Neurophysiology of Endocannabinoids and Marijuana

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DISCLOSURES

- I have no relevant financial or commercial interests to disclose.

Learning Objectives

At the end of this session, you should be able to:

- Describe the endogenous endocannabinoids and their production.
- The two cannabinoid receptors and their localization in the body.
- The biochemical fate of the endocannabinoids
- The major influences of endocannabinoid receptor activation.

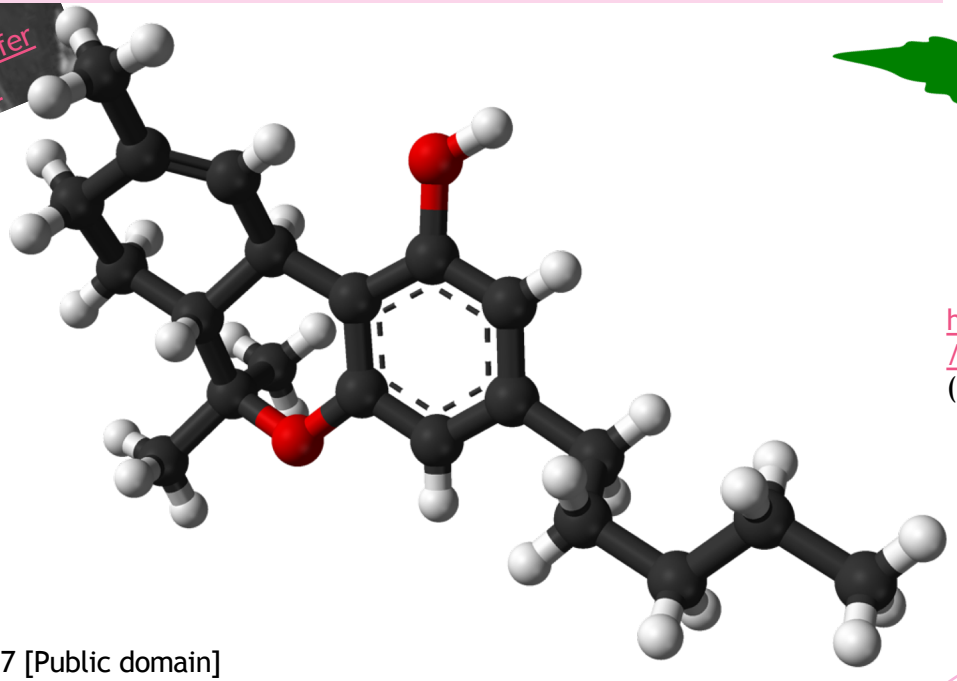
Case Study

- A 65-year old Caucasian woman presented with painless instability in her hip. Radiographic studies revealed severe arthritic deterioration in her left hip, requiring hip replacement surgery.
- After surgery, the patient reported minimal pain (1/10) on the night following the surgery. Two Tylenol relieved the pain. She required no additional pain relief (reporting pain levels of 0/10) throughout the remainder of her recovery.
- The patient's blood levels of arachidonoylethanolamine (AEA, anandamide) were 70% higher than controls, while 2-arachidonyl-glycerol (2-AG) levels were normal.

The Exogenous Cannabinoids

https://www.sbs.com.au/guide/article/2016/11/23/reefer-madness-laughing-old-marijuana-propaganda-films

Δ-9 Tetrahydrocannabinol



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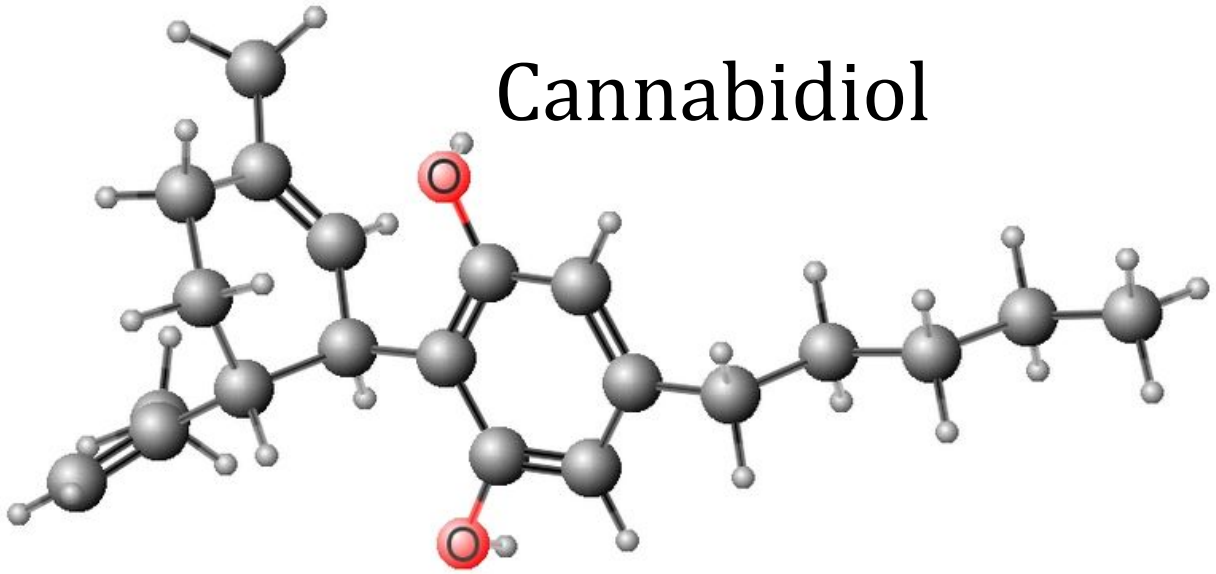
https://upload.wikimedia.org/wikipedia/commons/e/ef/Delta-9-tetrahydrocannabinol-from-tosylate-xtal-3D-balls.png

https://upload.wikimedia.org/wikipedia/commons/a/a8/Cannabis_leaf.svg Oren neu dag [CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0)]

The Exogenous Cannabinoids



https://www.kcur.org/post/man-behind-kansas-citys-fastest-growing-cbd-chain-gaining-notoriety-and-making-some-enemies#stream/0

https://upload.wikimedia.org/wikipedia/commons/b/b8/Cannabidiol_%28CBD%29_molecule_3D.JPG; Gotgot44 [CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0)]

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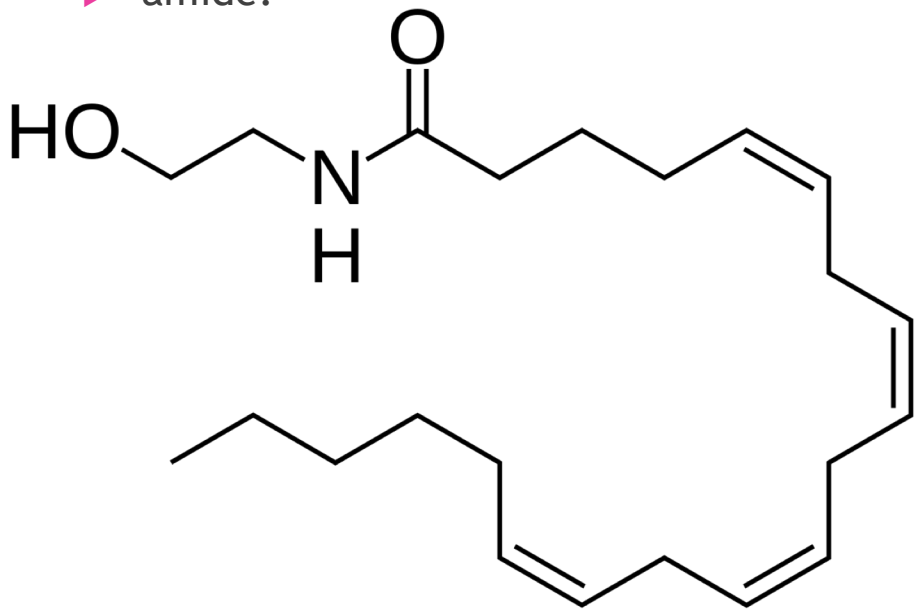
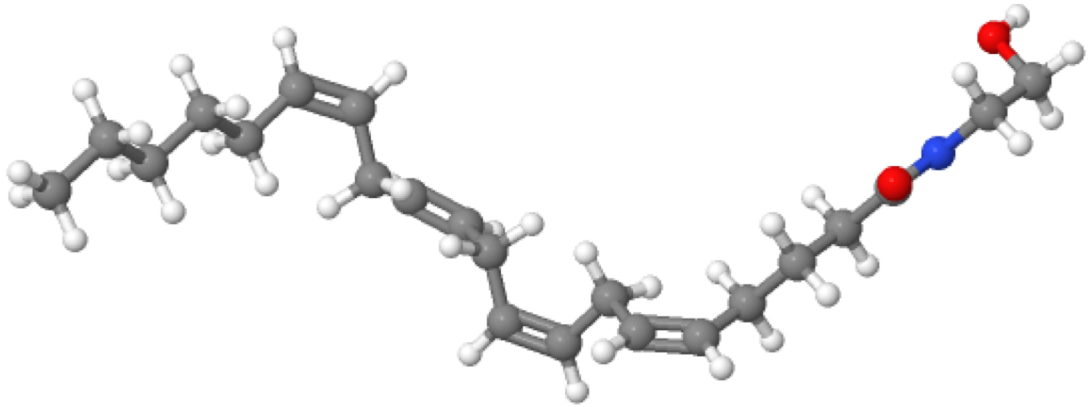
The Endogenous Cannabinoids

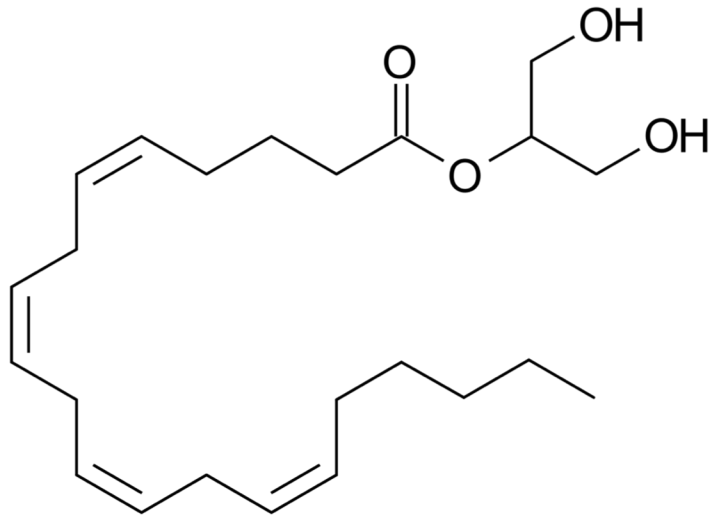
- **Arachidonoylethanolamine** (AEA, more commonly known as **anandamide**)
  - Ananda: Sanskrit for “joy, bliss, delight”
  - amide:





The Endogenous Cannabinoids

- **2-Arachydonoylglycerol (2-AG)**



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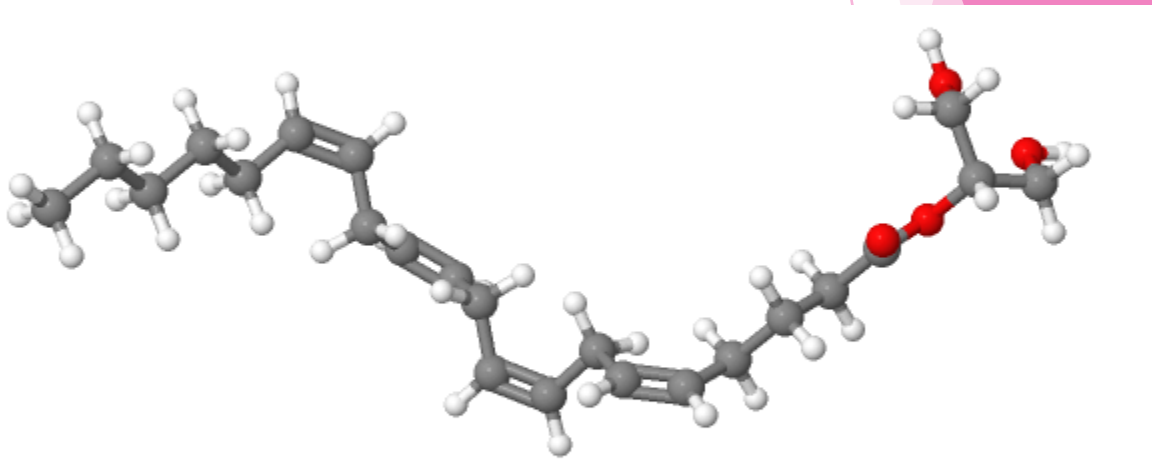

https://chemapps.stolaf.edu/jmol/jmol.php?model=O%3DC%28OC%28CO%29CO%29CCC%5CC%3DC%2FC%5CC%3DC%2FC%5CC%3DC%2FC%5CC%3DC%2FCCCCC

The Endogenous Cannabinoids

- The synthesis of Anandamide and 2-AG are separate pathways!
- Anandamide: Derived from N-arachidonoyl phosphatidyl ethanol (NAPE)
- 2-AG: Derived from arachidonoyl-containing phosphatidyl inositol bis-phosphate (PIP2)
- Notes:
  - Both contain Arachidonic Acid.
    - Source: ?

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    - Source: THE CELL MEMBRANE
    - Other uses: **PROSTAGLANDIN SYNTHESIS**

The Endogenous Cannabinoids

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- Notes:
  - 2-AG is major source for Arachidonic acid in certain tissues, especially brain.
    - Consequence?

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- Notes:
  - 2-AG is major source for Arachidonic acid in certain tissues, especially brain.
    - Consequence: **Pharmacologic manipulation of 2-AG production has wide reaching effects beyond those of the endocannabinoid system.**

Learning Objectives

At the end of this session, you should be able to:

- Describe the endogenous endocannabinoids and their production.
  - Anandamide
    - Aka AEA, Arachidonoylethanolamide
  - 2-AG
    - 2-arachidonoylglycerol
  - Both are arachidonic acid derivatives, 2-AG also serves as part of the pool providing arachidonic acid to other biochemical pathways (especially the prostaglandin).

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The Cannabinoid Receptors – CB1

Cannabinoid Receptor #1

- Neuronal location
- Activation associated with the psychoactive responses to the cannabinoids
- In humans, 472 amino acid peptide coupled to G-proteins.
  - 97-99% homology with the mouse and rat versions of the receptor.
  - Located on chromosome 6
  - Polymorphisms linked to occurrence of obesity, ADHD, schizophrenia, and depression in Parkinson's disease.
  - Can form a heterodimer with other NT receptors, including dopamine and orexin.

Hua, T., Vemuri, K., Pu, M., Qu, L., Han, G.W., Wu, Y., Zhao, S., Shui, W., Li, S., Korde, A., Laprairie, R.B., Stahl, E.L., Ho, J-H., Zvonok, N., Zhou, H., Kufareva, I., Wu, B., Zhao, Q., Hanson, M.A., Bohn, L.M., Makriyannis, A., Stevens, R.C., Liu, Z.J. [Public domain]
https://upload.wikimedia.org/wikipedia/commons/b/bc/Cannabinoid_CB1_Receptor.png

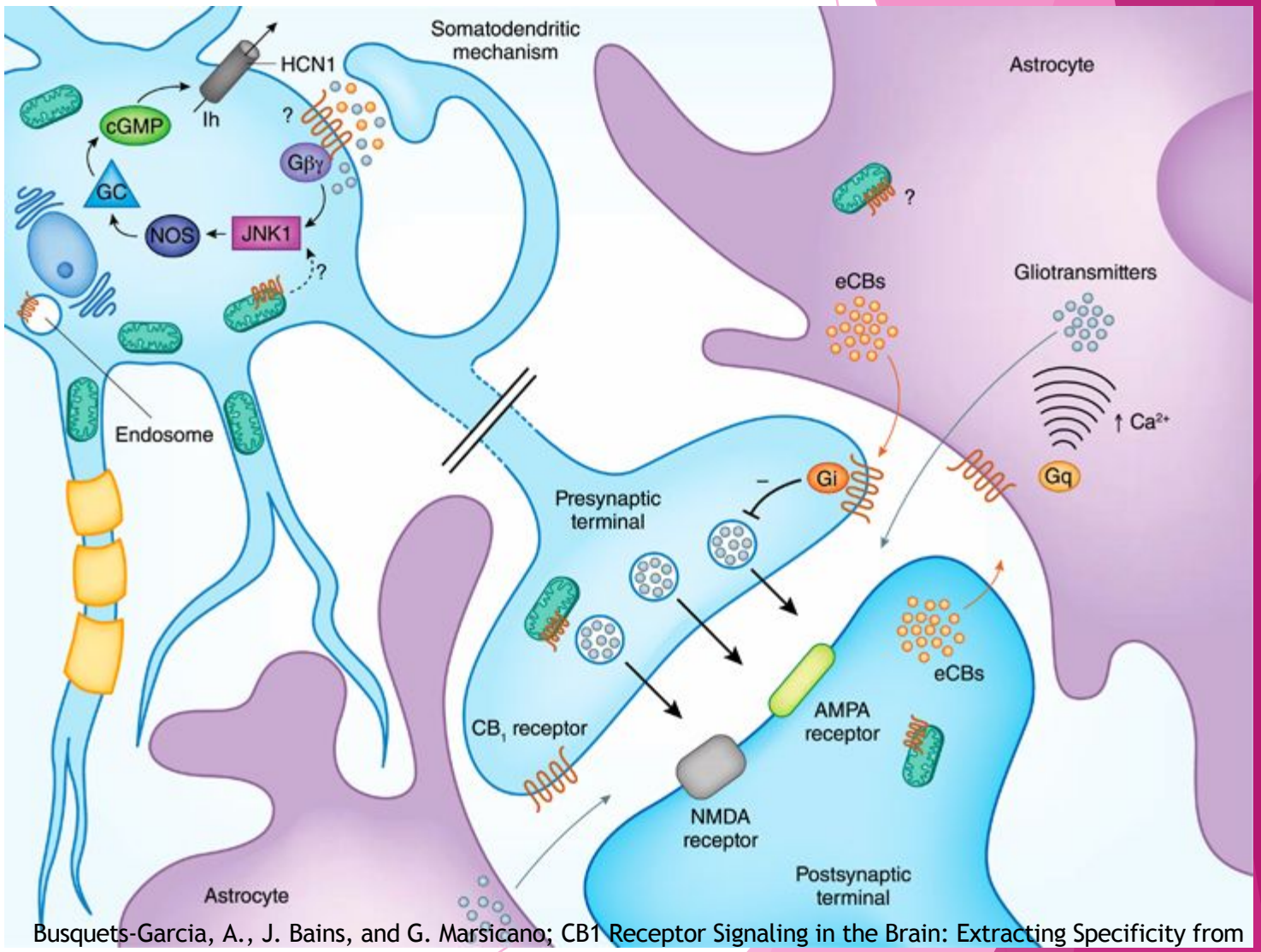
The Cannabinoid Receptors – CB1

CB1

- Distribution: **Central Nervous System Neurons**
  - "Uniform" distribution:
    - Striatum
    - Thalamus
    - Hypothalamus
    - Cerebellum
    - Lower brain stem (NTS)
  - "Non-uniform" (associated with specific neuron types)
    - Cortex
    - Amygdala
    - Hippocampus

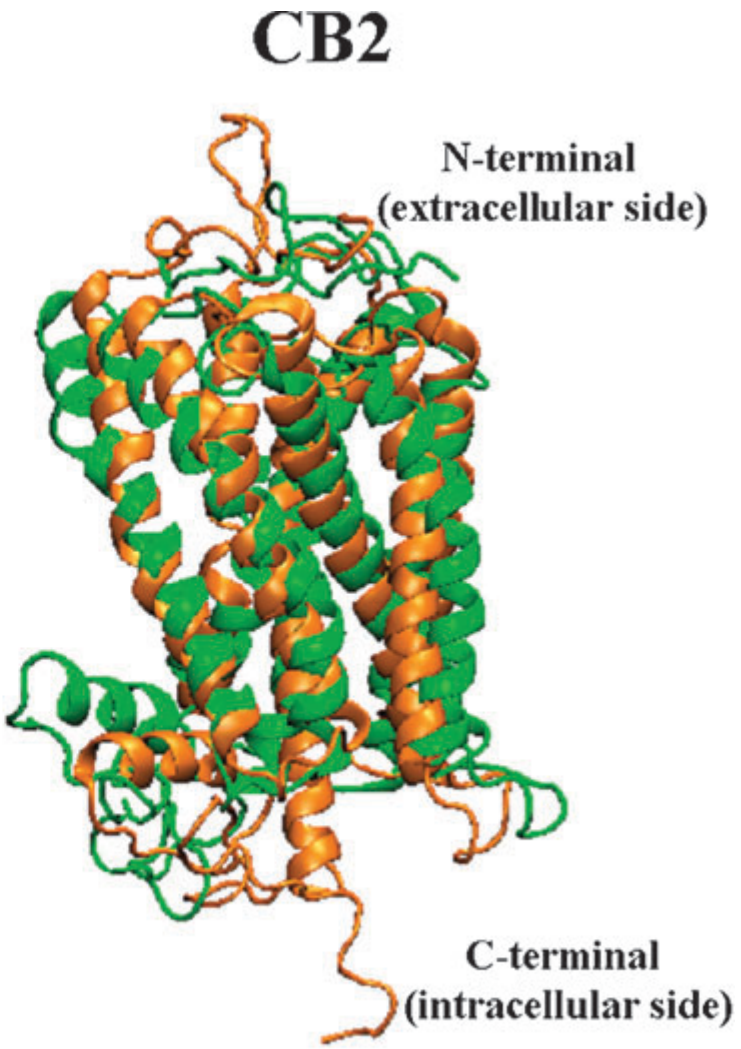
The Cannabinoid Receptors – CB1

- Synaptic location
  - Largely PRESYNAPTIC
  - Some sources will say “exclusively”.
  - Generally away from active zone (where the vesicles are).
  - Greater density at inhibitory synapses.
- Binds AEA and 2-AG with high affinity.



Busquets-Garcia, A., J. Bains, and G. Marsicano; CB1 Receptor Signaling in the Brain: Extracting Specificity from Ubiquity. Neuropsychopharmacology Reviews (2018) 43: 4–20

The Cannabinoid Receptors – CB2



Cannabinoid Receptor #2

- Peripheral location earliest report
- G-protein coupled
  - 360 amino acids
  - 44% homology with CB1.
  - 82% homology with the mouse version of the receptor.

The Cannabinoid Receptors – CB2

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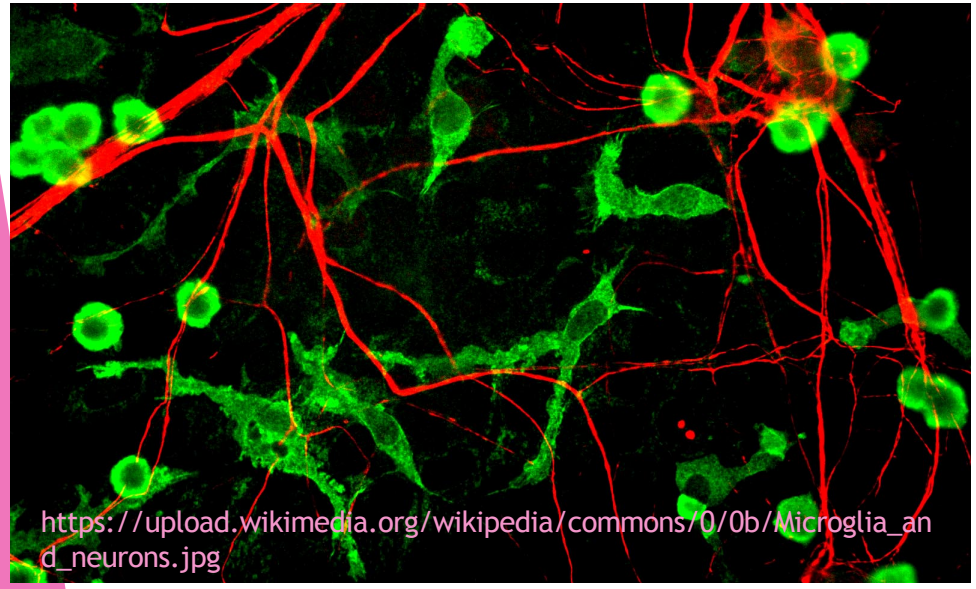
https://upload.wikimedia.org/wikipedia/commons/1/15/Macrophage.jpg



https://upload.wikimedia.org/wikipedia/commons/0/0b/Microglia_and_neurons.jpg

- Were initially reported as peripheral receptors, found primarily on macrophages
- More detailed studies do find them in the brain, but on the microglia.
- Neuronal location (dendrites and within soma) are also reported, usually associated with nerve injury.
- Highly inducible in response to injury or inflammation.
- Binds 2-AG better than AEA.

Case Study

- Physical examination reveals numerous small scars on her arms, most of which were the results of accidental burns. She reported that she was unaware of burns until she smelled her skin burning. Quantitative sensory testing revealed hyposensitivity to noxious heat stimuli in her hands and feet.

TRPV1

- Transient Receptor Protein Vanilloid 1
  - Ca^{++} - selective cation channel
  - Found on sensory neurons
  - Mediate thermoreception.
  - Name a famous vanilloid

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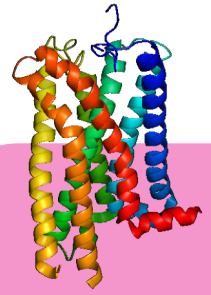
Capsaicin!

CASE STUDY

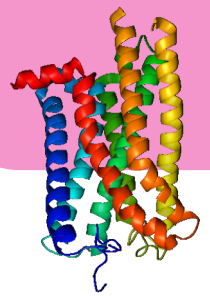
- The patient was also noted for her penchant for very (spicy) hot foods, reporting that she had little to no burning sensation with even those foods.

Capsaicin!

The Endocannabinoids – At the synapse



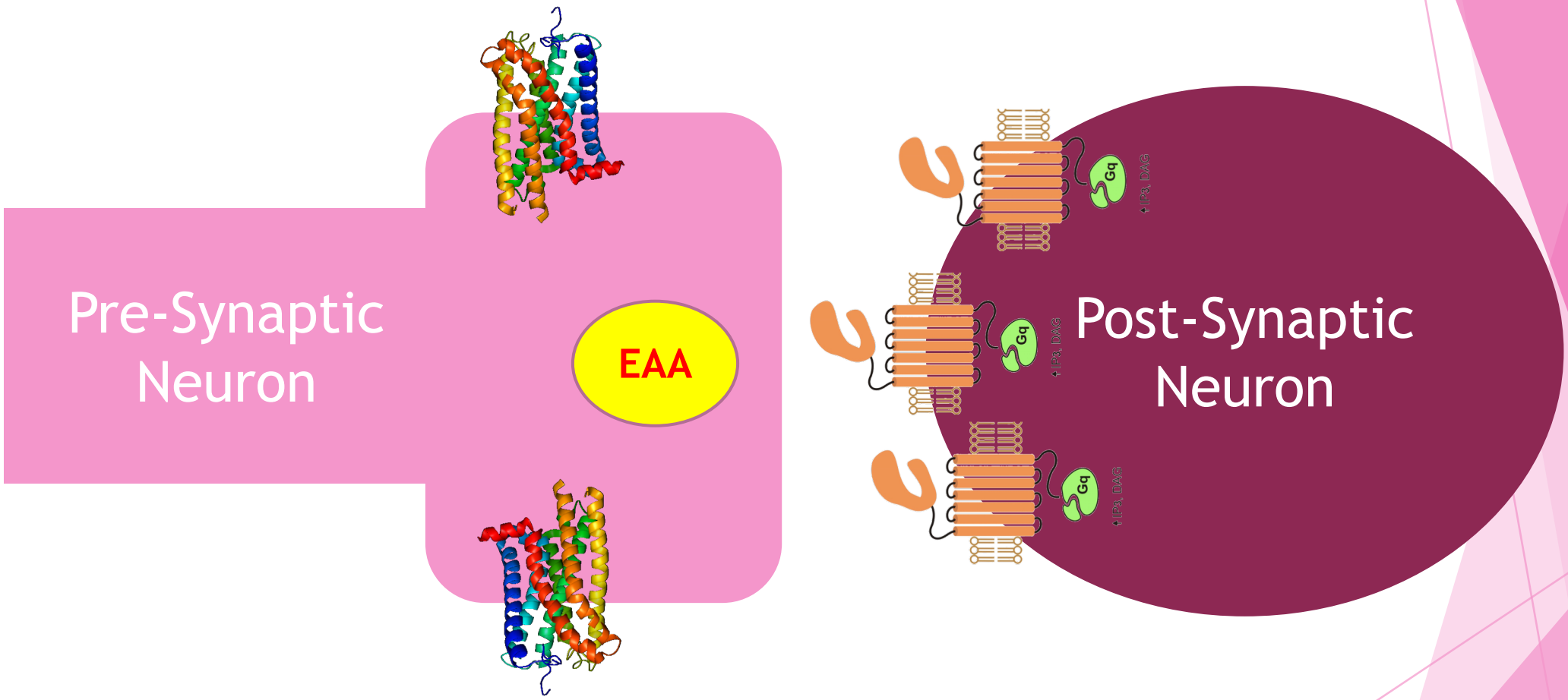
Pre-Synaptic Neuron



Post-Synaptic Neuron

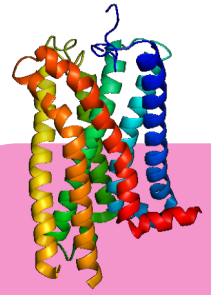
CB1 Receptors are located PRESYNAPTICALLY

The Endocannabinoids – At the synapse



Release of excitatory amino acids by the presynaptic neuron activates metabotropic or NMDA receptors on the post-synaptic cell.

The Endocannabinoids – At the synapse



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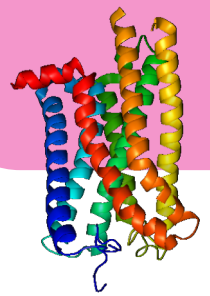
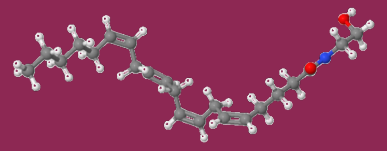
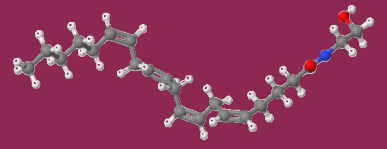


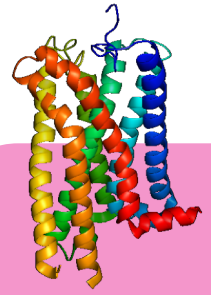


Post-Synaptic Neuron



Increased intracellular calcium in the post-synaptic cell activates endocannabinoid synthesis.

The Endocannabinoids – At the synapse



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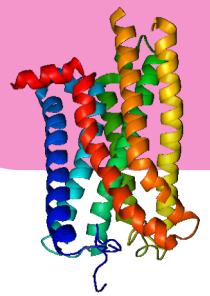
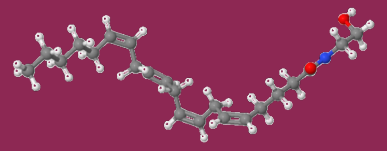
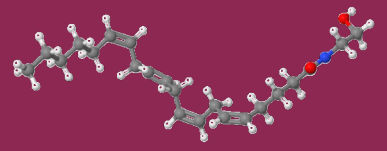




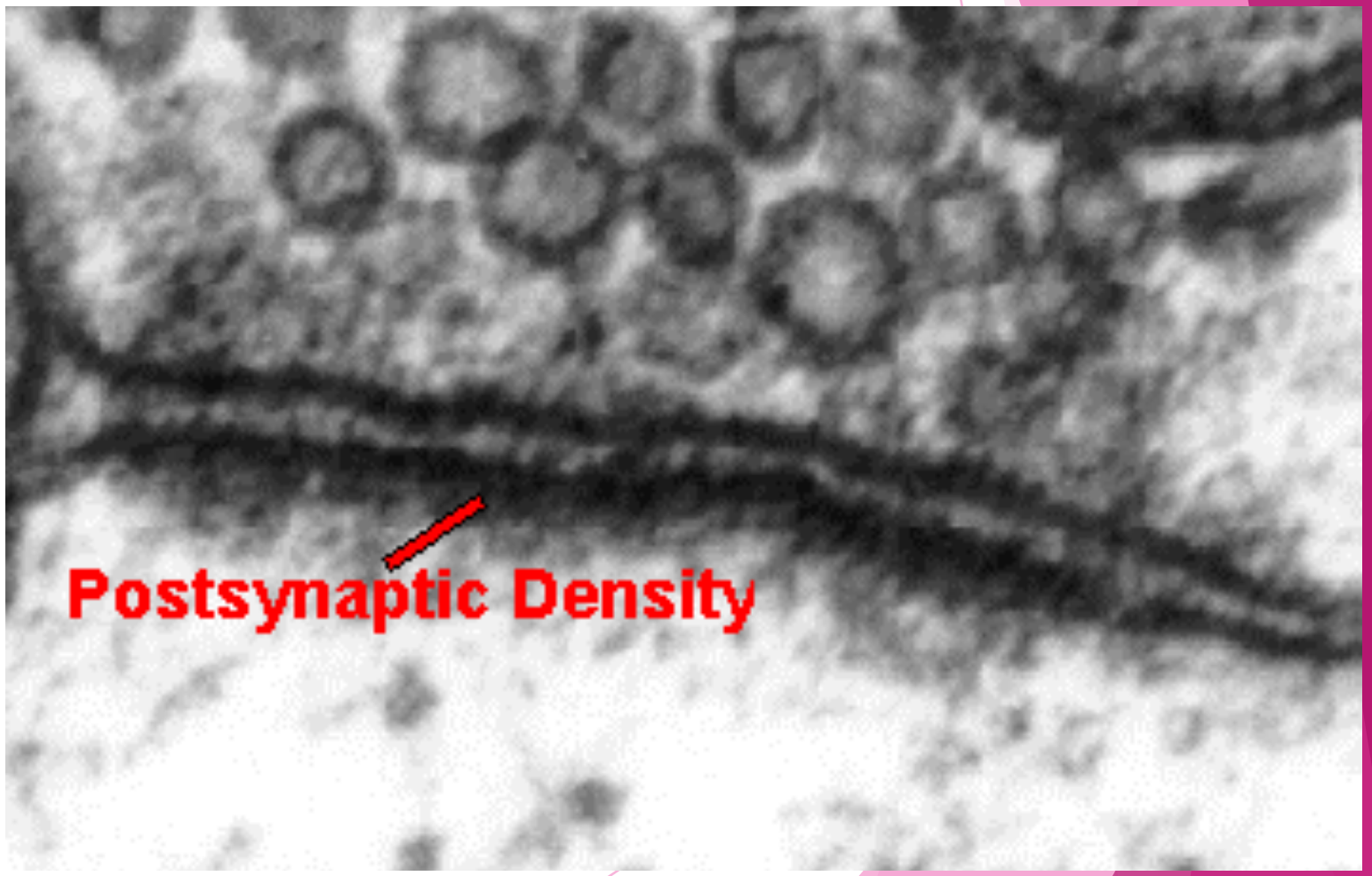
Post-Synaptic Neuron



The endocannabinoid (in this case, AEA) is released into the synaptic cleft, diffuses BACK to the pre-synaptic cell, binding to its receptors (CB1).

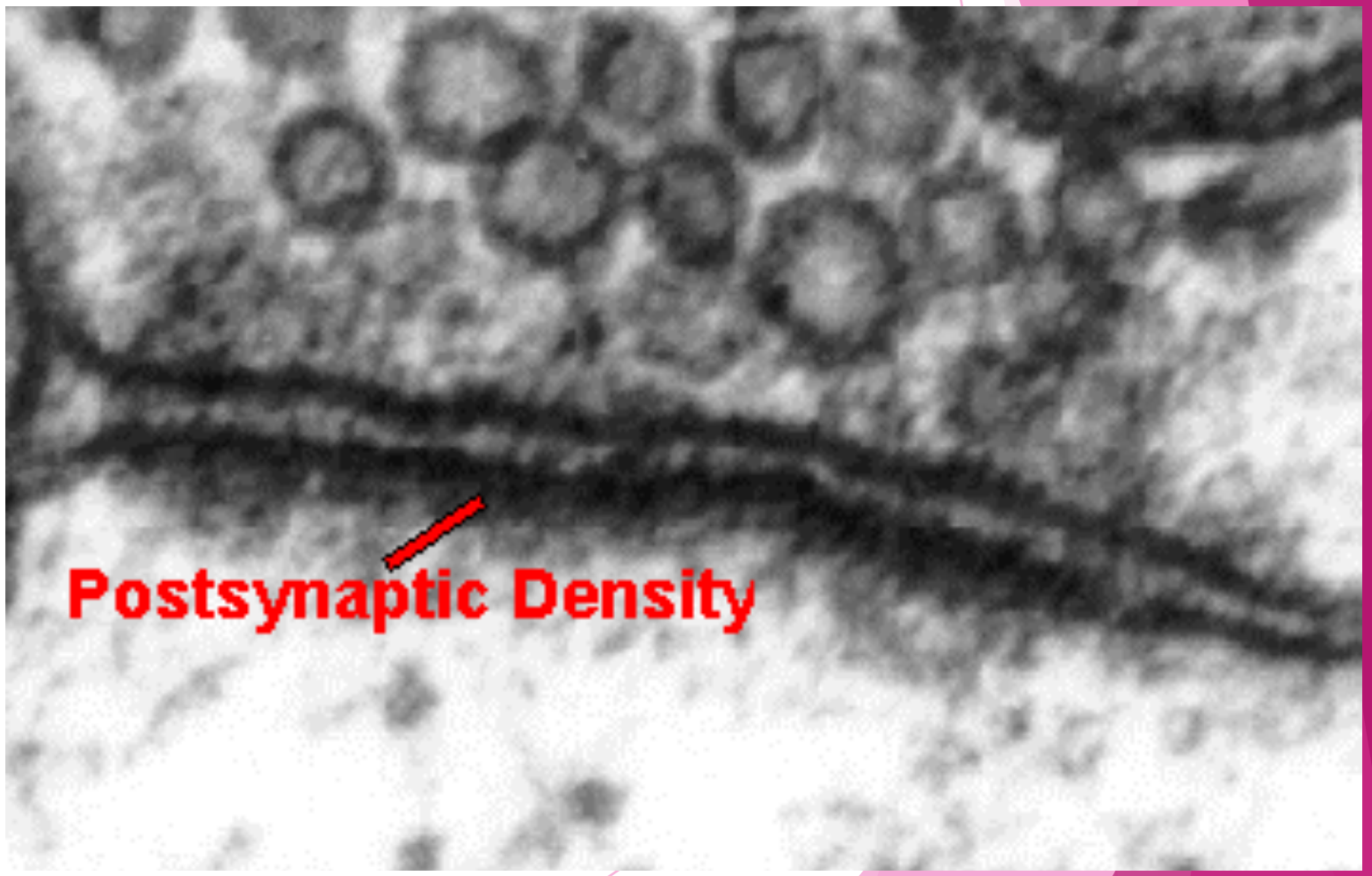
The Endocannabinoids – At the synapse

Activation of CB1 reduces the amount of neurotransmitter transported into the vesicles, reducing neurotransmitter release at the synapse.



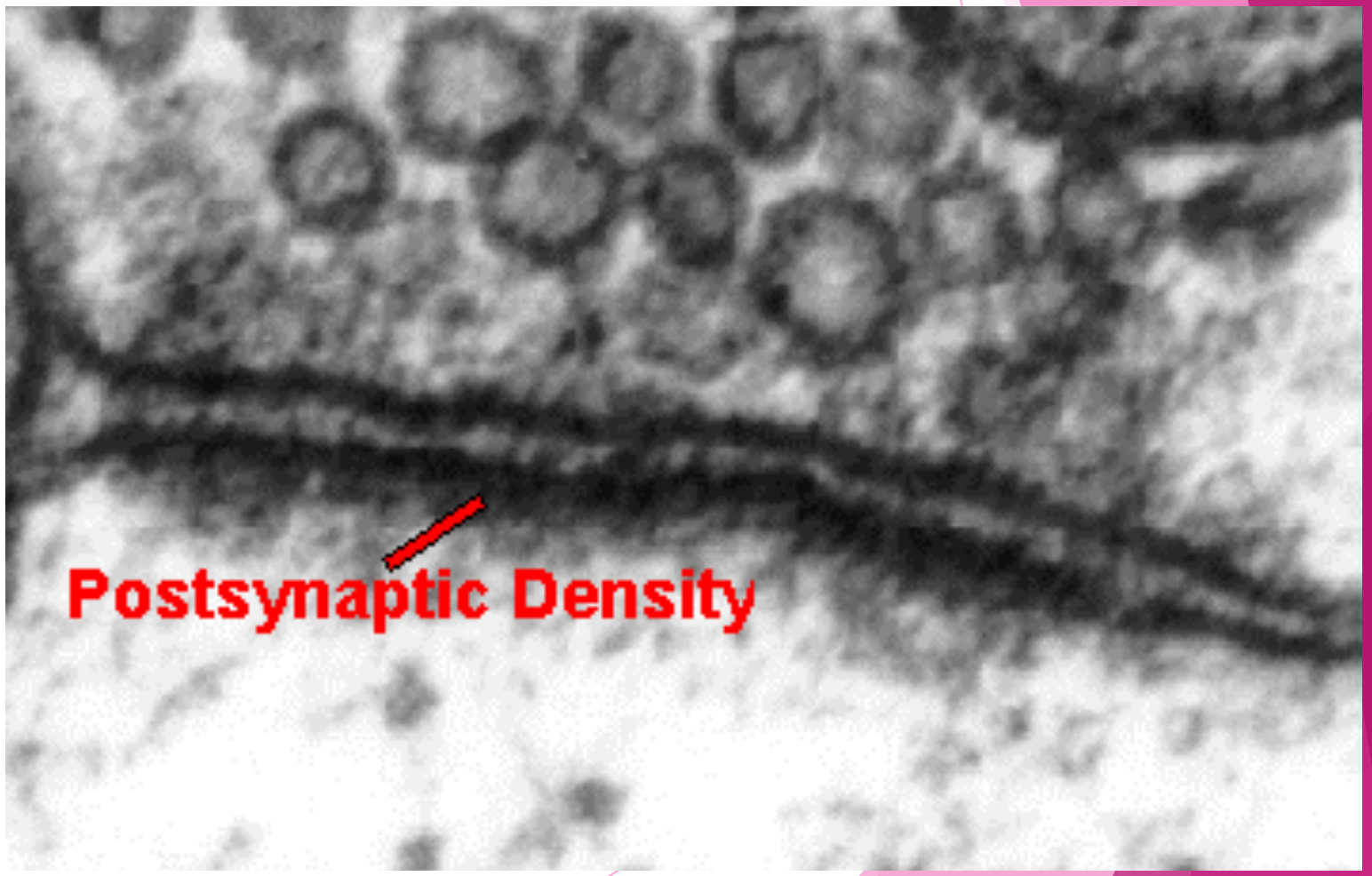
The Endocannabinoids – At the synapse

Endocannabinoids have been shown to reduce release of neurotransmitter at both EAA (glutamatergic) and GABA-ergic synapses.



The Endocannabinoids – At the synapse

This reduction in neurotransmission has been shown to lead to short- and long-term depression at these synapses.



Learning Objectives

At the end of this session, you should be able to:

- The two cannabinoid receptors and their localization in the body.
  - CB1
    - Neuronal
    - Located presynaptically
    - Modulates neurotransmitter release.
  - CB2
    - Immune
    - Located peripherally and centrally
    - Highly inducible in response to inflammation
    - Reduction in inflammation.

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The Endocannabinoids – At the synapse

- Degradation
  - Two different paths: Hydrolysis or Oxidation.
  - Hydrolysis – prevalent in neurons
    - Anandamide (AEA) and 2-AG are degraded via two separate pathways.
    - Anandamide (AEA)
      - Fatty Acid Amide Hydrolase (FAAH)
      - Two forms now known to exist.
    - 2-AG
      - Mono-acyl glycerol lipase (MAGL)
  - Oxidation via cyclooxygenase and lipoxygenase pathway (both AEA and 2-AG).

CASE STUDY

- Additional testing revealed a double mutation that led to a profound reduction in FAAH activity
  - The first was a common polymorphism that leads to a partial reduction in enzyme activity. Her father and son also shared this polymorphism.
  - The other was a *de novo* mutation in a FAAH pseudogene that led to near-complete inactivation of FAAH.

Case Study

- A 65-year old Caucasian woman presented with painless instability in her hip. Radiographic studies revealed severe arthritic deterioration in her left hip, requiring hip replacement surgery.
- After surgery, the patient reported minimal pain (1/10) on the night following the surgery. Two Tylenol relieved the pain. She required no additional pain relief (reporting pain levels of 0/10) throughout the remainder of her recovery.
- The patient's blood levels of arachidonoylethanolamine (AEA, anandamide) were 70% higher than controls, while 2-arachidonyl-glycerol (2-AG) levels were normal.

Learning Objectives

At the end of this session, you should be able to:

- The biochemical fate of the endocannabinoids
  - AEA and 2-AG are degraded by two different hydrolytic pathways.
    - FAAH: AEA
    - MAGL: 2-AG
  - Interesting note: believed that acetaminophen acts on this part of the pathway. Other NSAIDS have been reported to show activity.

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The Endocannabinoids – Physiologic Effects

- Modulation of Pain
  - Appear to act at several levels
  - In spinal cord: EAA as neurotransmitter between nociceptor and second-order neuron. Reduction in EAA release mediated by CB1 decreases post-synaptic response.
  - In brain: CB1 receptors found in the Periaqueductal Grey (PAG), Raphe nuclei, rostral ventrolateral medulla (RVLM), and amygdala
    - These are the areas associated with the descending modulation of pain produced by opiates.

The Endocannabinoids – Physiologic Effects

- Given this information about the patient, what additional signs do you suspect she experienced?

The Endocannabinoids – Physiologic Effects

- Memory
  - Differential effects on memory:
    - Short-term and working memory are impaired
    - Long-term memory and retrieval are not
  - Physiology reminder:
    - The physiologic substrate of short-term and working memory is long-term potentiation produced by EAA release in the hippocampus.
  - Endocannabinoids reduce EAA release in the hippocampus, which impairs the development of LTP.
  - Long-term memory is believed to be the result of physical changes in the synapses and retrieval is activation of those altered synapses…

The Endocannabinoids – Physiologic Effects

- Anxiety
  - Differential effects on anxiety:
    - At low-doses, generally considered anxiolytic
    - At high-doses, may be anxiogenic.
  - Physiology reminder:
    - Endocannabinoids are altering both EAA and GABA-ergic neurotransmission.
    - One is excitatory, the other inhibitory, so mixed effects are not un-expected
  - Endocannabinoids reduce EAA release in the amygdala and their release increases during anxiety-provoking experiences.
  - Blocking CB1 increases anxiety in animal models.
  - Blocking FAAH decreases anxiety in animal models.

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- The patient's blood levels of arachidonoylethanolamine (AEA, anandamide) were 70% higher than controls, while 2-arachidonyl-glycerol (2-AG) levels were normal.
- Using a standardized measure for anxiety, the patient scored a 0/29.

The Endocannabinoids – Physiologic Effects

- Depression
  - Consistently demonstrate an anti-depressant activity
  - Physiology reminder:
    - Mood is tightly linked to the monoamines, with serotonin and dopamine often grabbing the lion's share of attention.
  - Endocannabinoids have been shown to increase the release of monoamines in the relevant areas of the brain.
  - In humans, a clinical trial of a CB1 antagonist to treat obesity was halted due to an increase in suicidal ideation, suicide attempts, and depression.

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- Using a standardized measure for anxiety, the patient scored 0/21
- Using a standardized measure of depression, the patient scored 0/29

The Endocannabinoids – Physiologic Effects

- Feeding behavior
  - Consistently demonstrate stimulation of appetite.
  - Likely occurs at several levels:
    - Reward for food (VTA/Nucleus accumbens)
    - Hunger (lateral hypothalamus)
  - Endocannabinoids
    - Increased in the lateral hypothalamus during fasting.
    - Injection of 2-AG into the Nucleus Accumbens produced feeding behavior
  - As noted, blocking CB1 is associated with a decrease in appetite.

The Endocannabinoids – Physiologic Effects

- Neuroprotection
  - In response to trauma, ischemia, epilepsy
  - Physiology reminder: Excitotoxicity
    - Excess release of excitatory amino acids has been shown in response to ischemic injuries in the brain.
    - The high levels of calcium in the post-synaptic cells initiates a variety of responses that lead to the death of neurons in both injured and non-injured areas of the brain.
  - Remember – one of the major effects of the Endocannabinoids is to reduce EAA release from the presynaptic terminal.
  - This is one place where the decrease in GABA release probably isn't important – the EAA release is so overwhelming that the change in GABA is inconsequential.

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- Describe the endogenous endocannabinoids and their production.
- The two cannabinoid receptors and their localization in the body.
- The biochemical fate of the endocannabinoids
- The major influences of endocannabinoid receptor activation.

SELECTED REFERENCES

- Busquets-Garcia, A., J. Bains, and G. Marsicano; CB1 Receptor Signaling in the Brain: Extracting Specificity from Ubiquity. Neuropsychopharmacology Reviews (2018) 43: 4–20.
- Donvito, G., S.R. Nass; J.L. Wilkerson; Z.A. Currry, L.D. Schurman, S.G. Kinsey, A.H. Lichtman; The Endogenous Cannabinoid System: A Budding Source of Targets for Treating Inflammatory and Neuropathic Pain. Neuropsychoparmacology Reviews (2018); 43: 57-79.
- Habibm, A.M., A.L. Okorokov, M.N. Hill, J.T. Bras, M-C. Lee, S. Li, S.J. Gossage, M. van Drimmelen, M. Morena, H. Houlden, J.D. Ramirez, D.L.H. Bennett, D. Srivastava, and J.J. Cox. Microdeletion in a FAAH pseudogene identified in a patient with high anandamide concentrations and pain insensitivity. (2019) British Journal of Anaesthesia, 123 (2): e249-e253. doi: 10.1016/j.bja.2019.02.019
- Kano, M., T. Ohno-Shosaku, Y. Hashimotodani, M. Uchigashima, and M. Watanabe. Endocannabinoid-Mediated Control of Synaptic Transmission. (2009) Physiol Rev 89: 309–380; doi:10.1152/physrev.00019.2008
- Lu, Hui-Chen and K. Mackie; An Introduction to the Endogenous Cannabinoid System. Biol Psychiatry. (2016); 79(7): 516-525. doi:10.1016/j.biopsych.2015.07.028
- Mackie, K.; Mechanisms of CB1 receptor signaling: endocannabinoid modulation of synaptic strength; International Journal of Obesity (2006) 30, S19–S23
- Pacher, P., S. Batkai, and G. Kunos; The Endocannabinoid System as an Emerging Target of Pharmacotherapy. Pharmacol Rev. (2006); 58(3): 389–462
- Reggio, P.H.; Endocannabinoid Binding to the Cannabinoid Receptors: What Is Known and What Remains Unknown; (2010) Curr Med Chem.; 17(14): 1468–1486.
- Zou, S. and U. Kumar; Cannabinoid Receptors and the Endocannabinoid System: Signaling and Function in the Central Nervous System. Int. J. Mol. Sci. (2018) 19:833; doi:10.3390/ijms19030833