
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/15357597211062412
journals.sagepub.com/home/epi
 SAGE

In Vivo Endocannabinoid Dynamics at the Timescale of Physiological and Pathological Neural Activity

Farrell JS, Colangeli R, Dong A, George AG, Addo-Osafo K, Kingsley PJ, Morena M, Wolff MD, Dudok B, He K, et al. *Neuron*. 2021;109: 2398-2403.e4. doi:10.1016/j.neuron.2021.05.026.

The brain's endocannabinoid system is a powerful controller of neurotransmitter release, shaping synaptic communication under physiological and pathological conditions. However, our understanding of endocannabinoid signaling in vivo is limited by the inability to measure their changes at timescales commensurate with the high lability of lipid signals, leaving fundamental questions of whether, how, and which endocannabinoids fluctuate with neural activity unresolved. Using novel imaging approaches in awake behaving mice, we now demonstrate that the endocannabinoid 2-arachidonoylglycerol, not anandamide, is dynamically coupled to hippocampal neural activity with high spatiotemporal specificity. Furthermore, we show that seizures amplify the physiological endocannabinoid increase by orders of magnitude and drive the downstream synthesis of vasoactive prostaglandins that culminate in a prolonged stroke-like event. These results shed new light on normal and pathological endocannabinoid signaling in vivo.

Commentary

The endocannabinoid system is dynamically involved in the control of neurotransmitter release via retrograde synaptic signaling and has also been implicated in playing a role in seizure suppression.¹ Endocannabinoids (eCBs) are lipid-derived messengers that are synthesized post-synaptically. Endocannabinoids activate cannabinoid type 1 receptors (CB1), which are expressed throughout the brain at presynaptic terminals and act to reduce neurotransmitter release.² The prevalence of CB1 receptors in the brain, and more specifically the hippocampus, coupled with their unique signaling properties, makes the eCB system an excellent target for therapeutic treatment of epilepsy.^{1,3} While eCBs have been implicated in seizure control,⁴ little is known about their dynamics and the way in which eCBs act within the temporal lobe. One limitation in the study of eCB dynamics has been the lack of technology that permits eCB level monitoring in real-time, while simultaneously monitoring neural activity in vivo.

In their recent publication, Farrell et al.⁵ employed the use of a novel genetically encoded endocannabinoid sensor, GRAB-eCCB2.0 (GRAB), that allows for visualization of eCB dynamics in vivo. GRAB is sensitive to the 2 major eCBs known to be present in the CNS, anandamide (AEA) and 2-arachidonoylglycerol (2-AG). They combined this eCB sensor technology with two-photon calcium imaging to reveal never-seen-before coupling between neural activity and eCBs. These techniques were utilized to investigate these dynamics during physiological activity and seizure pathophysiology.

Farrell et al. began by tackling a gap in the field—that is, which eCBs are released under physiological conditions and what are the real-time dynamics? To answer this, they visualized eCBs in the CA1 cell layer of hippocampus during locomotion. It is well known that CA1 neurons fire bursts of action potentials, which elicit calcium transients, when animals traverse precise locations (place fields) on a linear track. Simultaneous imaging of calcium activity and eCBs revealed that changes in eCBs levels were activity-dependent and exquisitely localized such that eCB levels only increased in close proximity to the neurons that were active. Through a series of pharmacological manipulations, they found 2-AG is the primary player during physiological hippocampal eCB signaling during locomotion. As the eCB system is altered in epilepsy,³ the next question that emerges is whether eCB signaling during heightened activity, that is, seizures, is similar to what was observed under healthy conditions. This is especially pertinent when considering cannabinoid-based interventions in epilepsy.

Seizures were elicited via electrical kindling stimulation in the ventral hippocampus, which gave the experimenters precise control over when seizures would occur, making imaging tractable. Seizure activity was monitored electrographically, while simultaneously measuring eCB dynamics with two-photon calcium imaging. They found that there was a marked increase in 2-AG during seizures and these levels returned to baseline shortly after seizure termination. Consistent with the hyper-synchronization expected during a seizure, the authors observed that cells exhibited an increase in overall correlation during seizures, which in turn resulted in a decrease






in spatial specificity of 2-AG. The authors reported an increase in electrographic seizure duration following pharmacological inhibition of 2-AG synthesis, suggesting that 2-AG may act as a switch to suppress seizures. However, this result did not occur in all animals tested. While the observed heterogeneity weakens the conclusion that 2-AG acts to suppress electrically evoked seizures, these findings are consistent with previous work showing that 2-AG suppresses spontaneous seizures in a chronic model of epilepsy.⁴ On the other hand, another study pointed to AEA, rather than 2-AG, as being the eCB that mediates seizure control.¹ It is possible that Farrell et al. are biased toward observing 2-AG effects based on the imaging plane (pyramidal cell layer). As designed, they were blind to eCB dynamics in the distal dendritic tree. Future experiments with prisms allowing for transverse visualization of CA1 neurons could be enlightening.

Although a surge in 2-AG levels during seizures was shown to provide some level of protection, it is also known that 2-AG can have potentially harmful effects.⁶ Given that Farrell et al. observed elevated quantities of 2-AG during seizures orders of magnitude higher than seen during physiological conditions, a pressing question is whether 2-AG is doing more harm than good. 2-AG hydrolysis is known to initiate prostaglandin pathways, and high concentrations of prostaglandins promote vasoconstriction.⁷ As both rodents and humans have previously been shown to be susceptible to hypoxic episodes postictally,^{8,9} the experimenters explored whether there was a link between 2-AG induced neuro-inflammation and postictal hypoxia. To test this, they implemented a series of pharmacological interventions that inhibited specific enzymes involved in the 2-AG-prostaglandin pathway. They measured brain oxygen levels in freely behaving mice experiencing electrically evoked seizures with/without these interventions. Mice with intact 2-AG-prostaglandin pathways showed substantial postictal hypoxia. On the other hand, they found that inhibiting the synthesis of 2-AG or blocking later stages of the 2-AG-prostaglandin pathway was sufficient to reduce or prevent postictal hypoxia.

These findings highlight the duality of 2-AG. On one side, increased levels of 2-AG offer a source of protection from seizure activity and on the other side, activate a cascade of events resulting in postictal hypoxia. Ideally, therapeutics designed to leverage the endocannabinoid system would harness the seizure suppressing effects while avoiding promotion of hypoxia. Moving forward, integrating eCB imaging with a chronic model of temporal lobe epilepsy could provide exciting insight to altered dynamics in between seizures, possibly shedding light on mechanisms of comorbidities. Furthermore, these techniques could be leveraged to better understand the mechanisms of existing clinical therapeutics that are aimed at the endocannabinoid system. For example, cannabidiol (CBD), the non-psychoactive component of cannabis, has been shown to have anticonvulsant effects in the treatment of epilepsy, though little is known of the mechanisms.^{2,9} Cannabidiol has been reported to inhibit the degradation of AEA,¹⁰ one of the

major eCBs. As discussed above, Farrell et al. found that 2-AG was the predominant eCB involved in seizure control, but perhaps AEA becomes a prominent player in the presence of CBD. Given that GRAB is also sensitive to AEA, an exciting application of this new technique would be to investigate whether a role for AEA in seizure control is unveiled in the presence of CBD.

By Brittney L. Boubilil and Laura A. Ewell 

ORCID iD

Laura A. Ewell  <https://orcid.org/0000-0002-1638-426X>

References

- Marsicano G, Goodenough S, Monory K, et al. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science*. 2003;302:84-88. doi:10.1126/science.1088208.
- Soltész I, Alger BE, Kano M, et al. Weeding out bad waves: Towards selective cannabinoid circuit control in epilepsy. *Nat Rev Neurosci*. 2015;16:264-277. doi:10.1038/nrn3937.
- Goffin K, Van Paesschen W, Van Laere K. In vivo activation of endocannabinoid system in temporal lobe epilepsy with hippocampal sclerosis. *Brain*. 2011;134:1033-1040. doi:10.1093/brain/awq385.
- Sugaya Y, Yamazaki M, Uchigashima M, et al. Crucial roles of the endocannabinoid 2-arachidonoylglycerol in the suppression of epileptic seizures. *Cell Rep*. 2016;16:1405-1415. doi:10.1016/j.celrep.2016.06.083.
- Farrell JS, Colangeli R, Dong A, et al. In vivo endocannabinoid dynamics at the timescale of physiological and pathological neural activity. *Neuron*. 2021;109:2398-2403.e4. doi:10.1016/j.neuron.2021.05.026.
- Nomura DK, Morrison BE, Blankman JL, et al. Endocannabinoid hydrolysis generates brain prostaglandins that promote neuro-inflammation. *Science*. 2011;334:809-813. doi:10.1126/science.1209200.
- Dabertrand F, Hannah RM, Pearson JM, Hill-Eubanks DC, Brayden JE, Nelson MT. Prostaglandin E2, a postulated astrocyte-derived neurovascular coupling agent, constricts rather than dilates parenchymal arterioles. *J Cereb Blood Flow Metab*. 2013;33(4):479-482.
- Devinsky O, Cilio MR, Cross H, et al. Cannabidiol: Pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia*. 2014;55:791-802. doi:10.1111/epi.12631.
- Farrell JS, Gaxiola-Valdez I, Wolff MD, et al. Postictal behavioural impairments are due to a severe prolonged hypoperfusion/hypoxia event that is COX-2 dependent. *eLife*. 2016;5:e19352. doi:10.7554/eLife.19352.
- Leweke FM, Piomelli D, Pahlisch F, et al. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Transl Psychiatry*. 2012;2:e94. doi:10.1038/tp.2012.15.