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Imaging the endocannabinoid signaling system

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Neurons use a variety of compounds as intercellular signaling agents, including amino acids, peptides, amines, purines, or gases. In addition to these messengers, however, it has been increasingly recognized that lipids also have important signaling functions in the nervous system. Indeed, lipids account for half of the brain by dry weight, serving a variety of structural, modulatory and signaling roles in both neurons and glial cells (Piomelli et al., 2007). A prominent lipid neuromodulatory system in the brain utilizes endocannabinoids (eCBs), a diverse group of endogenous lipids (arachidonic acid derivatives) that bind to cannabinoid receptors (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995). The principal receptor of endocannabinoids, the cannabinoid type-1 receptor (CB_1), is a G-protein coupled receptor (GPCR) that is phylogenetically conserved across vertebrates and also present in some invertebrates (Elphick, 2012). Underlining its importance, the CB_1 is the most abundant GPCR in the brain, with CB_1 protein levels rivaling that of the ubiquitous GABA and glutamate receptors (Herkenham et al., 1990; Li et al., 2020). Widespread across central and peripheral regions, the eCB system is atypical compared to classical neurotransmitters in several ways: not only does it use lipids as messengers, but it also mediates retrograde transmission, from postsynaptic to the presynaptic compartments (Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Wilson et al., 2001). In addition, in contrast to classical neurotransmitters such as glutamate and GABA that are released from vesicles, eCBs are synthesized “on-demand” from membrane-enriched precursors and are mobilized by mechanisms distinct from vesicle fusion (Marsicano et al., 2003; Piomelli, 2003). eCBs acting on CB_1 are involved in regulating development, synaptic plasticity, and neuron-glial interactions (Chevaleyre et al., 2006; Harkany et al., 2006; Stella, 2004). While recent advances in super-resolution microscopy greatly accelerated our understanding of the molecular organization of transsynaptic macromolecular complexes that regulate glutamatergic neurotransmission and plasticity (Choquet and Hosy, 2020; Glebov et al.,

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2017, 2016; Nair et al., 2013; Tang et al., 2016; Willig and Barrantes, 2014), the precise molecular organization of the eCB system has remained considerably less understood in large part due to the unusual characteristics of lipid signaling.

How and where eCBs are liberated? How they cross the synaptic cleft? How is cannabinoid receptor distribution controlled, and how are the receptors coupled to their downstream targets? In this mini-review, we provide an overview of recent advances visualizing the nanoscale subcellular distribution and dynamics of molecular players of the endocannabinoid system, with a focus on CB_1 receptors in the mouse hippocampus and discuss outstanding questions that can be approached using superresolution methods.

Nanoscale distribution of CB_1 receptors

Seminal discoveries in molecular neuroanatomy and cellular neurophysiology revealed a now-canonical retrograde synaptic eCB system comprised of postsynaptic synthesizing enzymes, presynaptic receptors, and pre- or postsynaptic hydrolyzing enzymes (Fig. 1A) (Alger, 2002; Castillo et al., 2012; Freund et al., 2003; Kano et al., 2009; Katona and Freund, 2012; Mechoulam and Parker, 2011). The eCB system is involved in regulating numerous pathways across the peripheral and central nervous system. We will not attempt to summarize the vast literature on CB_1 distribution in different regions, cell types and organelles, as excellent reviews are available on this topic by others (Hu and Mackie, 2015; Lu and Mackie, 2021). Briefly, CB_1 expression has been reported in various regions from the spinal cord to the neocortex, with prominent expression in nociceptive fibers, the retina, olfactory blub, striatum, amygdala, hippocampus, prefrontal cortex, cerebellum, brainstem and hypothalamus. CB1Rs are present on various cell types including select excitatory (glutamatergic) and inhibitory (GABAergic) neurons, astrocytes, and potentially microglia and oligodendrocytes (Min and Nevian, 2012; Navarrete and Araque, 2008). Subcellularly, CB_1 can be expressed in the neuronal plasmalemma either presynaptically (in axons and axon terminals) or postsynaptically (in somata and dendrites). As discussed in detail below, the possible functional role of presynaptic CB_1 is in regulating neurotransmitter release, while postsynaptic CB_1Rs may modulate neuronal excitability. CB_1 is also found on intracellular membranes, most prominently mitochondria, where it may play a role in regulating energy homeostasis and ultimately synaptic transmission (Bénard et al., 2012; Busquets-Garcia et al., 2018). Finally, CB_1 is highly expressed in the developing brain, where it plays a role in axonal growth and pathfinding (Keimpema et al., 2011).

Despite this widespread expression, CB_1 is found at one very specific subcellular domain at much higher density than anywhere else on the neuronal surface, namely, on the axons of a particular class of cortical GABAergic interneurons identified by cholecystokinin (CCK) expression (Fig. 1B) (Katona et al., 1999; Marsicano and Lutz, 1999; Tsou et al., 1999). The development of single molecule localization microscopy methods, such as Stochastic Optical Localization Microscopy (STORM) (Betzig et al., 2006; Hess et al., 2006; Rust et al., 2006), enabled the investigation of CB_1 distribution with unprecedented detail.

The principles of superresolution microscopy methods, the comparisons of various approaches such as structured illumination microscopy, localization microscopy, and

expansion microscopy, have been reviewed elsewhere (Allen et al., 2013; Igarashi et al., 2018; Möckl and Moerner, 2020; Oddone et al., 2014; Sigal et al., 2018; Wassie et al., 2019). Briefly, STORM takes advantage of the photo-switching behavior of organic fluorescent dyes. Instead of creating an optical image of fluorophore distribution in the sample, it creates a computational reconstruction of the location of individual fluorophores iteratively with precision and accuracy that exceeds the resolution of conventional light microscopy by an order of magnitude, typically to 20–30 nm in the imaging plane and 60–70 nm along the z-axis (Huang et al., 2008), and down to ~2 nm with interferometric approaches (G. Wang et al., 2016). Various methods have been developed for engineering the shape of the point spread function (PSF) of individual fluorophores, allowing precise localization in three dimensions (3D) (Gustavsson et al., 2018; Huang et al., 2008; Pavani et al., 2009). In addition, multiplexed, simultaneous imaging of different labels has been developed, either by spectrally separating fluorophores, or by selectively controlling their PSF or their photoswitching properties (Bates et al., 2012, 2007; Shechtman et al., 2016; Zhang et al., 2015). Importantly, STORM (and the ever-expanding toolkit of single molecule localization methods) offers not only structural images with improved resolution, but by obtaining the spatial coordinates of individual labeled molecules, it also allows sophisticated analysis of intermolecular distances, as well as dynamics of molecular distribution (with temporally resolved imaging of live specimens) (Barna et al., 2015; Deschout et al., 2014; Dietz and Heilemann, 2019; Nicovich et al., 2017; Tasso et al., 2019).

Electron microscopy and superresolution microscopy methods have been used for mapping the subcellular localization of eCB receptors and enzymes (Cristino et al., 2017). As a first step towards the superresolution imaging of the eCB system, dual-color 3D STORM with correlated confocal microscopy (Barna et al., 2015) was applied to determine CB_1 distribution at the presynaptic axon terminals of identified, individually labeled CCK-expressing interneurons (Dudok et al., 2015). Confirming the previous electron microscopy studies, high CB_1 density was found on the axon terminal membrane surrounding synaptic active zones (Fig. 1C). The measured intramolecular distance distributions were similar to modeled distributions generated at random, suggesting that the receptors are homogenously distributed, as opposed to being organized in clustered or synaptic nanodomain-enriched patterns.

Voltage gated calcium channels (VGCCs) controlling action potential-dependent vesicle release are tethered to the presynaptic active zone via Rab3 interacting molecules (RIM proteins) (Fig 1A) (Kaeser et al., 2011). Given the fact that VGCCs and RIMs are downstream targets of retrograde synaptic CB_1 signaling (Chevaleyre et al., 2007; Guo and Ikeda, 2004; Heifets and Castillo, 2009; Mackie and Hille, 1992; Mato et al., 2008; Szabó et al., 2014), this homogenous distribution of CB_1 was unexpected. A potential resolution of this apparent contradiction is that CB_1-expressing CCK interneurons use mainly N-type (Cav2.2) VGCCs, as opposed to P type (Cav2.1) channels expressed in CB_1-negative interneurons and pyramidal cells. The presynaptic active zone protein bassoon selectively recruits P/Q, but not N type channels to the active zone (Davydova et al., 2014), and a resulting distinct distribution is a likely explanation of the more precise action potential - release coupling in synapses with P/Q type channels and loose coupling in synapses with N-type channels (Bucurenciu et al., 2008; Eggermann et al., 2012). Although

the superresolution localization of the N-type channels in cortical inhibitory synapses remains elusive due to the lack of suitable antibodies, electron microscopic studies using an epitope-tagged knockin mouse confirmed their frequent extrasynaptic localization in the dorsal root ganglia (Nieto-Rostro et al., 2018). Altogether, the mechanisms governing CB_1 and downstream VGCC distribution remain unclear. Interestingly, it is the ratio of CB_1 over the size of the active zone, rather than CB_1 density, that is correlated with CB_1 efficacy in suppressing neurotransmitter release, suggesting that the molecular stoichiometry of receptors and downstream targets in the microdomain around the synapse may be an important factor to consider (Dudok et al., 2015). With the recent development of a transgenic mouse line for the genetic targeting of CB_1-expressing basket cells (Dudok et al., 2021), cell type-specific nanoscale imaging of CB_1 and other molecular signaling elements of the eCB system at GABAergic synapses is becoming more feasible. In contrast to GABAergic synapses, glutamatergic axon terminals exhibit markedly lower CB_1 expression, which presents additional challenges for efforts to determine whether the organization of presynaptic eCB signaling is similar between cell types. Interestingly, despite its low density of expression on excitatory axon terminals (Katona et al., 2006; W. Wang et al., 2016), CB_1 is highly effective in suppressing glutamate release (Kreitzer and Regehr, 2001; Ruehle et al., 2013; Takahashi and Castillo, 2006), indicating that CB_1 and VGCCs may be targeted to precisely organized nanodomains at these synapses.

On the axons of CCK-expressing interneurons, high density CB_1 was reported not only on synaptic boutons, but also in the membranes of interconnecting axonal segments (Nyíri et al., 2005), where CB_1 is unlikely to regulate synaptic transmission. However, the function of this axonal CB_1 pool has remained unknown until recently. One of the first applications of STORM for neuroscience lead to the discovery of ring-like structures of actin and spectrin in axons (Xu et al., 2013). The quasi-1D periodic structure, termed membrane- associated periodic skeleton (MPS), also organizes transmembrane proteins such as ion channels and receptors into periodic distributions. Correlative STORM and electron microscopy revealed that MPS rings are made of two intertwined long actin filaments (Vassilopoulos et al., 2019). Multi-color STORM imaging of CB_1 and the MPS revealed that axonal CB_1 is organized into a periodic pattern aligned to the MPS (Fig. 1D) (Li et al., 2020; Zhou et al., 2019). Moreover, CB_1 agonist binding resulted in an increased recruitment of CB_1 to the MPS, inducing extracellular signal–regulated kinase (ERK) signaling. Disruption of the MPS prevented such molecular reorganization and downstream ERK signaling. CB_1 interactions with the cytoskeleton via Rho/ROCK signaling is functionally important in multiple processes, including the control of axonal growth, bouton formation, bouton size, and synapse stabilization (Berghuis et al., 2007; Hu et al., 2019; Liang et al., 2021; Monday et al., 2020; Njoo et al., 2015; Roland et al., 2014). Importantly, MPS disruption completely abolished the ERK activating effect of CB_1 agonists (Zhou et al., 2019). Given that MPS-like organization is observed not only in axons but also in dendrites (Han et al., 2017), these findings indicate that the cytoskeleton may be a general organizer of CB_1 and required for downstream signaling outside the axon as well.

Compared to axons, CB_1 expression in the somatodendritic (postsynaptic) membranes is very low. Accordingly, the nanoscale targeting and physiological role of dendritic CB_1 is even less understood. Despite its low abundance and restriction to only a subset of pyramidal

cells, dendritic CB_1 signaling via a purely postsynaptic pathway involving c-Jun N-terminal kinases (JNK) and hyperpolarization activated cyclic nucleotide gated potassium channel (HCN) 1 was found to be powerful enough to modulate synaptic plasticity and learning (Fig. 1E) (Maroso et al., 2016). Electron microscopic immunolocalization showed a strong gradient in HCN1 channel density along the somatodendritic domain (Lörincz et al., 2002), which is a likely cause of the observed several folds larger hyperpolarization-activated depolarizing "sag" currents in distal dendrites (Magee, 1998). Whether dendritic CB_1 follow this distribution is not known, and the molecular mechanisms that may shape their nanoscale distribution similarly remain elusive. Key mechanisms leading to CB_1 axonal enrichment are the preferential somatodendritic endocytosis (Leterrier et al., 2006; Simon et al., 2013) and kinesin-dependent axonal transport of CB_1 (Saez et al., 2020). An intriguing possibility is that dendritic CB_1 levels are only maintained in distal dendrites, where endocytosis is less efficient in removing surface CB_1.

Dynamics of CB_1 receptors

While nanoscale mapping of CB_1 distribution in fixed tissue samples contributed to our understanding of eCB signaling, a precise understanding of the mechanisms that determine this distribution requires dynamic observations in live samples. Tracking individual CB_1 trajectories using quantum dots (QDs) in cultured cells revealed that surface CB_1s are highly mobile and translocate between synapses (Mikasova et al., 2008). This mobility was activity dependent, as indicated by reduced speed upon CB_1 desensitization by agonists. Agonist-dependent endocytosis may be one of the factors regulating bouton surface CB_1 levels. STORM imaging experiments indicated that chronic treatment with the plant cannabinoid Δ9-tetrahydrocannabinol, or increasing the levels of the endogenous CB_1 agonist 2-AG by permanent genetic deletion of its hydrolyzing enzyme monoacylglycerol-lipase (MGL) caused CB_1 internalization, while acute elevation of 2-AG levels by pharmacological MGL inhibition did not cause such internalization (Dudok et al., 2015; Imperatore et al., 2015; Lee et al., 2015; Thibault et al., 2013). In addition to internalization, exclusion of desensitized receptors from synaptic terminals by lateral diffusion (Mikasova et al., 2008) may be a key mechanism for the fast regulation of synaptic CB_1 availability.

Improved bright QDs in combination with single molecule localization microscopy allowed analyzing CB_1 diffusion within synapses at nanoscale-level detail (Tasso et al., 2019). Trajectories of labeled CB_1 resulted in a dense coverage of the entire membrane surface over time (Tasso et al., 2019), indicating that in agreement with the observed homogenous distribution (Dudok et al., 2015), CB_1 diffusion is largely unrestricted in presynaptic membranes, including the synaptic active zone. Whether separate pools of synaptic and extrasynaptic/axonal CB_1 are maintained by tethering or corralling mechanisms, remains unknown. With the development of methods for studying glutamatergic receptor movements (Groc and Choquet, 2020), dynamic superresolution imaging with single particle tracking in tissue slices and ultimately *in vivo* is certainly within sight for the eCB field.

Imaging lipid ligands of CB_1 receptors

Our relatively limited understanding of lipid messengers stems from the challenging nature of analyzing and visualizing the exceedingly diverse family of eCBs (Bisogno et al., 2009; Piomelli et al., 2007). The structural similarity but functional specificity of closely related eCB analogs necessitated the use of sophisticated mass spectroscopy methods to distinguish between lipid species, and these biochemical approaches typically have limited spatiotemporal resolution. Due to the lack of tools to directly visualize eCB dynamics, the mechanisms of retrograde eCB transport are elusive and highly debated. As mentioned above, eCB mobilization and release is clearly distinct from the vesicular accumulation, storage, and release of non-lipid neurotransmitters such as glutamate, GABA, or neuropeptides. In vitro experiments suggested that the on-demand synthesis and release of eCBs occurs in less than 50 ms (Heinbockel et al., 2005), and eCBs can travel at least 20 microns in the extracellular space as evidenced by heterosynaptic expression of eCB-dependent synaptic plasticity (Ohno-Shosaku et al., 2000; Wilson and Nicoll, 2001). The sensitivity of the magnitude and duration of eCB-mediated plasticity to inhibition of presynaptic eCB hydrolysis suggests that eCB diffusion is limited by uptake and ultimately enzymatic degradation (Hashimotodani et al., 2007). While certain pieces of evidence suggest a role of either free diffusion, augmented transport by secreted peptide or protein carriers, or extracellular vesicles in eCB signaling, none of the proposed mechanisms explain all properties of eCB signaling, including the pharmacological sensitivity and temperature dependence of eCB transport (Chicca et al., 2012; Gabrielli et al., 2015; Haj-Dahmane et al., 2018; Niphakis et al., 2015), as recently reviewed (Kaczocha and Haj-Dahmane, 2021). To further complicate the picture, eCB signaling has a complex bidirectional relationship with nutrition, stress, and sex hormones, especially estrogen (Bisogno and Maccarrone, 2014; Gorzalka and Dang, 2012; Hill et al., 2009; Lafourcade et al., 2011; Santoro et al., 2021). Even within a single synaptic pathway in the hippocampus, 2-AG and anandamide were found to be differentially involved in eCB signaling in male and female mice (Ferraro et al., 2020; Tabatadze et al., 2015). To date, superresolution localization of the molecular members of the diverse and ever-expanding family of the eCB enzymatic pathways (Ahn et al., 2008) has been lagging behind compared to our relatively detailed understanding of CB_1 distribution.

Specific antibodies can enable selectively visualizing macromolecules including CB_1Rs through immunolabeling (Fig. 2A), however, antibodies are rarely available against lipids. Lipid receptors with specific binding sites evolved to distinguish lipid messengers from their precursors and related lipid species. Protein engineering enabled the development of genetically encoded neurotransmitter and neuromodulator reporters, based on either periplasmic binding protein- (PBP) or GPCR activation-based (GRAB) fluorescent sensors (Patriarchi et al., 2018; Sabatini and Tian, 2020; Sun et al., 2018). Using a fusion of a circularly permutated fluorescent protein sequence and a truncated GPCR ligand binding domain, GRAB sensors revolutionized the detection of bioactive compounds in live samples by standard fluorescent microscopy methods. Recently, a CB_1-based eCB-GRAB has been developed, and is being rapidly adopted for studying eCB dynamics in live cells, in vitro slices, and *in vivo* (Dong et al., 2020). GRABs are inserted in the plasmalemma and preserve

the binding site structure of the GPCRs form which they are derived, therefore eCB-GRAB allows sensing lipid eCB messengers in the same subcellular compartment where endogenous CB_1 is expressed (Fig. 2B). Using *in vivo* 2-photon eCB-GRAB and correlated calcium indicator imaging, hippocampal pyramidal cell eCB synthesis was studied at high spatiotemporal resolution under physiological conditions as well as during pathological network activity in a seizure model (Dong et al., 2020; Farrell et al., 2020). Importantly, pharmacological perturbation of the 2-AG enzymatic pathway was effective in preventing (by blocking synthesis) or elongating (by blocking hydrolysis) eCB-GRAB signals. These experiments also revealed a high degree of spatiotemporal specificity of eCB signaling, in line with the view that activity-dependent 2-AG production and mobilization is the principal eCB source for synaptic eCB signaling in the hippocampus. Note, however, that anandamide may be released in pathways outside the hippocampus, as demonstrated in the case of peripheral nerve fibers of the pain pathway, GABAergic neurotransmission in the amygdala, and glutamatergic neurotransmission in the limbic forebrain (Clapper et al., 2010; Morena et al., 2019; Patel et al., 2003). As single molecule localization methods such as universal point accumulation-for-imaging-in-nanoscale-topography (uPAINT) or photoactivated localization microscopy (PALM) were recently adapted for dynamic imaging of fluorescent proteins (Giannone et al., 2010; Li and Blanpied, 2016; Manley et al., 2008), and since eCB-GRAB appears to have a low baseline activity which supports the sparse detection of spatially isolated transients (Dong et al., 2020), application of current and future photoconvertible versions of engineered biosensors targeted to specific pre- or postsynaptic membranes for the superresolution localization of eCBs can be envisioned, with significant potential to shed new light on mechanisms of eCB production, transport and action. While eCB-GRAB allows indirectly visualizing endogenous ligands, fluorescently labeled ligands can allow visualizing endogenous binding sites (Fig. 2C). Indeed, a CB_1-specific fluo-cannabinoid agonist has been developed and used for superresolution imaging of CB_1Rs in cell cultures using an approach called pharmacoSTORM (Prokop et al., 2021). These novel approaches complement prior antibody-based labeling methods to expand the toolkit for visualizing CB_1 distribution (Fig. 2).

Future directions

One of the most intriguing developments in understanding the molecular organization of synaptic signaling is related to the discoveries that certain synaptic proteins are accumulated at saturating densities so that they form separated liquid phases that are necessary for their physiological function. While the role of phase transitions has been first recognized in the formation of membrane-less compartments, phase separation has recently also been described for post- and presynaptic molecules interacting with transmembrane proteins (Chen et al., 2020). A liquid phase formed by the scaffolding proteins SynGAP and PSD95 is critical for AMPA-type glutamate receptor synaptic targeting (Feng et al., 2019; Zeng et al., 2016). Similarly, a liquid phase formed by presynaptic synapsins is required for neurotransmitter vesicle clustering (Milovanovic et al., 2018, 2015; Pechstein et al., 2020; Wang and Kaeser, 2018), and synaptic active zone formation also requires phase separation (McDonald et al., 2020). Recently, a study using a biochemical model system of cell membranes, called supported lipid bilayers, suggested that the synaptic enrichment of

VGCCs is facilitated by condensates formed by RIM and RIM binding proteins (Wu et al., 2019). Given the role of RIMs and VGCCs as downstream targets of CB_1, it is possible that liquid/liquid phase separation has a significant role in eCB signaling, either in facilitating the transsynaptic transport of lipid messengers or coupling receptor signaling pathways, or perhaps both. Notably, transcriptomic evidence shows the co-expression of gamma synuclein (Sncg) in CB_1-expressing interneurons (Dudok et al., 2021; Tasic et al., 2016; Yao et al., 2021). Sncg has a role in lipid droplet fusion in adipose tissue, but an unknown role in the brain (Millership et al., 2013). Its homolog alpha synuclein is involved in the formation of SNARE complexes necessary for synaptic vesicle fusion. Based on these premises, one may hypothesize that presynaptic eCB signaling may involve the condensation of a separate liquid phase, perhaps with the involvement of Sncg or RIM, within which CB_1 and eCBs may reach concentrations that are much higher than expected from their average levels. Such hypothetical liquid phases would be highly amenable for study by superresolution optical methods, including CB_1 tracking by QDs, PALM or uPAINT, imaging of lipid biosensors, or fluorescently labeled ligands such as those already developed for CB_1 and CB_2 (Prokop et al., 2021; Spinelli et al., 2020). Importantly, such studies need to be carried out either *in vivo* or under conditions as closely matching the *in vivo* situation as possible.

Due to the critical dependence of both the sensitivity and resolution of single molecule localization on the number of photons detected over background, successful application of these methods often requires very high light intensities, the presence of chemical reducing agents in the medium, and in proximity of the sample to the coverglass. Therefore, implementing STORM and related methods in living tissue has remained challenging. Recent advances in photonics, however, suggest that the above-mentioned limitations may be overcome. A novel localization method called MINIFLUX allows precise localization with 22 times fewer photons compared to previous methods (Balzarotti et al., 2017), suggesting that the currently used high numerical aperture and low working distance lenses will not be required for future applications. Novel illumination strategies are reducing the light irradiance to levels that are compatible with live samples (Mau et al., 2021), while the improved sensitivity and reduced noise floor of scientific complementary metal-oxide-semiconductor (sCMOS) sensors allow imaging of reporters with lower photon yield (Mandracchia et al., 2020). Indeed, nanometer-precise localization deep (>30 microns) in a scattering sample has been achieved using modulated illumination (Jouchet et al., 2021), and superresolution structural imaging using structured illumination has been demonstrated in live mouse brain (Turcotte et al., 2019). With the rapid development of reporters, sensors, and imaging methods, dynamic nanoscale imaging of eCB transmission *in vivo* will likely be feasible in the not too distant future, opening up novel avenues for investigations into the mechanisms of eCB signaling.

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Highlights

- Superresolution microscopy can reveal nanoscale molecular distribution in neurons
- CB1 cannabinoid receptor distribution is subcellular domain-specific
- Dynamic CB1 distribution is regulated by and lateral diffusion and internalization
- Fluorescent biosensors can reveal endocannabinoid dynamics
- Endocannabinoid release is precisely regulated in space and time

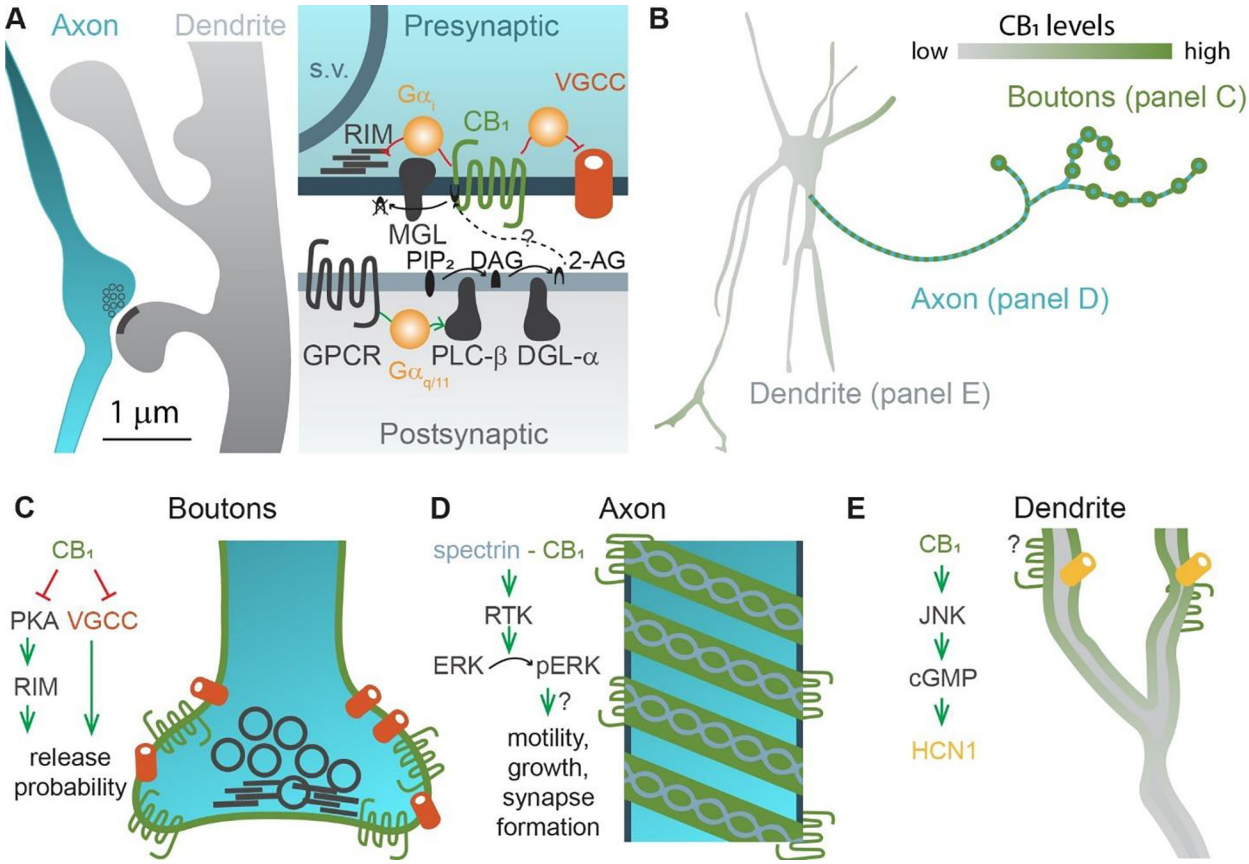

Figure 1: Compartment-specific subcellular distribution of CB_1 receptors

A) Left: Schematic drawing of an axon (blue) and a dendrite (gray). The scale bar denotes the approximate scale of synaptic structures. Right: pre- and postsynaptic localization of selected molecular components of retrograde eCB signaling. Postsynaptic GPCR (e.g. metabotropic glutamate receptor) activation activates an enzymatic cascade leading to the synthesis and release of 2-AG. Presynaptic CB_1 activation suppresses VGCCs and RIMs. The eCB signal is terminated by its presynaptic hydrolyzing enzyme, MGL. S.v.: synaptic vesicle; RIM: Rab3 interacting molecule; MGL: monoacylglycerol-lipase; VGCC: voltage gated calcium channel; GPCR: G-protein coupled receptor; PIP_2: Phosphatidylinositol 4,5 -bisphosphate; DAG: diacylglycerol; 2 -AG: 2-arachydonoylglycerol; PLC: phospholipase C; DGL: DAG lipase. Note that several molecular components, such as those involved in anandamide signaling, are not displayed for clarity. The precise mechanism of retrograde 2-AG transport is not understood.

B) Schematic drawing of a generalized neuron, displaying distinct CB_1 distribution at axons, synaptic boutons, and dendrites. Note that eCB signaling at each subcellular domains may be relevant in distinct cell types.

C) CB_1 distribution at synaptic (GABAergic) boutons. CB_1 receptors are distributed at random in high density. Downstream signaling results in the suppression of synaptic vesicle release probability. PKA: protein kinase A. Note that not all steps of the biochemical cascade are shown.

D) CB_1 distribution at extrasynaptic axonal segments. A membrane-associated periodic skeleton based on spectrin filaments organizes complexes of CB_1 and other GPCRs, RTKs and downstream signaling molecules. The precise molecular mechanism of axonal CB1 signaling is not yet fully understood. RTK: receptor tyrosine kinase; ERK: extracellular signal-regulated kinase.

E) Dendritic CB1 receptors are present at low levels and their distribution is presumably biased towards distal dendrites. Downstream signaling via JNKs activates HCN1 channels resulting in a hyperpolarizing current (h-current). JNK: c-Jun N-terminal kinases; cGMP: cyclic guanosine monophosphate; HCN: hyperpolarization activated cyclic nucleotide gated potassium channel. Note that not all steps of the biochemical cascade are shown, and that the distribution of CB_1 in the dendrites is hypothetical and will need to be demonstrated in future experiments.

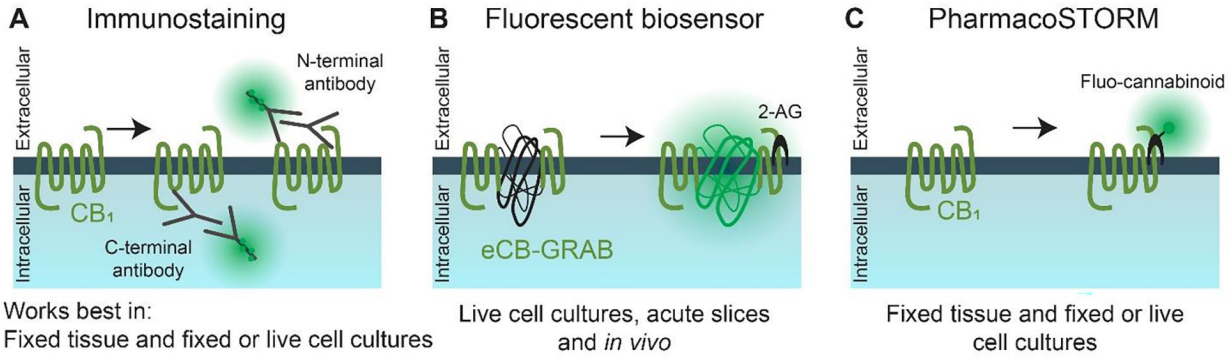

Figure 2: Approaches for fluorescently labeling CB_1 receptors for nanoscale imaging.

A) Antibody labeling with indirect immunostaining or with Q-dots reveals endogenous receptor localization, but may require cell fixation and permeabilization to access intracellular epitopes.

B) Fluorescent biosensors, such as eCB-GRAB, emit light after binding endogenous cannabinoids such as 2-AG, but require the expression of modified CB_1 Rs.

C) Fluorescently labeled ligands become detectable when immobilized by receptor binding and can activate or inhibit receptors depending on pharmacological profile.