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Spatial Encoding of GPCR Signaling in the Nervous System

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Abstract

Several GPCRs, including receptors previously thought to signal primarily from the cell surface, have been recently shown to signal from many intracellular compartments. This raises the idea that signaling by any given receptor is spatially encoded in the cell, with distinct sites of signal origin dictating distinct downstream consequences. We will discuss recent developments that address this novel facet of GPCR physiology, focusing on spatial segregation of signaling from the cell surface, endosomes, and the Golgi by receptors relevant to the nervous system.

Keywords

oligomers; membrane microdomains; clathrin; single-molecule; diffusion kinetics; endosome; Golgi; nanobodies; phosphoinositides; retention; lipid domains; microscopy

Introduction

The principles of the interplay between receptor signaling and membrane trafficking are currently being re-evaluated in the field. Trafficking of receptors, including that of G protein-coupled receptors (GPCRs) - the largest family of signaling receptors - was first described many decades ago [1]. GPCRs activated on the cell surface are rapidly sequestered in specialized endocytic domains and internalized from the cell surface [2,3]. Internalized GPCRs are transported to the endosomal network, from where they may be either recycled back to the surface or degraded [4]. These trafficking steps were primarily viewed as mechanisms to remove receptors from or add GPCRs to the cell surface, thereby regulating receptor access to extracellular signals and modulating cell sensitivity to signals [5]. While this is still valid, several GPCRs, including canonical receptors previously thought to signal primarily from the surface, are now known to signal from multiple intracellular compartments [6–8]. The downstream consequences of signaling (e.g., the genes that are activated) for any given receptor can differ based simply on the compartment in which

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receptors are located [9–11], suggesting that signaling is spatially encoded. We will discuss recent examples of receptors signaling from the plasma membrane, endosomes, and the Golgi apparatus that exemplify spatial encoding in pathways relevant to the nervous system.

Spatial encoding at the plasma membrane

The basal state of GPCRs in the plasma membrane is highly dynamic, and vary between different receptors. It is likely that receptors exist in an equilibrium of monomers, dimers, and higher order oligomers [12,13], but the physiological distribution and roles of these complexes are not clear. For example, single molecules of the neurotensin receptor NTSR1 ‘sample’ multiple dimer states, and transit between different dimer conformations rapidly [13]. Single molecule imaging of the dynamics of α 2- and β 2-adrenergic receptors (A2AR, B2AR) by total internal reflection fluorescence microscopy found 4 discrete states of protein movement, based on diffusion rate, for both receptors and their G proteins within the plasma membrane. Similarly, 4 diffusive states have also been shown by different group for the metabotropic glutamate receptor mGluR3 [14]. The diffusion of A2AR, B2AR, and the μ opioid receptor μ OR appear constrained by ‘fence’-like barriers created by actin and tubulin [15,16]. Relatedly, cytoskeletal factors like filamin A control the membrane dynamics and internalization of the somatostatin receptor SSTR2 [17]. In addition to protein barriers, receptor diffusion can also be regulated by lipid domains. The μ - and κ -opioid receptors (μ OR, κ OR) show different diffusion dynamics within the plasma membrane when analyzed by fluorescence correlation spectroscopy, although both are primarily localized at cholesterol-rich domains [18].

Receptors can redistribute in the plasma membrane in response to agonist in multiple ways. A2AR and B2AR do not change their diffusion dynamics following agonist addition, but their cognate G proteins G_i and G_s became more mobile after agonist addition and receptor-G protein interactions are shortened [15]. This shortening could reflect a higher likelihood of ‘productive’ interactions following receptor activation by agonist, although this was not tested. In contrast, mGluR3 slows its diffusion significantly after agonist addition [14]. Similarly, the μ OR redistributes in response to agonists, and this redistribution varies depending on the agonist. DAMGO, a synthetic variant of an endogenous opioid, but not morphine, an exogenous drug of abuse, causes μ OR to be reorganized into small clusters at the plasma membrane [19]. Interestingly, this was independent of receptor localization with clathrin, as DAMGO-activated receptors did not colocalize with endocytic markers until upwards of 60 minutes after agonist exposure [19]. While this observation is consistent with single molecule studies of A2AR and B2AR where receptor colocalization with clathrin was minimal at early time points [15], this contrasts with a large amount of evidence that supports rapid association between DAMGO-treated μ OR and clathrin-mediated endocytosis components [20–22]. Consistent with this, diffusion kinetics of metabotropic glutamate receptors was slowed by agonist-mediated clustering in clathrin-coated pits and was also modulated by inhibiting downstream G proteins G_i/Go by pertussis toxin [14]. Because endocytosis, and arguably oligomerization, are highly regulated by many environmental factors and cell states, the variable observations in the above studies could reflect differences in experimental and imaging setups, cell lines, and expression levels of the receptors.

The differential redistribution of GPCRs in the membrane can be correlated to changes in downstream signaling, although the exact mechanisms are still under debate. In the same study that observed differential clustering of μ OR, DAMGO, but not morphine, caused transient ERK1/2 activation in both the cytosol and nucleus in a β -arrestin-dependent but endocytosis-independent manner [19]. However, μ OR can activate ERK1/2 when localized to endocytic domains, with a magnitude depending on the time that receptors remained bound to arrestin. Importantly, this “endocytic dwell time” and corresponding ERK1/2 activation were significantly higher for DAMGO-activated μ OR than for morphine-activated μ OR [23]. A similar dependence of β -arrestin-dependent ERK1/2 activation on endocytic dwell times has also been shown for the cannabinoid receptor 1 (CB1R) [24]. β -arrestin can be activated catalytically by transient interactions with some GPCRs [25,26], allowing ERK1/2 signaling from endocytic domains independent of receptor association [27]. Molecular dynamics simulations suggest receptors can break a specific salt bridge between R175 and D296 residues on arrestin-1, causing activation [25]. This work extends earlier studies that identified unique β -arrestin conformations that mediate internalization and signaling [28,29]. Together, these findings separate the signaling and adaptor functions of β -arrestins, suggesting that arrestins could be catalytically activated effectors of GPCRs in addition to their roles in receptor trafficking. The variation in whether arrestin translocates to endocytic domains with or without a given GPCR also underscores the idea that the mechanisms that link receptor organization to signaling are receptor-specific.

Spatial encoding at endosomes

GPCR signaling from endosomes is now well documented for many receptors, and it has been discussed in several recent reviews [6,7] but its role in neuronal function is just starting to be addressed. Endosomal signaling was first described using MAPK activation as an output for the protease activated receptor PAR2 [30] and for the angiotensin receptor ATR1 [31]. For both these receptors, β -arrestin was required for MAPK activation at endosomes. In contrast, MAPK activation by the luteinizing hormone receptor [32,33] and the protease activated receptor PAR1 [34] on endosomes is independent of endosomal recruitment of β -arrestins, but dependent on specific scaffolding proteins that could directly activate the pathway. Endosomal activation of G proteins was first described for GPCRs of the class B family, such as the parathyroid hormone receptor [35], the thyroid stimulating hormone receptor [36], the vasopressin 2 receptor [37]. Recent studies using Nb80, a nanobody that recognizes B2AR in its active conformation, and Nb37, a nanobody that recognizes the nucleotide-free Gs protein presumably in the process of exchanging GDP for GTP, suggest that B2AR signals from endosomal domains [38]. Sequence-dependent localization of B2AR to specific microdomains, marked by a localized actin-based machinery, was required for endosomal G protein activation [10,39]. Active μ - and δ -opioid receptors too were localized to endosomes recently using a nanobody-based approach [40]. Generation of cAMP at endosomes induces expression of a distinct subset of genes, as identified by transcriptional profiling [9].

Endosomal signaling has also been linked both directly and indirectly to a number of pain pathologies [11] and has inspired interest in spatially restricted receptor activation as a therapeutic strategy [41]. Sustained signaling of the neurokinin receptor NK1R from

endosomes in spinal cord neurons caused nociception [42]. Newly designed membrane-anchored antagonists of NK1R that stay associated with the receptor throughout endocytic trafficking show better anti-nociception compared to traditional antagonists [42]. Similarly, a membrane-tethered antagonist against the CGRP-targeted calcitonin-like receptor (CLR) decreased nociception *in vivo* [43]. Although the specific impact of endosomal signaling to the physiological functions of GPCRs remains to be fully understood, this raises the exciting possibility that targeting endosomal vs. surface receptors can be a novel therapeutic strategy.

Spatial encoding at the Golgi

GPCR localization and signaling in the Golgi apparatus is an emerging paradigm. All newly synthesized GPCRs transit the Golgi on their way to the surface, but some of them localize to the Golgi at steady-state [44–46]. This localization could be a result of either retention of newly synthesized receptors or retrograde trafficking of GPCRs to the Golgi after internalization [47–50]. Intracellular localization of the δ opioid receptor (δ OR) in subsets of neurons has been known for many decades [44,51–55] [51,56–58]. Several studies indicate that newly synthesized δ OR is localized to a late- or post-Golgi compartment. This localization is regulated by a balance of Phosphoinositide 3 kinase Class 2 alpha and the phosphatase PTEN, which may control the levels of the phospholipid PI(3,4)P2 in the Trans Golgi Network [44,59]. Indirect readouts measuring DOR function suggest that surface levels of DOR in neurons can be regulated by acute NGF signaling, as well as by ROCK and LIM kinases that could modify the actin cytoskeleton, but the exact steps that these pathways regulate are still not clear [45,60]. Recently, Golgi localization have been described for other GPCRs, including the beta-1 adrenergic receptor (B1AR) and for the somatostatin receptor 5 [46,61]. For these receptors, Golgi localization may involve interactions of the C-terminal PDZ ligand with Golgi-localized PDZ adaptor protein PIST/GOPC/CAL [62,63]. δ OR has no known PDZ ligand, but it can interact with the COPI machinery that retrieves proteins back to earlier compartments [64]. This suggests that Golgi localization may be a result of constant retrieval to earlier compartments, consistent with the view that cargo transits the Golgi as a default mechanism. The exact mechanisms of Golgi retention of GPCRs and the regulation of these mechanisms by signaling are fundamental questions that remain to be answered.

The role of Golgi-localized GPCRs is still being explored. For primarily “single-use” GPCRs like DOR which are mostly degraded rather than recycled after activation and internalization [4,58], this intracellular store might act as a readily releasable pool of receptors for quick recovery of signaling competence [57,65–68]. Although this is true, recent work using nanobody-based biosensors suggests that GPCRs may also actively signal from in the Golgi. Intracellular δ OR is competent for signaling, as cell-permeable agonists such as SNC80 and ARM390 can switch δ OR into an active conformation, as reported by the nanobody sensor Nb33 that preferentially binds the active δ OR conformation [40]. Similar Golgi activation was observed even when morphine was used to activate exogenously expressed μ OR, although the receptor typically does not accumulate in the Golgi in neurons [40,44,58]. Nanobodies that recognize active B1AR and nucleotide-free Gs both are also recruited to the Golgi in response to hydrophobic agonists such as dobutamine, which can rapidly cross cellular membranes [46]. Nevertheless, the consequences of

activating Golgi GPCRs are still not known. Activation of Golgi-localized B1AR leads to the local recruitment of PKA to the Golgi membranes, similar to what has been observed with TSHR, suggesting that activated Golgi receptors can signal [46,50]. In an overexpressed system, Golgi DOR appears to contribute to cellular cAMP inhibition [40]. Both EPAC activation downstream of Gs signaling, as well as G $\beta\gamma$ signaling downstream of GPCRs produce PI4P hydrolysis by PLC ϵ and activation of nuclear PKD [69,70]. It is possible that GPCRs located at the Golgi can activate these pathways. Local activation of receptors, G proteins, and effectors could cause distinct downstream effects as observed with local cAMP generation at the endosomes [9], but the specific targets are still being identified.

Whether these receptors are active in the Golgi *in vivo* is a more difficult question to address. Catecholamines like norepinephrine may be actively transported across membranes by transporters such as OCT3 allowing them to activate adrenergic receptors [46]. No such mechanism has been identified for peptide agonists, and opioid peptides cannot activate Golgi localized receptors at short (~20 min) timescales [40]. Nevertheless, it is clear that intracellular localization is highly regulated, and that it can be correlated to opioid physiology. DOR traffics from intracellular pools in response to inflammatory signals and activation of surface DOR [45,52,57,68,71]. Actively relocating DOR from the Golgi to the surface by inhibiting PTEN increases the antihyperalgesic effects of SNC80 without increasing convulsive effects [44], suggesting that signaling from the Golgi and the surface could have separable effects. Whether these peptides can be transported by trafficking to access intracellular receptors over longer time scales, or whether receptors such as DOR are constitutively active in these locations in neurons, are exciting areas for future study.

Conclusions and Future Directions

The new paradigm that an activated GPCR signals in multiple distinct phases separated by location and time provides an exciting new explanation for how single receptors can induce pleiotropic signaling effects. Each agonist, based on the distinct trafficking profile that it induces, could generate a characteristic “barcode” of phases of signaling. The timing and extent of these phases could be controlled precisely by mechanisms that regulate the trafficking of GPCRs, as they are the primary means of transporting receptors between membrane domains and protein complexes that are necessary for signaling. While we have focused primarily on endosomes and the Golgi in this review, recent data suggest that GPCRs could signal from any intracellular organelle. A compelling example is the metabotropic glutamate receptor mGluR5, which is localized to the nuclear envelope in many neurons [72]. Intracellular signaling of mGluR5 could be associated with synaptic plasticity and neuropathic pain [73]. Many other GPCRs such as α -adrenergic, cannabinoid, serotonin, purinergic, and chemokine receptors can signal from intracellular organelles including the endoplasmic reticulum, the nuclear envelope, and mitochondria (excellently reviewed recently in [74]). As we further understand these receptor trafficking mechanisms and the signaling pathways that regulate these mechanisms, we might be able to leverage them to develop effective therapeutics that precisely control different aspects of receptor signaling by any given drug.

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References:

1. Pierce KL, Premont RT, Lefkowitz RJ: Seven-transmembrane receptors. *Nat Rev Mol Cell Biol* 2002, 3:639–650. [PubMed: 12209124]
2. Zastrow von M, Kobilka BK: Antagonist-dependent and -independent steps in the mechanism of adrenergic receptor internalization. *Journal of Biological Chemistry* 1994, 269:18448–18452. [PubMed: 7518433]
3. Santini F, Gaidarov I, Keen JH: G protein-coupled receptor/arrestin3 modulation of the endocytic machinery. *The Journal of Cell Biology* 2002, 156:665–676. [PubMed: 11839771]
4. Hanyaloglu AC, Zastrow MV: Regulation of GPCRs by Endocytic Membrane Trafficking and Its Potential Implications. *Annu. Rev. Pharmacol. Toxicol* 2008, 48:537–568. [PubMed: 18184106]
5. Hausdorff WP, Caron MG, Lefkowitz RJ: Turning off the signal: desensitization of beta-adrenergic receptor function. *FASEB J.* 1990, 4:2881–2889. [PubMed: 2165947]
6. Eichel K, Zastrow von M: Subcellular Organization of GPCR Signaling. *Trends in Pharmacological Sciences* 2018, 39:200–208. [PubMed: 29478570]
7. Sposini S, Hanyaloglu AC: Spatial encryption of G protein-coupled receptor signaling in endosomes; Mechanisms and applications. *Biochemical Pharmacology* 2017, 143:1–9. [PubMed: 28456515]
8. Vilardaga J-P, Jean-Alphonse FG, Gardella TJ: Endosomal generation of cAMP in GPCR signaling. *Nature Publishing Group* 2014, 10:700–706.
9. Tsvetanova NG, Zastrow von M: Spatial encoding of cyclic AMP signaling specificity by GPCR endocytosis. *Nat Chem Biol* 2014, doi:10.1038/nchembio.1665.
10. Bowman SL, Shiwarski DJ, Puthenveedu MA: Distinct G protein-coupled receptor recycling pathways allow spatial control of downstream G protein signaling. *The Journal of Cell Biology* 2016, 214:797–806. [PubMed: 27646272] • This study showed that B2AR needs to be in specialized actin-stabilized microdomains on the endosome to activate Gs protein. Access to this domain, and therefore the balance of surface to endosomal signaling, is determined by extracellular signals that regulate PKA phosphorylation of B2AR.
11. Jimenez-Vargas NN, Pattison LA, Zhao P, Lieu T, Latorre R, Jensen DD, Castro J, Aurelio L, Le GT, Flynn B, et al.: Protease-activated receptor-2 in endosomes signals persistent pain of irritable bowel syndrome. *Proc. Natl. Acad. Sci. U.S.A* 2018, 115:E7438–E7447. [PubMed: 30012612]
12. Calebiro D, Rieken F, Wagner J, Sungkaworn T, Zabel U, Borzi A, Cocucci E, Zurn A, Lohse MJ: Single-molecule analysis of fluorescently labeled G-protein-coupled receptors reveals complexes with distinct dynamics and organization. *Proceedings of the National Academy of Sciences* 2013, 110:743–748.
13. Dijkman PM, Castell OK, Goddard AD, Munoz-Garcia JC, de Graaf C, Wallace MI, Watts A: Dynamic tuneable G protein-coupled receptor monomer-dimer populations. *Nat Comms* 2018, 9:1710.
14. Yanagawa M, Hiroshima M, Togashi, Abe M, Yamashita T, Shichida Y, Murata M, Ueda M, Sako Y: Single-molecule diffusion-based estimation of ligand effects on G protein-coupled receptors. *Sci Signal* 2018, 11:eaao1917.
15. Sungkaworn T, Jobin M-L, Burnecki K, Weron A, Lohse MJ, Calebiro D: Single-molecule imaging reveals receptor-G protein interactions at cell surface hot spots. *Nature Publishing Group* 2017, 550:543–547. •• References 14 and 15 use single-molecule imaging to address basal organization and diffusion of different GPCRs on the plasma membrane. They report partially divergent observations which suggest that the organization is receptor-specific. Reference 15 extends their studies to the localization of G proteins, cytoskeletal components, and endocytic machinery in a cellular context.

16. Suzuki K, Ritchie K, Kajikawa E, Fujiwara T, Kusumi A: Rapid Hop Diffusion of a G-Protein-Coupled Receptor in the Plasma Membrane as Revealed by Single-Molecule Techniques. *Biophysical Journal* 2005, 88:3659–3680. [PubMed: 15681644]
17. Treppiedi D, Jobin M-L, Peverelli E, Giardino E, Sungkaworn T, Zabel U, Arosio M, Spada A, Mantovani G, Calebiro D: Single-Molecule Microscopy Reveals Dynamic FLNA Interactions Governing SSTR2 Clustering and Internalization. *Endocrinology* 2018, 159:2953–2965. [PubMed: 29931263]
18. Rogacki MK, Golfetto O, Tobin SJ, Li T, Biswas S, Jorand R, Zhang H, Radoi V, Ming Y, Svenningsson P, et al.: Dynamic lateral organization of opioid receptors (κ , μ wt and μ N40D) in the plasma membrane at the nanoscale level. *Traffic* 2018, doi:10.1111/tra.12582.
19. Halls ML, Yeatman HR, Nowell CJ, Thompson GL, Gondin AB, Covicristov S, Bunnett NW, Lambert NA, Poole DP, Canals M: Plasma membrane localization of the μ -opioid receptor controls spatiotemporal signaling. *Sci Signal* 2016, 9:ra16–ra16. [PubMed: 26861044]
20. Roman-Vendrell C, Yu YJ, Yudowski GA: Fast modulation of μ -opioid receptor (MOR) recycling is mediated by receptor agonists. *J. Biol. Chem* 2012, 287:14782–14791. [PubMed: 22378794]
21. Soohoo AL, Puthenveedu MA: Divergent modes for cargo-mediated control of clathrin-coated pit dynamics. *Molecular Biology of the Cell* 2013, 24:1725–1734. [PubMed: 23536704]
22. Laporte SA, Oakley RH, Zhang J, Holt JA, Ferguson SS, Caron MG, Barak LS: The beta2-adrenergic receptor/betaarrestin complex recruits the clathrin adaptor AP-2 during endocytosis. *Proceedings of the National Academy of Sciences* 1999, 96:3712–3717.
23. Weinberg ZY, Zajac AS, Phan T, Shiowski DJ, Puthenveedu MA: Sequence-Specific Regulation of Endocytic Lifetimes Modulates Arrestin-Mediated Signaling at the μ Opioid Receptor. *Molecular Pharmacology* 2017, 91:416–427. [PubMed: 28153854]
24. Flores-Otero J, Ahn KH, Delgado-Peraza F, Mackie K, Kendall DA, Yudowski GA: Ligand-specific endocytic dwell times control functional selectivity of the cannabinoid receptor 1. *Nat Comms* 2014, 5:4589. • References 23 and 24 show that opioid and CB1 receptors use specific sequences on their C-termini to extend the lifetimes of clathrin-coated pits. This extension provides time for arrestin-receptor complexes to activate MAPK.
25. Latorraca NR, Wang JK, Bauer B, Townshend RJL, Hollingsworth SA, Olivieri JE, Xu HE, Sommer ME, Dror RO: Molecular mechanism of GPCR-mediated arrestin activation. *Nature* 2018, 35:308.
26. Eichel K, Jullié D, Barsi-Rhyne B, Latorraca NR, Masureel M, Sibarita J-B, Dror RO, Zastrow von M: Catalytic activation of β -arrestin by GPCRs. *Nature* 2018, 459:356.
27. Eichel K, Jullie D, Zastrow von M: β -Arrestin drives MAP kinase signalling from clathrin-coated structures after GPCR dissociation. *Nat Cell Biol* 2016, 18:303–310. [PubMed: 26829388] • References 25, 26, and 27 show that the functions of arrestin as an endocytic adapter and as a signaling scaffold can be separated based on the interacting domains on the receptors and arrestin. This suggests that arrestin could be catalytically activated by receptors akin to G proteins.
28. Lee M-H, Appleton KM, Strungs EG, Kwon JY, Morinelli TA, Peterson YK, Laporte SA, Luttrell LM: The conformational signature of β -arrestin2 predicts its trafficking and signalling functions. *Nature* 2016, 531:665–668. [PubMed: 27007854]
29. Cahill TJ III, Thomsen ARB, Tarrasch JT, Plouffe B, Nguyen AH, Yang F, Huang L-Y, Kahsai AW, Bassoni DL, Gavino BJ, et al.: Distinct conformations of GPCR- β -arrestin complexes mediate desensitization, signaling, and endocytosis. *Proceedings of the National Academy of Sciences* 2017, 114:2562–2567.
30. DeFea KA, Zalevsky J, Thoma MS, Déry O, Mullins RD, Bunnett NW: beta-arrestin-dependent endocytosis of proteinase-activated receptor 2 is required for intracellular targeting of activated ERK1/2. *The Journal of Cell Biology* 2000, 148:1267–1281. [PubMed: 10725339]
31. Luttrell LM, Roudabush FL, Choy EW, Miller WE, Field ME, Pierce KL, Lefkowitz RJ: Activation and targeting of extracellular signal-regulated kinases by beta-arrestin scaffolds. *Proceedings of the National Academy of Sciences* 2001, 98:2449–2454.
32. Jean-Alphonse F, Bowman S, Chen S, Beard G, Puthenveedu MA, Hanyaloglu AC: Spatially restricted G protein-coupled receptor activity via divergent endocytic compartments. *J. Biol. Chem* 2014, 289:3960–3977. [PubMed: 24375413]

33. Sposini S, Jean-Alphonse FG, Ayoub MA, Oqua A, West C, Lavery S, Brosens JJ, Reiter E, Hanyaloglu AC: Integration of GPCR Signaling and Sorting from Very Early Endosomes via Opposing APPL1 Mechanisms. *CellReports* 2017, 21:2855–2867.
34. Grimsey NJ, Aguilar B, Smith TH, Le P, Soohoo AL, Puthenveedu MA, Nizet V, Trejo J: Ubiquitin plays an atypical role in GPCR-induced p38 MAP kinase activation on endosomes. *The Journal of Cell Biology* 2015, 210:1117–1131. [PubMed: 26391660]
35. Ferrandon S, Feinstein TN, Castro M, Wang B, Bouley R, Potts JT, Gardella TJ, Vilardaga J-P: Sustained cyclic AMP production by parathyroid hormone receptor endocytosis. *Nat Chem Biol* 2009, 5:734–742. [PubMed: 19701185]
36. Calebiro D, Nikolaev VO, Gagliani MC, de Filippis T, Dees C, Tacchetti C, Persani L, Lohse MJ: Persistent cAMP-signals triggered by internalized G-protein-coupled receptors. *PLoS Biol* 2009, 7:e1000172. [PubMed: 19688034]
37. Feinstein TN, Yui N, Webber MJ, Wehbi VL, Stevenson HP, King JD, Hallows KR, Brown D, Bouley R, Vilardaga J-P: Noncanonical control of vasopressin receptor type 2 signaling by retromer and arrestin. *J. Biol. Chem* 2013, 288:27849–27860. [PubMed: 23935101]
38. Irannejad R, Tomshine JC, Tomshine JR, Chevalier M, Mahoney JP, Steyaert J, Rasmussen SGF, Sunahara RK, El-Samad H, Huang B, et al.: Conformational biosensors reveal GPCR signalling from endosomes. *Nature* 2013, 495:534–538. [PubMed: 23515162]
39. Vistein R, Puthenveedu MA: Reprogramming of G protein-coupled receptor recycling and signaling by a kinase switch. *Proc. Natl. Acad. Sci. U.S.A* 2013, 110:15289–15294. [PubMed: 24003153]
40. Stoeber M, Jullié D, Lobingier BT, Laeremans T, Steyaert J, Schiller PW, Manglik A, Zastrow von M: A Genetically Encoded Biosensor Reveals Location Bias of Opioid Drug Action. *Neuron* 2018, 98:963–976.e5. [PubMed: 29754753] • Using nanobody sensors, the study showed that opioid receptors are in an active conformation on endosomes and the Golgi apparatus, and that this intracellular activation contributes to inhibition of cAMP by opioid agonists.
41. Thomsen ARB, Jensen DD, Hicks GA, Bunnett NW: Therapeutic Targeting of Endosomal G-Protein-Coupled Receptors. *Trends in Pharmacological Sciences* 2018, 39:879–891. [PubMed: 30180973]
42. Jensen DD, Lieu T, Halls ML, Veldhuis NA, Imlach WL, Mai QN, Poole DP, Quach T, Aurelio L, Conner J, et al.: Neurokinin 1 receptor signaling in endosomes mediates sustained nociception and is a viable therapeutic target for prolonged pain relief. *Sci Transl Med* 2017, 9:•• This groundbreaking study correlated endosomal signaling to specific physiological effects in vivo and showed that these effects could be modulated by drugs that specifically target endosomal signaling. This provides a key proof that we can exploit spatial encoding for therapeutic benefit.
43. Yarwood RE, Imlach WL, Lieu T, Veldhuis NA, Jensen DD, Klein Herenbrink C, Aurelio L, Cai Z, Christie MJ, Poole DP, et al.: Endosomal signaling of the receptor for calcitonin gene-related peptide mediates pain transmission. *Proc. Natl. Acad. Sci. U.S.A* 2017, 114:12309–12314. [PubMed: 29087309]
44. Shiwarski DJ, Tipton A, Giraldo MD, Schmidt BF, Gold MS, Pradhan AA, Puthenveedu MA: A PTEN-Regulated Checkpoint Controls Surface Delivery of δ Opioid Receptors. *J. Neurosci* 2017, 37:3741–3752. [PubMed: 28264976] •• This study showed that relocating the delta opioid receptor from the Golgi to the cell surface significantly improves antinociceptive effects of DOR-targeted agonists. This provided a key proof of principle that we can fine-tune the effects of any given drug by independently relocating receptors.
45. Mittal N, Roberts K, Pal K, Bentolila LA, Fultz E, Minasyan A, Cahill C, Pradhan A, Conner D, DeFea K, et al.: Select G-protein-coupled receptors modulate agonist-induced signaling via a ROCK, LIMK, and β -arrestin 1 pathway. *Cell Reports* 2013, 5:1010–1021. [PubMed: 24239352]
46. Irannejad R, Pessino V, Mika D, Huang B, Wedegaertner PB, Conti M, Zastrow von M: Functional selectivity of GPCR-directed drug action through location bias. *Nature Publishing Group* 2017, 13:799–806. •• This study used nanobody sensors to describe B1AR signaling on the Golgi apparatus. They showed that the ability of ligands to activate Golgi receptors correlated with the rate at which they could be transported across the membranes, either passively or actively using transporters.

47. Charfi I, Abdallah K, Gendron L, Piñeyro G: Delta opioid receptors recycle to the membrane after sorting to the degradation path. *Cell. Mol. Life Sci* 2017, 75:2257–2271. [PubMed: 29288293]
48. Adachi N, Hess DT, McLaughlin P, Stamler JS: S-Palmitoylation of a Novel Site in the β 2-Adrenergic Receptor Associated with a Novel Intracellular Itinerary. *J. Biol. Chem* 2016, 291:20232–20246. [PubMed: 27481942]
49. Ebersole B, Petko J, Woll M, Murakami S, Sokolina K, Wong V, Stagljar I, Lüscher B, Levenson R: Effect of C-Terminal S-Palmitoylation on D2 Dopamine Receptor Trafficking and Stability. *PLoS ONE* 2015, 10:e0140661. [PubMed: 26535572]
50. Godbole A, Lyga S, Lohse MJ, Calebiro D: Internalized TSH receptors en route to the TGN induce local Gs-protein signaling and gene transcription. *Nat Comms* 2017, 8:443.
51. Bao L, Jin S-X, Zhang C, Wang L-H, Xu Z-Z, Zhang F-X, Wang L-C, Ning F-S, Cai H-J, Guan J-S, et al.: Activation of delta opioid receptors induces receptor insertion and neuropeptide secretion. *Neuron* 2003, 37:121–133. [PubMed: 12526778]
52. Cahill CM, McClellan KA, Morinville A, Hoffert C, Hubatsch D, O'Donnell D, Beaudet A: Immunohistochemical distribution of delta opioid receptors in the rat central nervous system: evidence for somatodendritic labeling and antigen-specific cellular compartmentalization. *J. Comp. Neurol* 2001, 440:65–84. [PubMed: 11745608]
53. Roth BL, Laskowski MB, Coscia CJ: Evidence for distinct subcellular sites of opiate receptors. Demonstration of opiate receptors in smooth microsomal fractions isolated from rat brain. *Journal of Biological Chemistry* 1981, 256:10017–10023. [PubMed: 6268622]
54. Kim K-A, Zastrow von M: Neurotrophin-regulated sorting of opioid receptors in the biosynthetic pathway of neurosecretory cells. *J. Neurosci* 2003, 23:2075–2085. [PubMed: 12657666]
55. Wang H, Pickel VM: Preferential cytoplasmic localization of delta-opioid receptors in rat striatal patches: comparison with plasmalemmal mu-opioid receptors. *J. Neurosci* 2001, 21:3242–3250. [PubMed: 11312309]
56. Zhang X, Bao L, Arvidsson U, Elde R, Hökfelt T: Localization and regulation of the delta-opioid receptor in dorsal root ganglia and spinal cord of the rat and monkey: evidence for association with the membrane of large dense-core vesicles. *Neuroscience* 1998, 82:1225–1242. [PubMed: 9466442]
57. Pettinger L, Gigout S, Linley JE, Gamper N: Bradykinin controls pool size of sensory neurons expressing functional δ -opioid receptors. *J. Neurosci* 2013, 33:10762–10771. [PubMed: 23804098]
58. Gendron L, Cahill CM, Zastrow von M, Schiller PW, Piñeyro G: Molecular Pharmacology of δ -Opioid Receptors. *Pharmacological Reviews* 2016, 68:631–700. [PubMed: 27343248]
59. Shiwerski DJ, Darr M, Telmer CA, Bruchez MP, Puthenveedu MA: PI3K class II α regulates δ -opioid receptor export from the trans-Golgi network. *Molecular Biology of the Cell* 2017, 28:2202–2219. [PubMed: 28566554]
60. Bie B, Zhang Z, Cai Y-Q, Zhu W, Zhang Y, Dai J, Lowenstein CJ, Weinman EJ, Pan ZZ: Nerve growth factor-regulated emergence of functional delta-opioid receptors. *J. Neurosci* 2010, 30:5617–5628. [PubMed: 20410114]
61. Bauch C, Koliwer J, Buck F, Hönck H-H, Kreienkamp H-J: Subcellular sorting of the G-protein coupled mouse somatostatin receptor 5 by a network of PDZ-domain containing proteins. *PLoS ONE* 2014, 9:e88529. [PubMed: 24523912]
62. Koliwer J, Park M, Bauch C, Zastrow von M, Kreienkamp H-J: The golgi-associated PDZ domain protein PIST/GOPC stabilizes the β 1-adrenergic receptor in intracellular compartments after internalization. *J. Biol. Chem* 2015, 290:6120–6129. [PubMed: 25614626]
63. Wente W, Stroh T, Beaudet A, Richter D, Kreienkamp H-J: Interactions with PDZ domain proteins PIST/GOPC and PDZK1 regulate intracellular sorting of the somatostatin receptor subtype 5. *Journal of Biological Chemistry* 2005, 280:32419–32425. [PubMed: 16012170]
64. St-Louis É, Degrandmaison J, Grastilleur S, Génier S, Blais V, Lavoie C, Parent J-L, Gendron L: Involvement of the coatamer protein complex I in the intracellular traffic of the delta opioid receptor. *Mol. Cell. Neurosci* 2016, 79:53–63. [PubMed: 28041939]

65. Brismar H, Asghar M, Carey RM, Greengard P, Aperia A: Dopamine-induced recruitment of dopamine D1 receptors to the plasma membrane. *Proceedings of the National Academy of Sciences* 1998, 95:5573–5578.
66. Hein L, Ishii K, Coughlin SR, Kobilka BK: Intracellular targeting and trafficking of thrombin receptors. A novel mechanism for resensitization of a G protein-coupled receptor. *Journal of Biological Chemistry* 1994, 269:27719–27726. [PubMed: 7961693]
67. Perron A, Sharif N, Gendron L, Lavallée M, Stroh T, Mazella J, Beaudet A: Sustained neurotensin exposure promotes cell surface recruitment of NTS2 receptors. *Biochemical and Biophysical Research Communications* 2006, 343:799–808. [PubMed: 16564027]
68. Patwardhan AM, Berg KA, Akopain AN, Jeske NA, Gamper N, Clarke WP, Hargreaves KM: Bradykinin-induced functional competence and trafficking of the delta-opioid receptor in trigeminal nociceptors. *J. Neurosci* 2005, 25:8825–8832. [PubMed: 16192372]
69. Nash CA, Brown LM, Malik S, Cheng X, Smrcka AV: Compartmentalized cyclic nucleotides have opposing effects on regulation of hypertrophic phospholipase C ϵ signaling in cardiac myocytes. *J. Mol. Cell. Cardiol* 2018, 121:51–59. [PubMed: 29885334]
70. Malik RU, Ritt M, DeVree BT, Neubig RR, Sunahara RK, Sivaramakrishnan S: Detection of G Protein-selective G Protein-coupled Receptor (GPCR) Conformations in Live Cells. *Journal of Biological Chemistry* 2013, 288:17167–17178. [PubMed: 23629648]
71. Pradhan A, Smith M, McGuire B, Evans C, Walwyn W: Chronic inflammatory injury results in increased coupling of delta opioid receptors to voltage-gated Ca $^{2+}$ channels. *Mol Pain* 2013, 9:8. [PubMed: 23497324]
72. Sergin I, Jong YI, Harmon SK, Kumar V, O'Malley KL: Sequences within the C Terminus of the Metabotropic Glutamate Receptor 5 (mGluR5) Are Responsible for Inner Nuclear Membrane Localization. *Journal of Biological Chemistry*. 2017, 292:3637–3655. [PubMed: 28096465]
73. Vincent K, Cornea VM, Jong YJ, Laferrière A, Kumar N, Mickeviciute A, Fung JS, Bandegi P, Ribeiro-da-Silva A, O'Malley KL, et al. Intracellular mGluR5 plays a critical role in neuropathic pain. *Nature Communications*. 2016, 7:10604.
74. Jong YI, Harmon SK, O'Malley KL. GPCR signalling from within the cell. *British Journal of Pharmacology*. 2018, 175:4026–4035. [PubMed: 28872669]

Diversity in GPCR signaling from subcellular locations.

At the plasma membrane GPCRs can signal through both G proteins and arrestins. Receptors like NK1R and B2AR can couple to different G proteins depending on the circumstances, although how this selectivity is regulated is not well understood. Most GPCRs change their diffusion, clustering, and localization to endocytic domains in response to agonist. The rates of these changes are different between different agonists, which could in turn dictate effector coupling and signaling as has been described for μ OR. Many GPCRs also signal from endosomes once internalized. Some receptors like NK1R could couple directly to different effectors in endosomes compared to the plasma membrane to cause different physiological effects. B2AR couples to Gs in both locations but cause divergent transcriptional outputs depending on where it is activated. GPCR signaling from the Golgi has recently been described for B1AR and δ OR, two GPCRs with steady-state Golgi localization. The physiological consequences and *in vivo* relevance of receptors signaling at the Golgi are still being explored. Exciting future work on the mechanisms and signaling of receptors at different sites in their trafficking itinerary may allow us to develop therapeutic strategies that spatially restrict receptor activation in subcellular locations and therefore fine-tune the consequences of receptor signaling.