

## Model organisms in GPCR research

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## Abstract

The study of G protein-coupled receptors (GPCRs) has benefited greatly from experimental approaches that interrogate their functions in controlled, artificial environments. Working *in vitro*, GPCR receptorologists discovered the basic biological mechanisms by which GPCRs operate, including their eponymous capacity to couple to G proteins (De Lean et al., 1980), their molecular makeup including the famed serpentine transmembrane unit (Dixon et al., 1986), and ultimately their three-dimensional structure (Palczewski et al., 2000; Rasmussen et al., 2007). While the insights gained from working outside the native environments of GPCRs have allowed for the collection of low noise data, such approaches cannot directly address a receptor's native (*in vivo*) functions. An *in vivo* approach can complement the rigor of *in vitro* approaches: as studied in model organisms, it imposes physiological constraints on receptor action and thus allows investigators to deduce the most salient features of receptor function. Here, we briefly discuss specific examples in which model organisms have successfully contributed to the elucidation of signals controlled through GPCRs and other surface receptor systems. We list recent examples that have served either in the initial discovery of GPCR signaling concepts, or in their fuller definition. Further, we selectively highlight experimental advantages, shortcomings, and tools of each model organism.

## Introduction

GPCR pharmacology began in earnest with Raymond Ahlquist's conjecture that there must be two types of adrenotropic receptors in order to account for excitatory and inhibitory effects of the sympathetic adrenergic mediator, epinephrine. This conclusion was based on a set of experiments that characterized the impact of biogenic amines on a roster of vegetative functions in dogs, cats, rats, and rabbits (Ahlquist, 1948). Most interestingly, the proposal of adrenoceptor subtypes was achieved prior to the era of molecular biology, before receptors transformed from a physiological concept into a molecular fact.

Ahlquist's work illustrates one advantage of animal models in pharmacological research: the ability to learn about receptor functions on cellular, organ, and organismic states without full knowledge of the molecular underpinnings of these effects. What may seem to be an experimental shortcoming at first sight in fact reveals its potential when considering the complex biology of signaling pathways involving GPCRs. Many GPCRs are „orphaned“, that is, they lack identified agonistic or antagonistic ligands, the elements that control receptor activity. This lack precludes classical pharmacological analyses that rely on the ability to challenge the receptors with a stimulus. Also, downstream messaging cascades of many GPCRs are still unknown and can therefore not be readily assayed through available standard reporters. The advantage to *in vivo* model systems is that the signaling network used by a given receptor is completely present and set up in an optimal fashion, irrespective of whether all of its (main) components and their working conditions have been identified and characterized.

Clearly, the nature of questions about receptor signals that model organism research can answer differs from those addressed through canonical *in vitro* assays. Whereas the latter provides a means to quantitatively study individual receptor function, receptor research using animal models aids in defining their role at a qualitative level and in understanding how their actions are integrated into the complex physiology of an organism. This has largely been achieved using the modern repertoire of molecular genetic tools to develop animal models into platforms for genetic screening and molecular manipulation. The combination of genetically tractable model organisms with *in vivo* physiology and imaging provides a powerful system for linking the molecular details of receptor function to physiology. Hence, genetic modifications have added direct manipulation of single receptors at the molecular scale to Ahlquist's pharmacological strategy to interrogate the function of entire receptor populations.

The most popular animal models that contribute to understanding pieces of the signaling logic of GPCRs and other membrane receptor pathways are the nematode, *Caenorhabditis elegans*, the vinegar fly, *Drosophila melanogaster*, the zebrafish, *Danio rerio*, and the mouse. The 2014 Lorentz Center Workshop on *Exploring the biology of GPCRs - from in vitro to in vivo* saw several talks highlighting how work in these species provided entry points into delineating the function of individual GPCRs and membrane receptors, which by and large have remained uncharted

territory for classical pharmacology. These include projects on the Adhesion class GPCRs and their roles in the nervous system, aminergic and peptidergic GPCRs involved in circadian rhythm, sleep-wake cycle, eclosion, and male mating behavior. Further, several speakers provided an overview on recent technologies to interrogate receptor function *in vivo* including genetically encoded probes and optogenetic tools. Here, we briefly present the main aspects of these projects.

## **Animal models to discover and validate molecular concepts**

### *The ins and outs of Adhesion GPCR actions*

The Adhesion class of GPCRs (aGPCRs) was discovered through a genome wide bioinformatical search for the sequence fingerprint of their heptahelical transmembrane unit (Bjarnadóttir et al., 2004). Like all GPCRs, aGPCRs possess a 7-transmembrane (7TM) domain, but the class is defined structurally by a large extracellular N-terminal region that is separated from the 7TM by a GPCR autoproteolysis-inducing (GAIN) domain (Arac et al., 2012), which encompasses the GPCR proteolysis site (GPS). aGPCRs represent the second largest GPCR class; however, the appreciation of their biological roles lags behind that of all other GPCR classes at both physiological and pharmacological levels. Several findings, obtained through phenotypic analyses of null or hypomorphic mutants of aGPCR genes in animal models, have established that these receptors function during developmentally dynamic periods of organogenesis and are involved in cell differentiation, migration, and polarity, similar to Frizzled-type GPCRs (Schulte, 2010; Dijksterhuis et al., 2014). However, whether aGPCRs also function in postmitotic tissue, which signals they read out, and how they transduce these into intracellular messages, has only recently begun to unfold (see also Monk et al., this issue). Amongst others, two aGPCR homologs, Gpr126 (*ADGRG6*) and Latrophilin (*ADGRGL1-3*), have served as model receptors to dissect aGPCR signals.

Analysis of zebrafish *gpr126* mutants uncovered an essential role for this aGPCR in the development of myelinated axons in the peripheral nervous system (PNS). In the vertebrate PNS, the myelin sheath is made by specialized glial cells called Schwann cells and is required for rapid impulse propagation. Without Gpr126, Schwann cells can ensheath axons, but fail to spiral their membranes to generate the myelin sheath (Monk et al., 2009) Thus, animal models served to uncover a critical function of this aGPCR that would have been impossible to decipher in traditional heterologous cell systems. Intriguingly, myelin defects in *gpr126* mutants could be rescued by cAMP elevation, suggestive of G<sub>s</sub> coupling. These studies are discussed in more detail in Monk et al., this issue. At the Lorentz workshop, more recent advances in understanding how Gpr126 controls Schwann cell development and myelination were presented. The advent of rapid genome editing tools has afforded unprecedented advances in mutant generation to study

the function of genes *in vivo*. Using Transcription Activator-Like Effector Nucleases (TALENs), new *gpr126* mutant alleles were generated in zebrafish; their analysis demonstrated a function of the Gpr126 N-terminus in early Schwann cell development that is distinct from the signaling function of the C-terminus. Moreover, genetic analyses in both mouse and zebrafish supported a model in which interactions between the Gpr126 N-terminus and the extracellular matrix (ECM) protein Laminin-211 modulates receptor signaling, perhaps by physical removal of the N-terminus, to allow for 7TM signaling of the C-terminus and myelination (Fig. 1A) (Petersen et al., 2015).

Model organisms have also been indispensable for understanding the function of *Latrophilin/Cirl*, an aGPCR conserved from ancient metazoa to man, which was known for many years only as a biochemical binding target for black widow spider venom at neurons. Concrete evidence for the physiological role of the receptor emerged through studies in *C. elegans*. Removal of the latrophilin receptor *lat-1* causes severe developmental problems due to the loss of the polar alignment of neuroblasts along the anterior-posterior body axis of worm embryos, indicating that this aGPCR functions in the control of planar cell polarity signals (Langenhan et al., 2009). Secondly, *lat-1* mutants proved infertile, but the nature of stimuli perceived through the receptor protein remained elusive (Prömel et al., 2012). Recent findings on the function of the latrophilin homolog *dCirl* in *Drosophila melanogaster* have now provided insights into this matter. Latrophilin/CIRL is located in peripheral mechanosensory neurons, which perceive mechanical signals such as sound, touch and muscle stretch. A genomically engineered *Latrophilin/dCirl* null mutant exhibited a much reduced sensitivity towards these sensory inputs implicating them – similar to *Gpr126* – as mechanosensors (Fig. 1B) (Scholz et al., 2015). Genetic experiments further demonstrated that *Latrophilin/dCirl* activity may regulate the input-output function of mechanosensory nerve cells through the modulation of transient receptor potential (TRP) channels, which ultimately govern the electrical response of these neurons (Scholz et al., 2015). To devise a solid model on receptor functionality, the *in vivo* findings on the mechanosensitive nature of aGPCRs will require testing how mechanical stimuli translate into metabotropic signals under *in vitro* conditions.

#### *Polycystin proteins as atypical aGPCRs: lessons from C. elegans*

*C. elegans* is a powerful model for neurobiology. GPCRs constitute about 7% of the *C. elegans* genome, most encoding chemoreceptors (Bargmann, 1998; Fredriksson and Schiöth, 2005; Thomas and Robertson, 2008). With the exception of the neuropeptide GPCRs (Frooninckx et al., 2012), much of our knowledge of *C. elegans* GPCR function comes from forward screens to identify genes regulating animal behavior. ODR-10, the first *bona fide* olfactory GPCR, was identified in a screen for mutants with a specific defect in chemotaxis to diacetyl (Sengupta et al., 1996). A natural variation in the neuropeptide Y receptor *npr-1* gene regulates social feeding

behaviors (de Bono and Bargmann, 1998). Genetic analysis of *C. elegans* male mating behavior identified two types of transmembrane (TM) spanning receptors. The Secretin-like class 2 GPCR pigment dispersing factor receptor (PDFR-1) modulates the neural circuit that promotes mate searching and male sex drive (Barrios et al., 2012).

Intriguingly, the nematode homologs of the 11TM spanning molecule polycystin-1 (PC1) and the TRP channel homolog polycystin-2 (PC2) – LOV-1 and PKD-2 – are required for mate searching, response to hermaphrodite contact, and location of the hermaphrodite's vulva (Barr and Sternberg, 1999; Barr et al., 2001; Barrios et al., 2008). In both *C. elegans* and mammals, the polycystins localize to cilia and ciliary extracellular vesicles where they are thought to act in a signaling capacity (O'Hagan et al., 2014; Wood and Rosenbaum, 2015). In humans, abnormalities in polycystin trafficking or stability may underlie autosomal dominant polycystic kidney disease (Cai et al., 2014). The Barr lab has been using *C. elegans* as a model for studying mechanisms regulating the localization and functions of the polycystins in cilia and extracellular vesicles (Wang et al., 2014; O'Hagan et al., 2014). PC1 proteins, like aGPCRs, contain a GAIN domain, undergo autoproteolytic cleavage at a GPS into N-terminal and C-terminal regions (NTR and CTR), and can activate G-protein second messengers, endowing them with GPCR-like properties (Delmas et al., 2004; Yu et al., 2007; Prömel et al., 2013). LOV-1 possesses a GPS, and an NTR lacking TM domains remains associated with cilia and extracellular vesicles, suggesting that LOV-1 may be an atypical aGPCR (Barr and Sternberg, 1999; Wang et al., 2014; O'Hagan et al., 2014).

The ligands activating PC1 and LOV-1 in the mammalian kidney and *C. elegans* male sensory neurons remain a mystery. As noted above, aGPCRs may function in some capacity as mechanosensors; perhaps PC1/LOV-1 and PC2/PKD-2 act in a similar manner. The N- and C-termini of aGPCRs may have distinct functions, based on studies of Latrophilins and *Gpr126* in several model systems (Prömel et al., 2012; Patra et al., 2013; Petersen et al., 2015; Scholz et al., 2015). Ligands for aGPCRs are often ECM or membrane-associated proteins (Langenhan et al., 2013), which bind the N-terminus and may unmask a binding site in the C-terminus (Liebscher et al., 2014). In this model, the N-terminus of PC1 and LOV-1 may bind to an ECM molecule, which would prime the polycystin complex for signaling. Consistent with this hypothesis, the PC1 N-terminus interacts with ECM proteins *in vitro* (Weston et al., 2001), and in zebrafish the polycystins genetically interact to regulate ECM formation (Mangos et al., 2010). Future work using *C. elegans* can draw from aGPCR studies in model organisms allowing us to determine whether PC1/LOV-1 is an atypical aGPCR.

*GPCR signaling in the circadian clock: the functional importance of GPCR coupling diversity in vivo*

Circadian behavior requires an oscillating neuronal circuit that is both stable and flexible. In *Drosophila*, this circuit consists of ~150 neurons, organized into a number of distinct groups, which are kept in synchrony by the actions of Pigment Dispersing Factor (PDF), a peptide released by a population of ~16 ventrolateral (LNV) clock neurons.

The effects of PDF are mediated by a single GPCR, the *Drosophila* PDF receptor (PDFR), a member of the Secretin receptor (B1) family most closely related to mammalian receptors for calcitonin and CGRP (Hewes and Taghert, 2001; Mertens et al., 2004; Hyun et al., 2005; Lear et al., 2005). Interestingly, PDFR is not expressed in all clock neurons (Fig. 2). Indeed, not all members of a particular subset of clock neurons express the receptor (Im and Taghert, 2010) or respond to PDF application (Shafer et al., 2008). Given the behavioral importance of this peptide and the impracticality of biochemical experiments on such small subsets of cells, investigators have used genetic and behavioral strategies *in vivo* to examine PDFR signaling mechanisms. These studies have confirmed, and importantly, modified *in vitro* findings, and the differences between *in vitro* and *in vivo* conclusions illustrate the power and significance of studying GPCR signaling in its native context.

Functional expression of PDFR in mammalian and insect cell lines provided a provisional description of its signaling capabilities. As expected of a family B1 GPCR, PDFR activation elevates cAMP and calcium levels (Hyun et al., 2005; Mertens et al., 2005). RNAi studies revealed that PKA is normally activated by PDF activity and likely promotes stability and cycling of the essential clock proteins TIMELESS (Seluzicki et al., 2014) and PERIOD (Li et al., 2014). Further, GW182, which mediates miRNA-dependent gene silencing through its interaction with AGO1, modulates PDFR signaling by silencing the expression of DUNCE, a cAMP phosphodiesterase (Y Zhang and Emery, 2013). Zhang and Emery argue that GW182 is a novel light-dependent rheostat modulating the amount of PDF GPCR signaling. Using an RNAi screen of GPCRs coupled to inositol phosphate (IP)-stimulated calcium elevation, PDFR appears to be G<sub>q</sub>-coupled in some neuronal subsets (Agrawal et al., 2013). To measure cyclic nucleotide levels, a number of investigators have used a transgenic fluorescence resonance energy transfer (FRET) reporter built around the cAMP-binding domain of the molecule EPAC (de Rooij et al., 1998; Nikolaev et al., 2004; Shafer et al., 2008) and reported that most, but not all, circadian pacemaker neuron groups in the brain respond to PDF with elevations of cyclic nucleotides. PDFR was found to couple to different adenylyl cyclases (ACs) in different pacemakers neuron populations (Duvall and Taghert, 2012; 2013). Specifically, PDFR stimulates the orthologue of mammalian AC3 in small LNvs (which express a PDFR autoreceptor), whereas PDFR in dorsolateral neurons (LNds) couples to the orthologue of mammalian AC8 and at least one other (currently unidentified) AC. Furthermore, within small LNvs, PDFR is coupled to G<sub>α<sub>s</sub></sub> and AC3, while other small LNV G<sub>α<sub>s</sub></sub>-coupled GPCRs (those sensitive to dopamine or other neuropeptides) are coupled to ACs different from AC3 (Duvall and Taghert, 2012). The differential pairing of



peptide GPCRs to distinct AC isoforms in different neurons, and even within single identified neurons, provides a striking example of the localized sub-cellular domains within which GPCR signaling complexes must be assembled as multi-protein clusters, permitting discrete spatio-temporal communication (Dessauer, 2009).

The ability to use genetic tools with imaging and behavioral endpoints in *Drosophila* provides the opportunity to assess the importance of GPCR function *in vivo*. As *in vitro* biochemical and structural studies provide more information on the mechanisms by which GPCRs couple to different output pathways, genetic model systems can be used to confirm results in native contexts and provide functional relevance for the amazing diversity of GPCR functions.

### **Insect ecdysis and the complexities of *in vivo* studies on GPCRs**

One system that exemplifies the complexities of GPCR action is the control of insect ecdysis behavior, the shedding of the old exoskeleton during arthropod molting. This behavior consists of a sequence of behavioral routines and is typically followed by the inflation and hardening of the new exoskeleton (Ewer and Reynolds, 2002). Insects must perform this complex sequence of movements relatively flawlessly in order to survive and reproduce. While normally stereotyped, ecdysis sequences can also include checkpoints at which sensory inputs can delay the shedding process if needed.

Ecdysis is controlled by numerous peptide hormones (Ewer and Reynolds, 2002; Zitnan and Adams, 2012) that act on identified GPCRs. One of these (Ecdysis Triggering Hormone, ETH) acts as the principal trigger of the ecdysial sequence. Strikingly, ETH is expressed in all neurons that produce neuropeptides associated with the control of ecdysis, and exposing excised *Drosophila* nervous systems to ETH activates these different targets at different times (Y-J Kim et al., 2006). The parallel activation of a GABAergic inhibitory pathway may explain how ETH activation of neuronal elements occurs in succession rather than synchronously (Mena and Ewer, unpublished data). These findings illustrate one way in which the simultaneous activation of the ETHR GPCR can cause the expression of a complex sequence of neuronal activity.

In addition to such ETH-mediated inhibitory delays, the ecdysis system is also subject to delays mediated by sensory inputs (White and Ewer, 2014). For instance, adult flies will postpone wing inflation up to several hours if they find themselves physically constrained (Peabody et al., 2009). This inhibition is neurally mediated and prevents the release of the hormone Bursicon, which appears to feed back to modulate the sensory-mediated delay pathways (Luan et al., 2012). Novel tools to identify and manipulate neurons that express the Bursicon receptor have provided insight into this process (Diao and White, 2012). The receptor uses cAMP as a second messenger, and as shown at the Leiden meeting, optogenetic activation of Bursicon receptor-

expressing neurons using a photoactivatable AC overcomes the sensory-mediated delays (Diao and White, unpublished data).

Recently, the technique used to target Bursicon receptor-expressing neurons has been adapted for use with other genes and can now be used to quickly gain genetic access to cells that express other GPCRs in the intact fly (Diao et al., 2015). This will help determine the spatial relationships between known ligand release to corresponding receptor-carrying cell populations. Specifically in the case of the Bursicon receptor, the results should prove interesting. Indeed, some neurons produce both subunits of Bursicon (and therefore presumably make the heterodimeric hormone), but others synthesize only one subunit. Activation of Bursicon receptor by homodimers has not been observed, but the only signal transduction assayed thus far is cAMP. If the receptor exhibits bias and uses different downstream transduction pathways for different ligands, homodimeric activation may occur. Co-localization of Bursicon receptor with release sites for putative homodimers would lend support for the hypothesis of homodimeric activation.

This brief exposition demonstrates some of the advantages of the ecdysis system in exploring how GPCRs and their ligands orchestrate with great precision, yet with some flexibility, complex sequences of behaviors and physiological changes. Nonetheless, much remains to be learned about signaling within the ecdysis network. In particular, the signal transduction mechanisms employed by the GPCRs and their effects on neuronal physiology have only been superficially characterized. The use of novel genome engineering tools, most notably CRISPR (Doudna and Charpentier, 2014), will render more animals amenable to genetic manipulation and recruit new models. This will allow for direct comparisons between GPCR action in *Drosophila* vs. other insects, paving the way for an understanding of the evolution of GPCR action.

## Tools for *in vivo* GPCR research

### *Metabotropic signal sensors for in vivo studies*

The two main intracellular messengers modulated through G protein activation are calcium and cAMP. Several FRET-based genetically encoded sensors for both messengers are available, which allow the monitoring of intracellular concentration changes in living cells with high spatial and temporal resolution (van Unen et al., this issue). cAMP sensors based on EPAC (Nikolaev et al., 2004; Ponsioen et al., 2004; Calebiro and Maiellaro, 2014) and genetically encoded calcium indicators D3cpV (Palmer and Tsien, 2006) and GCaMPs (Tian et al., 2009; Akerboom et al., 2013) are popular examples.

Several mouse lines with transgenic FRET sensor expression have been generated (Hara et al., 2004; Calebiro et al., 2009; Grienberger and Konnerth, 2012). However, their utility in monitoring GPCR activity *in vivo* is still limited by low signal-noise ratios and the requisite isolation of organs

or cells before experimentation. Moreover, GPCR ligand delivery in intact animals remains a challenge for these investigations.

In contrast, genetically encoded cAMP and calcium probes can be targeted to specific cell populations with sub-cellular specificity in some animal models, allowing the study of how receptor localization and presence of partner proteins contribute to GPCR function. Further, the optical isolation of the structure of interest for calcium or cAMP level monitoring dampens noise levels and allows for more precise kinetic measurements of GPCR messenger activation even under *in vivo* conditions.

An emerging animal model for such visualization of GPCR activity *in vivo* is *Drosophila* (Shafer et al., 2008; Shang et al., 2011; Duvall and Taghert, 2012; Pérez et al., 2013). Through the use of binary expression systems such as UAS/GAL4, probe expression can be confined to individual tissues and an ever enlarging collection of small cell clusters and single cells (del Valle Rodríguez et al., 2012) (Fig. 3 A,B). Indeed, GAL4-directed expression of an aequorin transgene in *Drosophila* was the first example of a calcium reporter animal (Rosay et al., 1997). More recent studies have expressed genetically encoded fluorescent reporters in *Drosophila* LNV clock neurons (Cao et al., 2013). Other studies in *Drosophila*, which have also exploited the power of genetically encoded sensors of GPCR activity, elucidated the role of dopamine and octopamine receptors in olfactory memory formation by monitoring calcium and cAMP signals in the Mushroom Body (MB), the part of the fly brain where olfactory memory is processed (Tomchik and Davis, 2009; Gervasi et al., 2010). Interestingly, depending on which GPCR was stimulated through its cognate ligand, MB metabotropic activity differed substantially. Activation of octopamine receptors by octopamine elicited a generalized cAMP signal in the region of the MB involved in appetite modulation. In contrast, dopamine stimulation triggered a localized cAMP signal in parts of the MB that modulate aversive learning. This demonstrates how knowledge of cellular location and dynamics of GPCR-generated signals in the nervous system helps to unravel complex brain functions, unlocking an organ- and behavior-specific context to GPCR function.

#### *Optogenetics and DREADDS for studying GPCR function in vivo*

The advent of optogenetics has transformed the ability of biologists to selectively interrogate neural circuits, cell types, and pathways critical for behavior and disease. Several recent advances are underway to utilize the advantages of light's spatial-temporal characteristics to selectively engage GPCR signaling in a cell-type selective manner *in vivo*.

Investigators have turned to utilizing vertebrate and non-mammalian rhodopsin receptors to mimic G-protein signaling both *in vitro* and *in vivo* using a variety of approaches (Zemelman et al., 2002; Schroll et al., 2006; Zhang et al., 2007). A recent study showed that bovine rhodopsin and class A GPCRs could be made into receptor chimeras, composed of extracellular and

hydrophobic light-sensitive rhodopsin domains, and intracellular loops targeted to couple to specific G-protein pathways (Airan et al., 2009). The authors used  $\beta_2$ -adrenergic and  $\alpha_1$ -adrenergic receptor intracellular loop and carboxy-tail components fused to bovine rhodopsin to achieve  $G\alpha_s$  and  $G\alpha_q$  signaling, respectively (Fig. 3C). This technique allows experimenters to utilize fiber optic or wireless LED technology (Yizhar et al., 2011; T-I Kim et al., 2013) to activate GPCR signaling within selected cell types in the mammalian brain of awake behaving animals. Elegant extensions of this approach have also been utilized in modifications of vertebrate rhodopsin with components of the 5HT1a or 5HT2c serotonin receptor for examining neural circuits and signaling in anxiety behavior (Spoida et al., 2014; Masseck et al., 2014). Recent work has also shown that chimeric opsin-wildtype receptors can be used to optically mimic opioid receptor signaling (Siuda et al., 2015) (Fig. 3C). Other studies used opsin GPCRs in cellular models for achieving remarkable spatial-temporal control of signaling gradients and cell migration (Karunaratne et al., 2015). These approaches allow the experimenter to precisely control the spatial components of subcellular GPCR signaling without activating receptor signaling in other microdomains or at different cellular stations (O'Neill and Gautam, 2014). Using plant cryptochrome domains (CRY2/CIB1), one can light-trigger recruitment of regulators of G-protein signaling or Gbg, allowing for the selected sequestering of GPCR signaling. Similar approaches have used short-wavelength opsins and invertebrate opsins from jellyfish to achieve subcellular spatial-temporal control.

Another line of technology to interrogate GPCR signals *in vivo* regards the recent wide adoption of designer receptors exclusively activated by designer drug (DREADDs; Sternson and Roth, 2014). DREADDs are activated by a pharmacologically inert ligand, clozapine-nitric-oxide (CNO), which displays low or no affinity for endogenous GPCRs, and which can engage  $G\alpha_s$ ,  $G\alpha_i$ , and  $G\alpha_q$  pathways (Fig. 3D). Additional variants are currently being explored for synaptic targeting (Stachniak et al., 2014) and more selective spatial control and multi-plexing using additional chemogenetic substrates based on reagents used with kappa-opioid receptors (Vardy et al., 2015). DREADD knockin animal models, including *Drosophila* and mice, are currently being used by the GPCR research community for engaging cell-type selective G-protein signaling. For example, the selective expression of DREADDs in the *Drosophila* heart clarified the signal pathway of the 5-HT receptor in the control of heartbeat (Majeed et al., 2013; Becnel et al., 2013). DREADD/CNO pharmacology does not provide spatial-temporal control; however, there is no need for complex fiber/LED systems or expensive microscopy, and this approach has therefore gained a wide user base.

Future work in developing these tools focuses on how can we utilize opsin and DREADD techniques to mimic endogenous receptor function. Given that they are artificial constructs being introduced via viral or genetic means, it is generally unknown how well they recapitulate the spatial-temporal dynamics of the endogenous receptor systems experimenters are dissecting.

Comparing kinetics, signaling pathway efficacy, and expression profiles of these tools *in vivo* will be needed as the field grows and matures. Nevertheless, as animal models become more tractable, using rhodopsin and DREADD receptors *in vivo*, in conjunction with side-by-side pharmacological analyses, will allow for a clearer dissection of GPCR signaling as it relates to physiology and behavior.

## Conclusions

Classic GPCR investigations have traditionally utilized elegant, reductionist *in vitro* approaches. These studies are enhanced and provided with a physiological context when considered along with *in vivo* analyses. As genome editing becomes routine, model organisms can give clear answers regarding biological functions of GPCRs in question. Moreover, continued advances in imaging and receptor manipulation will further our understanding of receptor function – even at sub-cellular resolution – in a living organism. The deepest understanding of GPCRs can come from concomitant investigations using *in vivo* and *in vitro* approaches, and future work can be directed to solving current outstanding problems including biological relevance of receptor dimerization, biased signaling, and questions of GPCR structure/function.

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## **Authorship Contributions**

Wrote or contributed to the writing of the manuscript: Langenhan, Barr, Bruchas, Ewer, Griffith, Maiellaro, Taghert, White, Monk

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Missing: Monk et al. and van Unen et al. both this issue of Mol Pharmacol

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## Footnotes

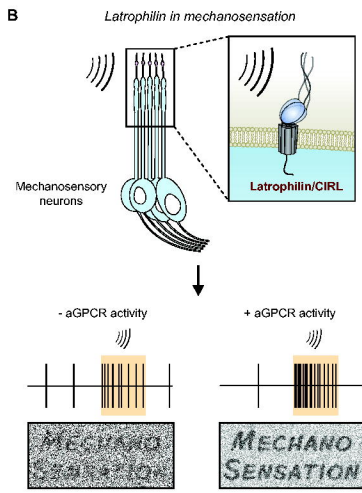
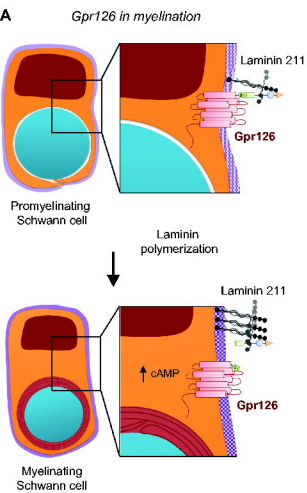
Work in our laboratories was supported by: Deutsche Forschungsgemeinschaft [FOR 2149/TP1 and TP3] and [SFB 1047/A5] to T.L.; National Institute on Drug Abuse Exceptional Unconventional Research Enabling Knowledge Acceleration Grant [R01 DA037152] to M.R.B., Fondo Nacional de Desarrollo Científico y Tecnológico [FONDECYT 1141278] and the Centro Interdisciplinario de Neurociencia de Valparaíso [P09-022-F], which is supported by the Millennium Scientific Initiative of the Ministerio de Economía, Fomento y Turismo, to J.E.; National Institute of General Medicine [R01 GM54408] to L.C.G.; National Institute of Mental Health [R01 MH067122] to P.H.T.; the Intramural Research Program of the National Institute of Mental Health to B.H.W.; National Institute of Neurological Disorders and Stroke [R01 NS079445] and National Institute of Child Health and Human Development [R01 HD080601] to K.R.M.

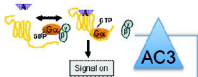
## Figure legends

**Fig. 1. Adhesion GPCRs as mechanically activated receptors.** (A) Early in Schwann cell development, Laminin-211 interacts with the N terminus of Gpr126 in a conformation that prevents cAMP accumulation through suppression of basal signaling, potentially allowing the Schwann cell to remain immature. Following Laminin 211 polymerization in the basal lamina (purple shadow), Laminin 211 facilitates an active conformation of the receptor, perhaps by physical removal of the N terminus, which exposes a cryptic ligand (noted by „S“) (Petersen et al., 2015). (B) The aGPCR *Latrophilin/Cirl* is present in mechanosensory neurons of *Drosophila*, where it regulates their sensitivity towards mechanical stimulation and maintains a physiological signal-to-noise ratio. Loss of *Latrophilin/Cirl* results in numbness, amblyacousia and proprioception deficits (Scholz et al., 2015). See also Monk et al., this issue.

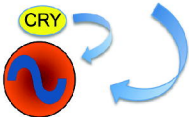
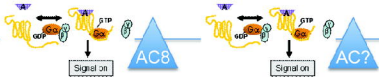
**Fig. 2. Cellular context dictates the nature of GPCR signaling.** The neuropeptide Pigment Dispersing Factor (PDF) activates a GPCR in target neurons of the circadian neural circuits in the *Drosophila* brain. M and E pacemakers refer to neurons that are biased to involvement in either a morning or evening bout of locomotor activity. *In vivo* observations indicate PDF-R signalosome components differ in the different target neurons: PDF-R associates with AC3 in M cells, but in the LNs E cell subgroup, PDF signaling relies of AC78C (AC8) and at least one other (currently unidentified) AC. Figure originally published by Duvall and Taghert (2013) and reproduced here with permission from the *Journal of Biological Rhythms*.

**Fig. 3. Modern tools for investigating GPCR signaling in vivo.** (A) Representation of intact (right) and dissected (left) *Drosophila* larva expressing the FRET-based sensor for monitoring cAMP changes, Epac1-camps. (B) YFP and time-resolved pseudocolor FRET images of *Drosophila* motoneurons expressing Epac1-camps. The application of agonist mediates the activation of the endogenous receptors, which results in production of cAMP. This cAMP increase is revealed by a loss in FRET caused by a conformational rearrangement of the sensor, induced by cAMP binding. Scale bar = 10  $\mu$ m. (C) Cartoon depicting chimeric “Opto-XR” approach whereby rhodopsin cDNA is fused with wildtype GPCR cDNA intracellular loops and tail to generate a photo-sensitive receptor system capable of spatiotemporal engagement of canonical GPCR signaling pathways such as Gq, Gs, and Gi or arrestin recruitment in selected cell types when combined with viral and genetic approaches *in vivo*. (D) Cartoon representing chemogenetic GPCRs termed Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), which selectively respond to the ligand clozapine-nitrous oxide (CNO) to engage canonical GPCR signaling pathways in selected cell types when also combined with viral or mouse genetic approaches.





M Pacemaker



E Pacemaker



