Neurotransmitter signaling through heterotrimeric G proteins: insights from studies in *C. elegans*[*]

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Abstract

Neurotransmitters signal via G protein coupled receptors (GPCRs) to modulate activity of neurons and muscles. *C. elegans* has ~150 G protein coupled neuropeptide receptor homologs and 28 additional GPCRs for small-molecule neurotransmitters. Genetic studies in *C. elegans* demonstrate that neurotransmitters diffuse far from their release sites to activate GPCRs on distant cells. Individual receptor types are expressed on limited numbers of cells and thus can provide very specific regulation of an individual neural circuit and behavior. G protein coupled neurotransmitter receptors signal principally via the three types of heterotrimeric G proteins defined by the G alpha subunits $G\alpha_o$, $G\alpha_q$, and $G\alpha_s$. Each of these G alpha proteins is found in all neurons plus some muscles. $G\alpha_o$ and $G\alpha_q$ signaling inhibit and activate neurotransmitter release, respectively. $G\alpha_s$ signaling, like $G\alpha_q$ signaling, promotes neurotransmitter release. Many details of the signaling mechanisms downstream of $G\alpha_q$ and $G\alpha_s$ have been delineated and are consistent with those of their mammalian orthologs. The details of the signaling mechanism downstream of $G\alpha_o$ remain a mystery. Forward genetic screens in *C. elegans* have identified new molecular components of neural G protein signaling mechanisms, including Regulators of G protein Signaling (RGS proteins) that inhibit signaling, a new $G\alpha_q$ effector (the Trio RhoGEF domain), and the RIC-8 protein that is required for neuronal $G\alpha$ signaling. A model is presented in which G proteins sum up the variety of neuromodulator signals that impinge on a neuron to calculate its appropriate output level.

1. Introduction

The nervous system functions through the use of neurotransmitters that act as chemical signals between cells. Small-molecule neurotransmitters such as acetylcholine, glutamate, and GABA (gamma aminobutyric acid) are released from vesicles clustered at synapses. Neuropeptides (secreted proteins of <50 amino acids) are released at synaptic and non-synaptic sites from a different class of vesicles, known as dense-core vesicles for their appearance in the electron microscope (Merighi et al., 2011). Certain small-molecule neurotransmitters such as serotonin can be released from either class of vesicle. All these types of neurotransmitters act on neurons and muscles to generate dynamic patterns of activity that constitute thoughts and behaviors. A principal objective in neuroscience is to understand the molecular mechanisms by which neurons and muscles respond to neurotransmitters. This objective is important to advance our basic science understanding of the brain, but also is of great medical significance since many pharmaceuticals used to treat psychiatric disorders act by mimicking, antagonizing, or altering the levels of naturally-occurring neurotransmitters (Conn and Roth, 2008), and drugs of abuse also act by altering neurotransmitter signaling (Joffe et al., 2014).

1.1. Neurotransmitters signal by gating ion channels and by activating G protein coupled receptors

Neurotransmitters signal via two distinct classes of receptors, known within the neuroscience field as ionotropic and metabotropic receptors. Ionotropic receptors are neurotransmitter-gated ion channels, and most small-molecule neurotransmitters each have a number of such receptors (Lemoine et al., 2012). Binding of neurotransmitter to an ionotropic receptor favors channel opening, and communication between neurons using ionotropic receptors can occur in less than a millisecond (Sabatini and Regehr, 1996). Metabotropic receptors are known outside the neuroscience field as G protein coupled receptors (GPCRs) because they activate intracellular signaling proteins called heterotrimeric G proteins. All small-molecule neurotransmitters have G protein coupled receptors, as do most neuropeptides (Hall, 2004; Beaulieu and Gainetdinov, 2011; Pytliak et al., 2011; Hoyer and Bartfai, 2012; Vaidya et al., 2013; Kruse et al., 2014). An individual small-molecule neurotransmitter might have up to a dozen different GPCRs. There are over 100 neuropeptide genes in both humans and *C. elegans,* and each organism also has about an equal number of GPCRs that are likely to be neuropeptide receptors, as they are similar to the few G protein coupled neuropeptide receptors that have been characterized so far (Li and Kim, 2008; Li and Kim, 2010; Frooninckx et al., 2012; Hoyer and Bartfai, 2012). Binding of a neurotransmitter to a GPCR, as opposed to an ionotropic receptor, leads to slower and longer lasting effects. For example, GCPR signaling can involve biochemical amplification of a signal (e.g., production of a pool of second messenger) that is much slower than the

rapid voltage changes induced by opening ion channels, and GPCR signaling can result in changes to the transcriptional program and structure of a neuron that last days or longer (Kandel, 2004). Whereas ionotropic receptors mediate signaling underlying such prosaic neural functions as the knee-jerk response, GPCRs mediate signaling underlying more poetical functions of the brain, such as feelings of pleasure and love that result from dopamine and oxytocin (Love, 2014), the psychedelic effects of hallucinogens (Fantegrossi et al., 2008), and the regulation of mood by serotonin (Donaldson et al., 2013).

1.2. *C. elegans* as a model system for studies of neural signaling

This review focuses on insights into the molecular mechanisms and biological functions of neurotransmitter signaling through GPCRs that arise from studies in the model organism *C. elegans*. But why study neurotransmitter signaling in the worm? Signaling through ionotropic receptors has already been studied in a sophisticated manner in other species using electrophysiological techniques to analyze ion channel activity. Such electrophysiological studies have often used model organisms such as slug, squid, leech, or crab that have large neurons easily accessible to electrodes. GPCRs activate intracellular signaling pathways that can have indirect effects on ion channel activity, and electrophysiological studies in the same model organisms have given important insights into how GPCR signaling modulates the function of neural circuits (Bailey and Kandel, 2008; Marder, 2012). However, methods for studying intracellular signaling pathways have been most highly developed outside of the neuroscience field, with the greatest successes coming from applying a combination of biochemistry and genetics, techniques not easily applied in the model organisms suited to electrophysiology. A remarkable body of biochemical studies of signaling by heterotrimeric G proteins, mostly in non-neuronal mammalian cells, has been in progress for decades and has resulted in many Nobel prizes (Cori and Cori, 1947; Sutherland, 1971; Krebs, 1992; Fischer 1992; Gilman 1994; Rodbell 1994; Kobilka, 2013; Lefkowitz 2013). Prior to the studies in *C. elegans* described in this review, neural G protein signaling had not been seriously studied using genetic approaches. Thus, despite the excellence of the body of biochemical work on G protein signaling, there remained significant gaps in understanding the molecular mechanisms of this type of signaling in neurons, and also in achieving a big-picture understanding of how and why such signaling is used to control the activity of neural circuits.

As described in this review, the molecular mechanisms of neural G protein signaling are strongly conserved between humans and *C. elegans*, and *C. elegans* provides two advantages that complement the past electrophysiological and biochemical work on neural G protein signaling. First, the power of forward genetics in *C. elegans* has allowed new neural signaling proteins to be discovered. Second, the simplicity of the *C. elegans* nervous system, combined with the use of genetics, has allowed biological functions to be assigned to signaling by specific neurotransmitters acting through specific GPCRs on individual identified neurons. Enough such biological functions of neural G protein signaling have now been described that the long-elusive big-picture understanding of the whole purpose of this mode of neurotransmission is beginning to emerge.

1.3. The heterotrimeric G protein activity cycle

Here I outline the activity cycle for a generic heterotrimeric G protein as a prelude to diving more deeply into how specific GPCRs, G proteins, and their downstream signaling pathways regulate neural function in *C. elegans*. The G protein activity cycle proceeds through five states labeled in Figure 1. Most steps in the cycle were discovered and characterized through biochemical studies of mammalian proteins, but the RGS and RIC-8 proteins that catalyze the transitions between states 4 and 5 were discovered through studies in *C. elegans* and subsequently characterized in mammals.

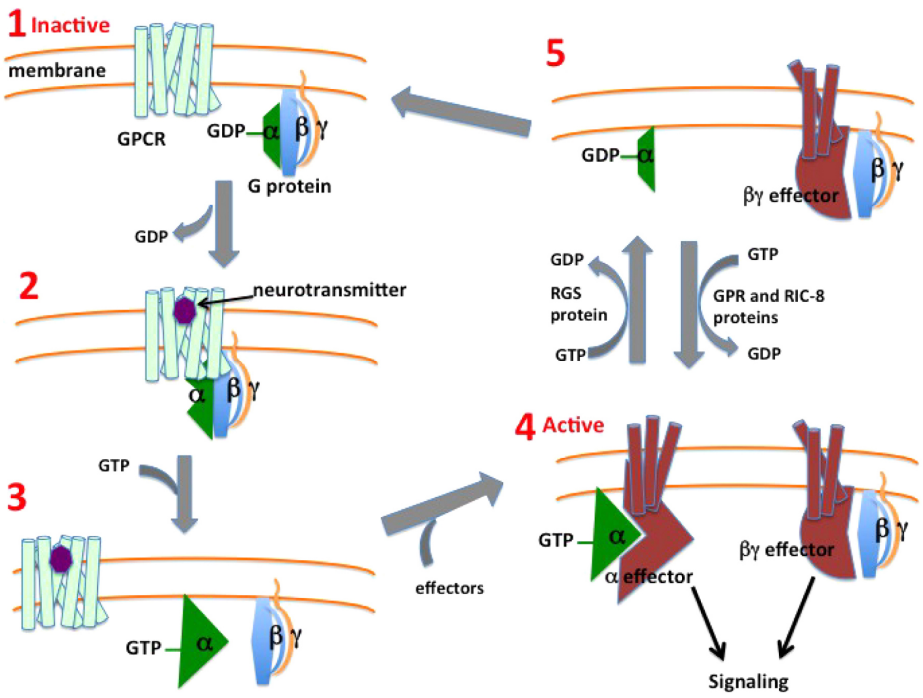


Figure 1. Activity cycle for a generic heterotrimeric G protein. In the inactive state (1), the G protein is an αβγ heterotrimer bound to GDP. When neurotransmitter binds the receptor (2), it assumes an active conformation that can bind the G protein heterotrimer and induce a conformational change in Gα that releases GDP. GTP can then bind the open nucleotide site (3), inducing another conformational change in Gα that releases both the receptor and Gβγ. The separate Gα-GTP and Gβγ complexes can then associate with their respective effector proteins (4), which are typically transmembrane protein complexes, activating those effectors for downstream signaling. Gα signaling is terminated when a Regulator of G protein Signaling (RGS) protein induces Gα to hydrolyze GTP (5), and Gβγ signaling is terminated when Gβγ re-associates with Gα-GDP (1). The RIC-8 non-receptor nucleotide-exchange protein, working with GPR domain proteins, can prolong signaling by converting Gα-GDP (5) back to Gα-GTP (4). RIC-8 is also a chaperone required for Gα folding and stability, which may be its principal function.

1.3.1. State 1, the inactive state

GPCRs are integral membrane proteins with seven transmembrane helices. Heterotrimeric G proteins have α, β, and γ subunits and are peripheral membrane proteins tethered to the inner leaflet of the plasma membrane by lipid modifications (Hepler and Gilman, 1992; Wedegaertner et al., 1995). The Gα subunit changes conformation depending on whether it is bound to GDP, GTP, or no guanine nucleotide, and these conformational changes allow Gα to alter its association with other proteins (Noel et al., 1993; Coleman et al., 1994; Lambright et al., 1994; Wall et al., 1995). In its GDP-bound inactive state, Gα is typically bound to Gβγ to form an inactive G protein heterotrimer.

1.3.2. State 2, activated GPCR promotes release of GDP by the Gα

Neurotransmitter binding induces conformational shifts in GPCRs that have recently been delineated by X-ray crystallographic studies (Katritch et al., 2013). GPCRs and G proteins are able to diffuse laterally due to the fluid nature of biological membranes, and a neurotransmitter-bound, activated receptor can thus collide with an inactive G protein heterotrimer. This interaction induces a large conformational change in Gα that allows the release of bound GDP (Fung et al., 1981; Brandt and Ross, 1986; Rasmussen et al., 2011). The active GPCR and nucleotide-free G protein heterotrimer form a stable complex that dissociates in the next step of the G protein cycle.

1.3.3. State 3, GTP binding induces dissociation of GPCR, Gα, and Gβγ

The open nucleotide-binding site on Gα can bind GTP, which causes Gα to undergo another conformational change that causes Gα-GTP to dissociate from both the receptor and Gβγ (Noel et al., 1993; Coleman et al., 1994; Lambright et al., 1994; Wall et al., 1995). The release of GDP and subsequent GTP binding by Gα is referred to as "nucleotide exchange". The receptor, which remains bound to neurotransmitter, can diffuse laterally in the membrane and proceed to promote nucleotide exchange on additional G proteins. Thus the active receptor can function as an enzyme to catalytically promote conversion of inactive Gαβγ-GDP to the active signaling species Gα-GTP and Gβγ (Ross, 2014).

1.3.4. State 4, Gα-GTP and Gβγ signal by forming stable complexes with effectors

The separated Gα-GTP and Gβγ complexes are able to stably bind to and regulate activity of other proteins to promote responses in the cell, and these other proteins are collectively termed G protein "effectors". Effectors have been identified for Gβγ (Reuveny et al., 1994; Herlitze et al., 1996) and for the Gα isoforms $G\alpha_s$ (Sunahara et al., 1996), $G\alpha_q$ (Kadamur and Ross, 2013; Rohas et al., 2007; Williams et al., 2007), $G\alpha_{12}$ (Siehler, 2009), and $G\alpha_i$ (Taussig et al., 1994). Most G protein effectors are transmembrane protein complexes. Some G protein effectors are enzymes that catalyze the production of second messengers, small molecules that can diffuse in the cell and evoke responses. Gα proteins bind their effectors via the same "switch" regions they use to bind Gβγ. Gα must be in its GTP-bound conformation to bind and activate effectors, but must be in its GDP-bound conformation to bind Gβγ. Thus Gα serves as a molecular switch that alternates between an active GTP-bound state and an inactive GDP-bound state. Gβγ can only activate its effectors once it has dissociated from Gα.

1.3.5. State 5, inactivation of Gα via GTP hydrolysis, and reactivation via receptor-independent nucleotide exchange

Gα subunits of heterotrimeric G proteins have a slow but significant intrinsic GTPase activity, such that purified Gα protein can hydrolyze bound GTP to GDP with a half time on the order of a few minutes. A key contribution of *C. elegans* and yeast genetics was the discovery of a class of "regulator of G protein signaling" (RGS) proteins (Koelle and Horvitz, 1996; Dohlman et al., 1996) that serve as GTPase activators for Gα proteins, speeding up the hydrolysis reaction by orders of magnitude (Berman et al., 1996; Tesmer et al., 1997). Physiologically, Gα signaling is typically inactivated with the help of an RGS protein (Figure 1).

A second key contribution of *C. elegans* genetics was the discovery of RIC-8, a soluble protein that promotes G protein signaling (Miller et al., 2000), sometimes with the help of other proteins containing the G protein regulatory (GPR) motif that binds Gα proteins (Colombo et al., 2003; Gotta et al., 2003; Srinivasan et al., 2003; Hofler and Koelle, 2011). Genetic studies in *C. elegans* show that GPR proteins and RIC-8 promote G protein activity, and the biochemical activities of these proteins *in vitro* suggest that they may do so by reactivating Gα-GDP as illustrated in Figure 1. However, studies in mammalian cells show that RIC-8, in addition to catalyzing nucleotide exchange *in vitro*, also acts in living cells as a chaperone to promote folding and stability of Gα proteins (Gabay et al., 2011; Chan P. et al., 2013). The *C. elegans* genetic data are consistent with the alternative model that GPR and RIC-8 proteins are simply required for Gα folding and stability.

The G protein activity cycle is completed when Gα-GDP re-associates with Gβγ to re-form the inactive Gαβγ heterotrimer. Gβγ is sequestered in the heterotrimer so that it can no longer associate with its effectors.

2. Neurotransmitters and receptors that signal through heterotrimeric G proteins

In this section, I describe analysis of the surprisingly large set of neurotransmitters and neural G protein coupled receptors present in *C. elegans*. Each neurotransmitter and each receptor is expressed in a very specific and limited set of neurons, and as a result each affects a very specific and limited set of behaviors. An important result from *C. elegans* is that a neurotransmitter can signal via a GPCR expressed on cells that are not postsynaptic to the neurons that release that neurotransmitter. Thus neurotransmitters travel through tissue to signal at sites distant from their site of release. The pattern of neurotransmitter signaling via GPCRs is thus not determined by the synaptic wiring of the nervous system, but rather by the specific expression patterns of the neurotransmitters and their GPCR receptors. The widespread nature of extrasynaptic neurotransmitter signaling forces us to expand our notion of a neural circuit to a unit consisting of neurons that function together but that may lack direct anatomical connections.

2.1. GPCRs for small-molecule neurotransmitters

Most major small-molecule neurotransmitters used in humans are also found in *C. elegans* (exceptions are norepinephrine, histamine, and possibly glycine and ATP). Table 1 and Table 2 summarize studies of a set of 28 *C. elegans* GPCRs for small-molecule neurotransmitters. This includes four serotonin receptors, four dopamine receptors, three octopamine receptors, three tyramine receptors, three acetylcholine receptors, two GABA receptors that function together as an obligate dimer, three apparent glutamate receptors, and six additional predicted receptors with sequence similarity to small molecule neurotransmitter GPCRs that have yet to have ligands assigned to them or to be studied genetically. Many of these 28 GPCRs are produced in multiple isoforms via alternative splicing of their RNA transcripts

2.1.1. Identification of ligands for individual GPCRs

Putative small-molecule neurotransmitter GPCRs were first identified in *C. elegans* as homologs of mammalian neurotransmitter receptors (e.g., Komuniecki et al., 2004). Such analyses can make predictions for the ligand that might activate a GPCR homolog, but these are weak predictions. For example, the *C. elegans* receptors SER-2, SER-3, and SER-6 were originally assigned their names due to similarity with serotonin receptors, but later proved experimentally to be receptors for other biogenic amine neurotransmitters (Table 1). Thus, assigning a ligand to a GPCR requires experimental evidence. Three lines of such experimental evidence are described below, and Table 1 lists which of these lines of evidence are available for each of 22 *C. elegans* receptors.

The first line of experimental evidence is listed in Table 1 as "binding studies". Here, a GPCR is expressed in heterologous cells inducing a binding activity in their membranes for a radiolabeled ligand. Since characterizing binding by many different radioligands is inconvenient, typically a single radioligand with binding activity is identified (e.g., ^{3}H-LSD, which binds with high affinity to most biogenic amine receptors), and then the ability of many unlabeled neurotransmitters and pharmaceuticals to compete off the radioligand is measured as a "K_i". Such studies measure the relative binding affinity of different ligands, but do not determine if an individual ligand is an agonist (activator) or antagonist (inhibitor) for the receptor. Based on such binding data, a GPCR is considered likely to be a receptor for the neurotransmitter that appears to bind it with highest affinity.

The second line of experimental evidence listed in Table 1 is "heterologous cell signaling". Here, a GPCR is expressed in heterologous cultured cells, typically mammalian cells or *Xenopus* oocytes, potential ligands are applied in the medium, and activation of downstream signaling is measured (e.g., through use of fluorescent Ca^{2+} indicators, electrophysiological recording of G protein regulated ion channels, etc.). This method allows the concentration of a ligand that gives half-maximal response (EC_{50}) to be measured, and can determine if a ligand is an agonist or antagonist. Further, it provides evidence for the type of Gα protein activated by the receptor (see Section 2.2.2.). Based on such heterologous cell signaling studies, a GPCR is considered likely to be a receptor for the neurotransmitter that activates it with the lowest EC_{50}.

The third line of experimental evidence listed in Table 1 is genetic studies in *C. elegans*. In many cases, a mutant for a GPCR renders worms insensitive to the effects of a specific neurotransmitter, either applied exogenously or released endogenously via optogenetic stimulation. Another type of genetic study demonstrates that a GPCR mutant shows behavioral defects similar to those of a mutant lacking a particular neurotransmitter. Such genetic studies provide evidence that GPCR functions *in vivo* as a receptor for a specific neurotransmitter.

Table 1 shows that for the 22 GPCRs listed as assigned a ligand, the number of experimental lines of evidence for that assignment varies from zero to three, and the quality of each piece of evidence can also vary widely. Clearly, the more experimental evidence that is available, the more confident a ligand assignment will be.

Both *C. elegans* and mammals have multiple GPCRs for individual neurotransmitters. A large number of pharmaceuticals have been developed that activate or antagonize specific mammalian receptor isoforms. It is tempting to try to use *C. elegans* as a model system to investigate the functions of specific neurotransmitter receptor isoforms to better understand the actions of these important drugs. However, when the sequences of *C. elegans* neurotransmitter receptors are lined up with those of mammalian receptors, individual *C. elegans* receptors cannot be unambiguously assigned as orthologs of specific mammalian receptors. For example, the *C. elegans* serotonin receptors are more similar to mammalian serotonin receptors than they are to mammalian receptors for other neurotransmitters, but an additional *C. elegans* receptor (named SER-2) is also most similar to mammalian serotonin receptors in sequence and turned out be a tyramine receptor (Rex et al., 2004). By sequence analysis, the bona fide *C. elegans* serotonin receptors do not unambiguously match up with individual mammalian serotonin receptor isoforms. Furthermore, when *C. elegans* serotonin receptors are expressed in cultured cells or *Xenopus* oocytes, their profiles of binding and activation by drugs do not match up to the pharmacology of specific mammalian serotonin receptor isoforms (Komuniecki et al., 2004). Thus, while *C. elegans* generally provides an excellent model for studying small-molecule neurotransmitter signaling through G proteins, there are limits to the level of conservation of receptors between humans and worms.

An important issue in assigning ligands to GPCRs is the potential that a single GPCR might physiologically mediate signaling by more than one type of neurotransmitter. The heterologous cell signaling studies cited in Table 1 show that certain receptors have significant affinities for more than one biogenic amine neurotransmitter. For example, the dopamine receptor DOP-3 is activated in heterologous cell signaling studies by dopamine with an EC_{50}

of 27 nm, but also by tyramine with an EC_{50} of 500 nm (Sugiura et al., 2005). Binding studies show significant affinities for more than one biogenic amine by the receptors DOP-1, DOP-2 (Suo et al., 2002), OCTR-1 (Wragg et al., 2007), SER-2 (Rex and Komuniecki, 2002), TYRA-2 (Rex et al., 2005), and TYRA-3 (Wragg et al., 2007). Since the synaptic concentrations of released neurotransmitters are thought to go above one millimolar (Barberis et al., 2011), the high nanomolar or micromolar binding affinities of certain receptors for a secondary neurotransmitter could be biologically meaningful. Studies in *C. elegans* could resolve this issue. Given the complete wiring diagram in *C. elegans*, along with the known identifies of neurons that release specific neurotransmitters and that express specific GPCRs, there is the potential to identify synapses at which a GPCR is exposed to a high concentration of one of its lower-affinity ligands. Genetic analysis could then determine if this results in physiologically significant signaling.

2.1.2. Identification of G proteins activated by individual GPCRs

Sequence comparison of *C. elegans* GPCRs to homologous mammalian GPCRs can be used to predict to which Gα protein a particular *C. elegans* GPCR might couple (e.g., Wragg et al., 2007). However, a more definitive assignment requires experimental evidence. Two lines of such experimental evidence are described below, and Table 1 lists the lines of evidence for Gα protein coupling available for each of 22 *C. elegans* receptors.

The first line of experimental evidence is listed in Table 1 as "heterologous cell" studies. Here, a GPCR is expressed in heterologous cultured cells for ligand activation studies, as described above in Section 2.1.1. In addition to identifying an activating ligand, these studies can determine what type of Gα protein is activated by the GPCR in heterologous cells. For example, activation of Ca^{2+} release generally indicates $G\alpha_q$ signaling, activation of cAMP production generally indicates $G\alpha_s$ activation, and inhibition of cAMP production and/or sensitivity to pertussis toxin generally indicates $G\alpha_{i/o}$ activation.

The second line of experimental evidence listed in Table 1 comes from genetic studies in *C. elegans*. Here, a *C. elegans* GPCR mutant can be used to demonstrate that a receptor acts in a particular cell type to support a particular behavior, and genetic studies can similarly show that a particular Gα protein is also required in the same cell type for the same behavior.

Table 1 shows that 20 of 22 GPCRs assigned to a ligand are also assigned coupling to a specific Gα protein, with the number of experimental lines of evidence for that Gα assignment varying from zero to two. Gα assignments from heterologous cell studies generally agree with assignments from *C. elegans* genetics in cases where both lines of evidence are available, bolstering confidence in assignments from the many cases with only one line of evidence. An interesting case is that of the dopamine receptor DOP-2, which was assigned to $G\alpha_{i/o}$ in heterologous cell studies, and for which *C. elegans* genetics suggests that three different $G\alpha_o$-related Gα proteins may mediate DOP-2 signaling in worms (Suo et al., 2003; Suo et al., 2009; Correa et al., 2012; Pandey and Harbinder, 2012; Mersha et al. 2013).

2.2. Cataloging GPCRs for neuropeptides

At present at least 119 *C. elegans* genes encoding over 250 neuropeptides have been cataloged (Li and Kim, 2008; Li and Kim, 2010). Identifying the receptors for all these neuropeptides is a major challenge. Of the >1,000 putative GPCRs encoded in the *C. elegans* genome, most are chemosensory receptors, and a much smaller subset are likely to be neuropeptide receptors. For tables itemizing these receptors, I refer readers to several recent reviews that have cataloged the likely *C. elegans* neuropeptide receptors by looking for GPCRs most similar to known neuropeptide receptors (Altun, 2011; Frooninckx et al., 2012; The neuronal genome of *Caenorhabditis elegans*). These cataloging efforts generally predict about 150 genes encoding neuropeptide receptors, with individual genes often producing a number of differentially spliced isoforms.

Only a relatively small number of putative neuropeptide receptors have been assigned as receptors for specific neuropeptide(s)—one recent count put the number of such "deorphanized" neuropeptide receptors at 23 (Frooninckx et al., 2012). The quality of the data varies for each receptor, but a definitive assignment of a peptide to a receptor should include data showing the peptide binds the receptor with high affinity and specificity, as well as *C. elegans* genetic data demonstrating that the peptide functions via the receptor to regulate a specific behavior. An example of such a definitive assignment is work showing that the sensory BAG neurons release two different peptides encoded by the *flp-17* gene which then act via the EGL-6 receptor on the HSN motor neuron to inhibit egg-laying behavior (Ringstad and Horvitz, 2008).

Can *C. elegans* neuropeptides or neuropeptide receptors be matched up with mammalian orthologs? In the case of the neuropeptides, the sequences are so short that there just is not enough information content in them to make such an analysis possible. In the case of the receptors, their information-rich sequences do allow such an analysis. Mammalian neuropeptide receptors can be broken down into several subfamilies based on sequence relationships, and many worm receptors can similarly be fitted into the same families (Altun, 2011; Frooninckx et al., 2012; The neuronal genome of *Caenorhabditis elegans*). Most *C. elegans* putative neuropeptide receptors cannot be definitively assigned as orthologs of specific mammalian receptors simply based on sequence analysis. However, based on sequence similarity as well as additional functional data, some worm receptors have been described as models for specific mammalian receptors. Thus *C. elegans* NPR-1 is similar to the mammalian neuropeptide Y receptor (de Bono and Bargmann, 1998), *C. elegans* PDFR-1 is similar to the *Drosophila* pigment dispersing factor receptor and mammalian vasoactive peptide and calcitonin receptors (Janssen et al., 2008), and *C. elegans* NPR-17 is similar to mammalian opioid receptors (Cheong et al., 2015).

2.3. Neural GPCRs are each expressed in very restricted sets of cells

An important result from analysis of G protein coupled neurotransmitter receptors in *C. elegans* is that each receptor tends to be expressed on a small number of specific neurons, allowing that receptor to mediate very specific effects on behavior. *C. elegans* hermaphrodites have just 302 neurons, which, due to symmetries and repeated structures in the anatomy, can be classified into 118 types. Each neuron can be identified by its unique position and morphology within the animal. Further, all the synaptic connections of each neuron with other neurons and muscles have been mapped, making *C. elegans* the only animal for which a complete neural wiring diagram is available (White et al., 1986). Because of these features, identifying all the specific neurons that express a neurotransmitter and its receptor is uniquely possible in *C. elegans*, and this information, once obtained, can be interpreted using the wiring diagram to obtain unique insights into neural signaling. In this section I begin a discussion of this area of investigation.

The expression patterns of a significant subset of *C. elegans* neural GPCRs have been studied. These experiments generally involve creating transgenic worms in which the promoter and other regulatory sequences for a GPCR gene are used to drive expression of the green fluorescent protein (GFP), and specific cells expressing GFP are identified by fluorescence microscopy. Data for the expressions patterns of 22 GPCR receptors for small-molecule neurotransmitters are summarized in Table 2, and expression patterns for 27 of the ~150 putative neuropeptide receptors are also available (The neuronal genome of *Caenorhabditis elegans*). The quality and completeness of the work varies greatly for each receptor. In some cases, only relatively short promoter regions were used to construct the GFP reporter transgenes, raising questions as to whether the results obtained fully represent the expression patterns of the endogenous GPCR genes. In other cases, only a cursory analysis of the fluorescently labeled cells is presented, with little or no attempt to identify the GFP-expressing cells.

Despite these caveats, an important generalization can be made about the expression patterns of neural GPCRs: an individual neurotransmitter receptor type tends to be expressed in a limited set of specific cells. This can be illustrated with examples from receptors for which relatively high-quality expression data are available and which typify the results obtained with other receptors. A GFP reporter for the EGL-6 neuropeptide receptor was expressed in just three types of neurons (HSN, SDQ, DVA) and one type of glial cell (GLR) (Ringstad and Horvitz, 2008). A GFP reporter for the NPR-1 neuropeptide receptor was expressed in approximately 20 neuron cell types (Coates and de Bono, 2002). A reporter for the NPR-4 neuropeptide receptor was expressed in five neuron types, the intestine, and the rectal gland cell, while a reporter for the NPR-5 neuropeptide receptor was expressed 13 neuron types and in all body wall muscles (Cohen et al., 2009). Expression patterns of G protein coupled receptors for small-molecule neurotransmitters (Table 1) show similar characteristics to those seen in the above examples of neuropeptide receptors—each receptor is typically expressed in a limited number of neuron types plus sometimes additional non-neuronal cell types. For example, a reporter for the TYRA-2 tyramine receptor is expressed in about 14 neuron types (Rex et al., 2005). Reporters for the SER-2 tyramine receptor are expressed in a total of 24 neuron types, plus body wall muscles, pharyngeal muscles, and the excretory gland (Tsalik et al., 2003).

2.4. Neurotransmitters signal extrasynaptically through GPCRs

The predominant model of neural signaling has been that synapses, specialized physical connections between neurons, are the sites of neurotransmitter signaling between neurons. A presynaptic neuron releases neurotransmitter from vesicles clustered at a presynaptic terminus, flooding the narrow synaptic cleft with neurotransmitter, which binds to neurotransmitter receptors clustered on the postsynaptic membrane. The idea that synapses are central to

understanding neural function inspired the landmark achievement of mapping all the synaptic connections in the *C. elegans* nervous system (White et al., 1986), and is behind current efforts, for example within the BRAIN initiative, to map synaptic connections within more complex nervous systems (Jorgenson et al., 2015).

While ionotropic neurotransmitter signaling may typically be restricted to synapses, studies in *C. elegans* provide ample evidence that neurotransmitter signaling through G protein coupled receptors may be predominantly extrasynaptic. The concept of extrasynaptic neurotransmission (also called volume transmission) originated in 1986 based on studies of mammalian brain (Agnati et al., 1986). Since then, many studies in mammalian brain demonstrated the release of neurotransmitters from extrasynaptic sites, the ability of neurotransmitters to diffuse through the extracellular space, and the localization of G protein coupled neurotransmitter receptors to extrasynaptic sites (Agnati et al., 2010). Indeed, given the submicromolar affinities of many GPCRs for their neurotransmitter ligands (see Table 1 for examples), it would make little sense for such receptors to function at synapses where neurotransmitter concentrations can rise to the millimolar level (Barberis et al., 2011) and may never be reduced to low enough concentrations to allow high-affinity receptors to become unliganded. Despite this evidence, it has been difficult to provide genetic evidence demonstrating the functional significance of extrasynaptic signaling in mammalian brain. In contrast, *C. elegans* is ideally suited to such work. In this system, it is possible to use rigorous genetic experiments to define the specific neurons that release a neurotransmitter to induce a particular behavioral response, and to also define the specific neurons that express the G protein coupled receptor that functionally receives the neurotransmitter signal to execute the response. Remarkably, results of such analyses show over and over again that the neurotransmitter releasing neuron and the receptor-expressing receiving neuron are not synaptically connected. Ironically, the complete synaptic wiring diagram of the *C. elegans* nervous system has thus been the key tool for demonstrating that synapses are often not needed to mediate neurotransmitter signaling via G protein coupled receptors. Here I summarize the evidence for extrasynaptic neurotransmitter signaling in *C. elegans*.

2.4.1. Neurotransmitters are released from extrasynaptic sites

Neuropeptides and some small-molecule neurotransmitters are released from dense-core vesicles, which are distinct from the small-clear vesicles clustered at presynaptic termini. In *C. elegans* neurons that make synapses dense-core vesicles are excluded from the synaptic active zones where synaptic vesicles are released, although many are localized nearby (Hammarlund et al., 2008). Furthermore, *C. elegans*, like the human brain, has specialized neuroendocrine cells that synthesize and release neurotransmitters but that do not form synaptic connections with any other cells. The uv1 neuroendocrine cells release tyramine and neuropeptides to inhibit egg-laying behavior (Jose et al., 2007), and the NSM neuroendocrine cells release serotonin to regulate locomotion (Sawin et al., 2000; Gürel et al., 2012). The extrasynaptic neurotransmitter release sites of NSM have been studied in some detail (Nelson and Colón-Ramos, 2012). The documented functions of neurotransmitter release from uv1 and NSM provide one set of evidence that neurotransmitters do signal extrasynaptically. Some additional cells in *C. elegans* do not form any synapses, yet express neuropeptide genes, so any functions of peptides secreted from these cells must also be extrasynaptic. Cells in this category include the CAN cell, intestinal cells, muscle cells, and hypodermal cells (White et al., 1986; Li and Kim, 2008; Li and Kim, 2010).

2.4.2. Neurotransmitters can signal through GPCR receptors on cells not postsynaptic to their release sites

The specific *C. elegans* neurons that synthesize and release individual small molecule neurotransmitters or neuropeptides have been mapped in considerable detail (see WormBook chapter GABA; Rand, 2007; Chase and Koelle, 2007; Li and Kim, 2008; Li and Kim, 2010; Jafari et al., 2011; Serrano-Saiz et al., 2013; Periera et al., 2015). Thus when the expression pattern of a G protein coupled receptor for a specific neurotransmitter is determined, we can use the synaptic wiring diagram of *C. elegans* to see if the cells that produce that neurotransmitter are presynaptic to the cells that express its receptor. In many cases, the answer is no.

G protein coupled receptors for small-molecule neurotransmitters are expressed on cells not postsynaptic to neurons that release the corresponding neurotransmitter. This was first noted for the dopamine receptors DOP-1 and DOP-3, which are expressed, among other places, on ventral cord motor neurons (Chase et al., 2004). Dopamine is released from exactly three types of neurons in *C. elegans*, and none of these neurons make synapses onto the ventral cord motor neurons. Genetic studies show that dopamine regulates locomotion via the DOP-1 and DOP-3 receptors, and the locomotion defects of *dop-1* and *dop-3* mutants can be rescued be re-expressing the receptors specifically in ventral cord motor neurons (Chase et al., 2004). These experiments rigorously demonstrate that dopamine receptors are not only found on cells distant from dopamine release sites, but that they also function to mediate dopamine signaling in these distant cells.

Since those initial studies of dopamine signaling, similar experiments have demonstrated extrasynaptic signaling by other small-molecule neurotransmitters. One striking case is that of the neurotransmitter tyramine, which is released from only the neuroendocrine uv1 cells (which make no synapses) and from the RIM interneuron, which makes synapses only onto four other types of neurons and onto neck muscles (White et al., 1986). When *C. elegans* is touched on the head, tyramine released from RIM mediates a complex escape response. One aspect of this response is mediated by tyramine signaling at synapses from RIM onto neck muscles using an ionotropic receptor (Pirri et al., 2009). However, another aspect of the escape response is mediated by tyramine signaling from RIM through a G protein coupled receptor. This GPCR, SER-2, is expressed on GABAergic motor neurons, which are not post-synaptic to RIM, and defects in the escape response seen in *ser-2* mutants are rescued by cell-specific re-expression of *ser-2* in GABAergic motor neurons, demonstrating that SER-2 acts in these neurons to mediate extrasynaptic signaling by tyramine that originates from RIM (Donnelly et al., 2013).

There have now been similar genetic demonstrations that neuropeptides signal onto cells not postsynaptic to the neurons that release them. For example, FLP-17 neuropeptides are released from BAG sensory neurons and signal via the EGL-6 neuropeptide receptor on the HSN motor neurons, which are not postsynaptic to BAG, to inhibit egg laying (Ringstad and Horvitz, 2008). The *C. elegans* defecation motor program is coordinated by NLP-40 peptides released from intestinal cells, which being non-neuronal make no synapses, and these NLP-40 peptides signal onto GABAergic motor neurons via the AEX-2 peptide receptor (Wang et al., 2013). Beyond these rigorous genetic demonstrations that specific peptides signal extrasynaptically via particular neuropeptide receptors, there are additional data showing that some neuropeptide receptors are expressed on cells that do not receive any synapses, so that any signaling they mediate on these cells must be extrasynaptic. These receptor-expressing cells include intestinal, hypodermal, and glial cells (The neuronal genome of *Caenorhabditis elegans*).

2.5. The genetics of G protein coupled neurotransmitter receptors

Null mutants or RNAi knockdowns for many *C. elegans* G protein coupled neurotransmitter receptors have been analyzed, and the main finding is that phenotypic defects are very hard to detect in them. Most of the time no defects are observed, and the few mutant phenotypes that have been described for neural GPCRs are typically not gross behavioral defects obvious upon inspection of individual animals, but rather narrow behavioral defects detectable only when populations of worms are put through specific phenotypic assays.

An initial effort at identifying neural GPCR phenotypes used RNAi to knock down expression of 60 G protein coupled neurotransmitter receptors (Keating et al., 2003) and simply looked for uncoordinated movement or defects in reproduction. Knockdown of seven of the receptors had detectable effects on the frequency and/or amplitude of body bends, while knockdown of five receptors had some effect on the production of progeny. Later large-scale RNAi screens for genes that affect acetylcholine release/signaling identified hits in five GPCR genes, of which three were validated using genetic knockout mutations (Sieburth et al., 2005; Vashlishan et al., 2008). It is difficult to interpret some of these RNAi results: not all the defects seen with RNAi were reproduced with genetic mutations, the RNAi results did not always reproduce in different labs, and the phenotypes that were observed have not been characterized in much detail. Negative results from RNAi for GPCRs are also difficult to interpret since gene expression in *C. elegans* neurons is not always efficiently knocked down by RNAi, even in mutant backgrounds that enhance neuronal RNAi (Poole et al., 2011).

Mutations in genes encoding GPCRs have almost never been generated in forward genetic screens, despite the large number of GPCR genes in the worm genome. Since Brenner's first screen for uncoordinated mutants (Brenner, 1974), *C. elegans* mutants for thousands of genes have been generated in genetic screens, including a large number with defects in neural structure and/or function. Included in this trove are many mutants for heterotrimeric G proteins and other signaling proteins that act downstream of neural GPCRs (Perez-Mansilla and Nurrish, 2009), making the absence of neural GPCR mutants even more conspicuous.

Considering the few neural GPCR mutants that have arisen in forward genetic screens is instructive. The EGL-6 neuropeptide receptor was identified by a rare gain-of-function mutation that inhibits egg laying (by increasing EGL-6 signaling), yet knockout mutations of EGL-6 do not produce detectable egg-laying defects (Ringstad and Horvitz, 2008). Loss-of-function mutations in G protein coupled serotonin and dopamine receptors have arisen in forward genetic screens, but in these cases the phenotype screened for was resistance to the paralysis induced by applying these neurotransmitters to worms at high concentrations (Chase et al., 2004; Gürel et al., 2012). These mutants do not have obvious behavioral defects when animals are not bathed in a neurotransmitter, although defects have been detected when populations of mutant animals are put through very specific behavioral assays

known to depend on serotonin or dopamine. There is perhaps only a single example of a neural GPCR for which loss-of-function mutants have been recovered from a forward genetic screen and for which the mutant phenotype is easily observable in single animals: mutants lacking AEX-2, the receptor for NLP-40 neuropeptides, are defective in the expulsion step of defecation (Mahoney et al., 2008; Wang et al., 2013).

C. elegans mutants for many G protein coupled neurotransmitter receptors are available as a result of large-scale efforts to produce gene knockouts for all *C. elegans* genes (*C. elegans* deletion mutant consortium, 2012). However, phenotypic defects have been described for only a small subset of these GPCR mutants. The cases in which phenotypic defects have been detected have generally come when either the neurotransmitter that activates the GPCR was known, so that specialized assays for behaviors dependent on that neurotransmitter could be assayed for defects, or in cases in which the expression pattern of the GPCR was known, so that specialized assays for behaviors dependent on neuron(s) that express the receptor could be tested for defects. An example that illustrates both strategies comes from the work of Harris et al. (2009), which analyzed how *C. elegans* backs away from the odor of dilute octanol, a response known to depend on both the neurotransmitter serotonin and on a neural circuit containing specific sensory neurons and interneurons. This information was used to focus in on two G protein coupled serotonin receptors, one expressed in the interneurons, another in the sensory neurons, and further experiments showed that knockouts and knock downs of these receptors resulted in specific behavioral defects in response to octanol. The two serotonin receptors studied by Harris et al. (2009) are part of the set of 22 receptors for small molecule neurotransmitters listed in Table 2, which are the most intensively studied GPCRs in *C. elegans.* As shown in Table 2, at this point mutant defects have been described for 20 of these receptors. The situation is less encouraging in the case of neuropeptide receptors, with mutant phenotypic defects described for only a few out of this large set of ~150 receptors.

Considering the totality of available genetic studies of *C. elegans* G protein coupled neurotransmitter receptors, it appears that the knockout phenotypes for these proteins are generally so narrow that they will not be easily detected. Because individual receptors are expressed in only a few types of neurons, it is reasonable to expect that just the very specific behaviors affected by those neurons will be affected, and that specialized behavioral assays will be required to detect these defects. Another possible reason that GPCR mutant phenotypic defects are rarely detected could be that the large family of GPCRs contains many functionally redundant receptors, such that knocking out one receptor will not give obvious defects unless its redundant partners are also knocked out simultaneously. Several examples support the idea that neurotransmitter receptors can function redundantly. Two serotonin receptors appear to be co-expressed on the vulval muscles and both appear to promote activity of these muscles (Hapiak et al., 2009). Serotonin released from the NSM neurosecretory cells inhibits locomotion, and two different serotonin receptors expressed on largely non-overlapping sets of neurons redundantly mediate this effect (Gürel et al., 2012). Neuropeptides encoded by FLP-18 modulate locomotion, and three different receptors, NPR-1, NPR-4, and NPR-5, expressed in different sets of cells, redundantly mediate these effects of FLP-18 (Stawicki et al., 2013). FLP-18 signaling is quite complex, because other behavioral effects of FLP-18 peptides are mediated non-redundantly by NPR-1, and for these effects FLP-18 and FLP-21 neuropeptides both seem to activate NPR-1 (Choi et al., 2013). If such complex functional relationships between neurotransmitters and receptors are widespread, this will make assigning functions to receptors using knockout mutations very challenging.

A tool that may prove generally useful in genetically characterizing redundant or subtle GPCR functions is transgenic overexpression of GPCRs. One example comes from studies of the EGL-6 neuropeptide receptor. Loss-of-function mutants for EGL-6 do not result in obvious defects under standard lab growth conditions. However, using a transgene carrying many copies of *egl-6* genomic DNA to presumably overexpress the receptor does result in an obvious egg-laying defective phenotype, apparently due to increased activation of the overexpressed EGL-6 receptor by the FLP-10 and FLP-17 neuropeptides (Ringstad and Horvitz, 2008). Another example comes from studies of the NPR-17 neuropeptide receptor. NPR-17 mediates an effect of NLP-3 peptides to modulate how *C. elegans* backs away from aversive stimuli, and a transgene carrying many copies of the *npr-17* gene causes a gain-of-function phenotype opposite that of an *nlp-3* or *npr-17* knockout, and that depends on the presence of a wild-type NLP-3, suggesting that the overexpressed NPR-17 receptor causes increased NPR-17 signaling (Harris et al., 2010). If transgenically overexpressing a GPCR is generally able to induce gain-of-function phenotypes, this could be a valuable approach for identifying subtle or redundant functions of GPCRs.

2.6. Heterodimerization of G protein coupled neurotransmitter receptors

A major area of current research on mammalian GPCRs concerns the homo- and hetero-oligomerization of these receptors (González-Maeso, 2011). It appears that in many cases, two different types of GPCRs can exist and function *in vivo* as a heterodimeric complex, and that a heteromeric receptor can have very different signaling

properties than homomers of its subunits, including the ability to bind to different ligands. While GPCR heteromerization has so far been studied mainly using biochemical and biophysical methods, genetic analysis has much to contribute to understanding the physiological significance of this phenomenon. Heteromerization also potentially vastly complicates the challenge of understanding GPCR function: if *C. elegans* has ~150 G protein coupled neurotransmitter receptor genes, how many types of heteromeric GPCRs might actually be the functional units *in vivo*?

There have so far been only a few efforts to analyze GPCR heteromer function in *C. elegans*. One example is that of the G protein coupled receptor for the neurotransmitter GABA. In mammals this $GABA_B$ receptor is an obligate heteromer between the $GABA_{B1}$ and $GABA_{B2}$ subunits (Kaupmann et al., 1998). *C. elegans* has orthologs of each subunit, known as GBB-1 and GBB-2, respectively. Knockouts of either GBB-1 or GBB-2 can block all G protein coupled responses to GABA in worms (Schultheis et al., 2011), and GBB-1 and GBB-2 act together to alter responses to the drug aldicarb (Dittman and Kaplan 2008; Vashlishan et al., 2008), consistent with the idea that the $GABA_B$ receptor is an obligatory heterodimer in worms just as it is in mammals. A second example comes from studies of signaling onto sensory neurons, not by a neurotransmitter, but by the mixture of small molecules known as ascarosides that collectively make up the worm dauer pheromone. The functional receptor for the specific ascaroside isoform ascr#2 appears to be a heteromer of the GPCRs DAF-37 and DAF-38 (Park et al., 2012). ascr#2 binds directly to DAF-37, and DAF-37 is essential for response to ascr#2 but is not involved in the response to other ascaroside isoforms. Genetic studies show that DAF-38 assists but is not essential for response to ascr#2, and similarly assists response to other ascaroside isoforms that do not signal through DAF-37, suggesting DAF-38 may heteromize with and assist signaling by a several different GPCRs that bind directly to different ascaroside isoforms. These studies demonstrate the potential of *C. elegans* genetic studies to sort out the intricacies of how GPCR subunits function together as heteromeric receptors *in vivo*.

2.7. Deorphanizing neural G protein coupled neurotransmitter receptors

One of the principal issues in studying neural GPCRs is identifying the specific neurotransmitters that activate them. At this point, 22 of 28 GPCRs encoded in the worm genome that seem likely to be receptors for small-molecule neurotransmitters have been matched with their activating ligands (Table 1), but about 85% of the ~150 putative neuropeptide receptors remain "orphans", that is receptors with unknown activators (Frooninckx et al., 2012).

The general strategy to deorphanize neuropeptide receptors is to express them in heterologous cells, to apply synthetic versions of each neuropeptide encoded in the genome, and to test which specific peptides can activate receptor signaling. There are a number of cell types and signaling assays that have been used successfully for this purpose (Mertens et al., 2004). This strategy has been applied to a number of *C. elegans* neuropeptide receptors, for example, EGL-6 (Ringstad and Horvitz, 2008) and NPR-1 (Rogers et al., 2003 Kubiak et al., 2003). The reverse strategy has also been used, screening through a set of *C. elegans* neuropeptide receptors to identify those that can be activated by a particular neuropeptide of interest (Cohen et al., 2009). The results of receptor deorphanization experiments are complex: often a single receptor can be activated by multiple different peptides, the same peptides can activate multiple different receptors, the results can vary depending on the cell type and assay system used, and the EC_{50} values measuring the potencies with which peptides activate receptors vary from the nanomolar to micromolar ranges (Peymen et al., 2014).

C. elegans provides the opportunity to analyze mutants for the receptors and peptides putatively matched by such deorphanizing experiments to determine if they actually function together *in vivo*. Genetic approaches can also be used to match peptides and their receptors, for example, screening RNAi knockdowns of many neuropeptide genes to find those that phenocopy a particular orphan receptor mutant (Cohen et al., 2009). Such genetic studies have generated results that are similar in complexity to those from using signaling assays in cultured cells for receptor deorphanization. Thus it appears that *in vivo* individual receptors can be activated by multiple peptides and individual peptides can act through multiple receptors (Choi et al., 2013; Stawicki et al., 2013).

The gold standard in matching neuropeptides with receptors is to combine studies of receptor activation by purified peptides in cultured cells with genetic studies in *C. elegans*. For the most part, it has been possible to achieve consistent results between the two approaches, generating strong confidence in the results, although there have been occasional puzzling exceptions (Cheong et al., 2015). Overall, the data matching neuropeptides to their cognate receptors remains sparse and we have a long way to go to fully match up these signaling molecules.

2.8. Studies of receptor desensitization in *C. elegans*

In order for signaling by an activated GPCR to terminate, the receptor must eventually be inactivated. One mechanism for terminating receptor activity is to clear neurotransmitter from the extracellular space so that it no longer remains bound to the receptor. There are enzymes that degrade neurotransmitters and transporters that take them back up into cells that function for this purpose (Zimmerman and Soreq, 2006; Kanner and Zomot, 2008). Another mechanism for terminating signaling involves G protein coupled receptor kinases (GRKs) that specifically recognize and phosphorylate active GPCRs, and arrestin proteins that bind phosphorylated receptors. Phosphorylation and arrestin binding can block the ability of receptors to activate G proteins and also cause cells to internalize receptors from the cell surface, down-regulating signaling (Kohout and Lefkowitz, 2003). These mechanisms, referred to as receptor desensitization, have been extensively studied in mammalian cells. Studies in *C. elegans* of GRK and arrestin homologs have identified specific functions of these proteins in sensory neurons (Fukuto et al., 2004; Palmitessa et al., 2005, Pereira and van der Kooy, 2012; Singh and Aballay, 2012), but have so far failed to show any striking effects of GRK or arrestin on neurotransmitter signaling in the rest of the nervous system.

2.9. The overall landscape of neurotransmitter signaling through GPCRs

It is interesting to extrapolate from the existing data on *C. elegans* G protein coupled neurotransmitter receptors to consider what the overall landscape of neural signaling through GPCRs may look like. If we imagine that the ~175 neural GPCRs in *C. elegans* are each expressed on average in 10 of the 118 neural cell types, then a typical neuron would express about 15 GPCRs, about two of which might be small-molecule neurotransmitter receptors, with the remainder being neuropeptide receptors. This typical neuron would then face the task of simultaneously sensing the levels of ~15 neurotransmitters in its extracellular space and executing appropriate responses to the dynamic mix of these signals it receives over time. All the GPCRs in this typical neuron may signal through about three types of Gα proteins, which do not act separately but rather collaborate to modulate neurotransmitter release from the neuron (see Section 3). Thinking about neurotransmitter signaling through GPCRs this way, it does not make sense to consider the action of a single neurotransmitter at a time, which is the way that we currently investigate neural signaling. Rather, it may be more appropriate to investigate how a single neuron computes appropriate responses to the entire mix of neurotransmitters in its environment. A prerequisite to such an investigation would be to know all the GPCRs that are present on that neuron, and we currently do not have that information for even a single *C. elegans* neuron.

Perhaps the most striking result from analysis of *C. elegans* neural GPCRs is that neurotransmitters signal extrasynaptically through these receptors. Thus neurons that have no physical connections can signal each other and work together to control specific behaviors. The predominant model for understanding nervous system function has been that the functional ensembles of neurons that control thoughts and behaviors are circuits defined by the synaptic connections between the neurons in the ensemble. We have had the synaptic wiring diagram for the *C. elegans* nervous system for almost 30 years (White et al., 1986) and it has proven insufficient to allow us to understand the neural control of behaviors in this organism. Now that we understand the widespread nature of extrasynaptic neurotransmitter signaling, we need to expand our understanding of a neural circuit to be a functional ensemble of neurons that signal each other but that may lack direct anatomical connections. The patterns of signaling via GPCRs are not determined by the synaptic wiring of the nervous system, but rather by the specific expression patterns of neurotransmitters and their GPCR receptors. Thus to help understand neural circuit function, we need to supplement the existing synaptic wiring diagram with an additional diagram in which the specific cells that express each neurotransmitter and its cognate receptor(s) are defined. This goal, while ambitious, is potentially achievable in the *C. elegans* system, perhaps aided by new cell-specific RNAseq technologies (Spencer et al., 2014).

3. The mechanism of signaling by neural heterotrimeric G proteins

In this section, I focus on genetic studies in *C. elegans* of signaling by the neural G proteins $G\alpha_o$, $G\alpha_q$, and $G\alpha_s$ that mediate signaling by G protein coupled neurotransmitter receptors. The G proteins themselves and their downstream signaling pathways are strongly conserved comparing mammalian brain and *C. elegans*. Genetic studies in *C. elegans* show that all three types of Gα proteins signal to regulate neurotransmitter release. *C. elegans* genetic screens have been used to discover new signaling molecules that regulate neural G protein signaling, including Regulators of G protein Signaling (RGS proteins) that help terminate signaling, the RIC-8 protein that is required for signaling, and a new $G\alpha_q$ effector (Trio's RhoGEF domain). The *in vivo* studies of neural signaling in *C. elegans* suggest a model in which the heterotrimeric G proteins present in a neuron act to integrate signaling through the multiple GPCRs present on the neuron to produce its appropriate output level.

3.1. Heterotrimeric G proteins that mediate neurotransmitter signaling

I will focus here on the G protein subunits that mediate neurotransmitter signaling, and refer readers to an earlier review for a table listing all Gα, Gβ, and Gγ subunits in *C. elegans*, and for discussion of the large family of specialized *C. elegans* Gα proteins found in sensory neurons that mediate chemosensation (see WormBook chapter Heterotrimeric G proteins in *C. elegans*).

In *C. elegans*, as in mammals, there are multiple G protein α, β, and γ subunits that can potentially combine to form a larger number of heterotrimeric combinations. In mammals there are few functional differences between the various β and γ subunits (Khan et al., 2013). In *C. elegans* only one β subunit homolog is actually used in G protein heterotrimers (Zwaal et al., 1996; Chase et al., 2001; Robatzek et al., 2001; van der Linden et al., 2001), and only one γ subunit is expressed outside the chemosensory neurons (Jansen et al. 2002). Thus in *C. elegans*, as in mammals, functional diversity among G protein heterotrimers rests primarily on which α subunit is used. Mammals have multiple members of each of four families of α subunits, while *C. elegans* has just one member for each of these four α families (Jansen et al., 1999). The four conserved *C. elegans* α subunits, with their corresponding mammalian orthologs and percent sequence identities to them, are: GOA-1 ($G\alpha_o$, >80%), EGL-30 ($G\alpha_q$, >80%), GSA-1 ($G\alpha_s$, 66%), and GPA-12 ($G\alpha_{12}$, 52%). These worm Gα proteins are each more similar to their mammalian orthologs than they are to each other. Below I will use the mammalian names to refer to the worm Gα proteins, for example using $G\alpha_o$ to refer to its *C. elegans* ortholog GOA-1. The worm $G\alpha_o$, $G\alpha_q$, and $G\alpha_s$ proteins are each widely expressed in most or all neurons, plus some muscle and other cells (Mendel et al., 1995; Ségalat et al., 1995; Park et al., 1997; Korswagen et al., 1997; Lackner et al., 1999; Bastiani et al., 2003). $G\alpha_{12}$, in contrast, is expressed in only a small subset of neurons plus some muscle and hypodermal cells (van der Linden et al., 2003; Yau et al., 2003).

I note as an aside that while GOA-1 appears to be the $G\alpha_o$ ortholog in *C. elegans*, there are also two more distantly related $G\alpha_o$ homologs that have some functional redundancy with GOA-1. GPA-16 is co-expressed with GOA-1 in some neurons (Jansen et al., 1999) and in early embryonic cells, where these two Gα proteins function redundantly to control mitotic spindle positioning during asymmetric cell divisions (reviewed in Polarity establishment, asymmetric division and segregation of fate determinants in early *C. elegans* embryos). GPA-7 is another $G\alpha_o$-related protein that shows expression in many neurons (Jansen et al., 1999). The functions of GPA-7 have not been carefully investigated, but in one study GPA-7 and GOA-1 were shown to function redundantly to mediate dopamine signaling in *C. elegans* male copulatory neurons and muscles (Correa et al., 2012;).

Studies of mammalian GPCRs demonstrate that any one receptor may activate $G\alpha_o$, $G\alpha_q$, $G\alpha_s$, or $G\alpha_{12}$, but will generally not couple to more than one of these Gα types (Moreira, 2014). Currently it is not possible to predict from the sequence of a GPCR which Gα protein it will activate, so this must be determined experimentally (see Section 2.1.2).

C. elegans $G\alpha_{12}$, like mammalian $G\alpha_{12}$, can activate its effector protein RGS-RhoGEF protein (RHGF-1 in *C. elegans*) to in turn activate the small GTPase Rho, and genetic work in *C. elegans* shows this can regulate a pathway involving diacylglycerol and protein kinase C to increase neurotransmitter release (van der Linden et al., 2003; Yau et al., 2003; Hiley et al., 2006). However, these results all arise from studies of worms expressing constitutively active mutants of $G\alpha_{12}$, and no defects have yet been observed in worms carrying loss of function mutations in $G\alpha_{12}$ or its effector RHGF-1. Thus the normal physiological functions of the $G\alpha_{12}$ pathway in *C. elegans* remain to be elucidated. I will not further consider $G\alpha_{12}$ signaling here, but see Perez-Mansilla and Nurrish (2009)) for a detailed review of *C. elegans* $G\alpha_{12}$ signaling.

3.2. Introduction to the genetics of $G\alpha_o$, $G\alpha_q$, and $G\alpha_s$

Before delving into the details of the signaling pathways for the three major neural G proteins, I begin with an overview of what these pathways do and a description of the genetic approaches in *C. elegans* that have been used to study them.

3.2.1. A simplified overview: $G\alpha_o$, $G\alpha_q$, and $G\alpha_s$ signaling regulate neurotransmitter release

The simplified take-home message from genetic analysis of $G\alpha_o$, $G\alpha_q$, and $G\alpha_s$ signaling in *C. elegans* neurons is that $G\alpha_o$ signaling inhibits neurotransmitter release, while $G\alpha_q$ and $G\alpha_s$ signaling activate neurotransmitter release or promote synaptic activity (Figure 2). $G\alpha_o$ and $G\alpha_q$ signaling appear to affect the

localization of specific molecules at presynaptic release sites that regulate the neurotransmitter release machinery itself (Lackner et al., 1999; Nurrish et al., 1999; Chan et al., 2012), while it remains less clear how Gαs signaling promotes neurotransmitter release (Reynolds et al., 2005). A single neuron expresses many GPCRs (see Section 2), so at any one time all three pathways may be active simultaneously, and indeed there may be receptors for different neurotransmitters activating a single type of Gα protein simultaneously. Thus Gαo, Gαq, and Gαs sum up signaling by the several GPCRs active on a neuron, and their three downstream signaling pathways function together to compute an appropriate efficiency for the neurotransmitter release machinery in the neuron.

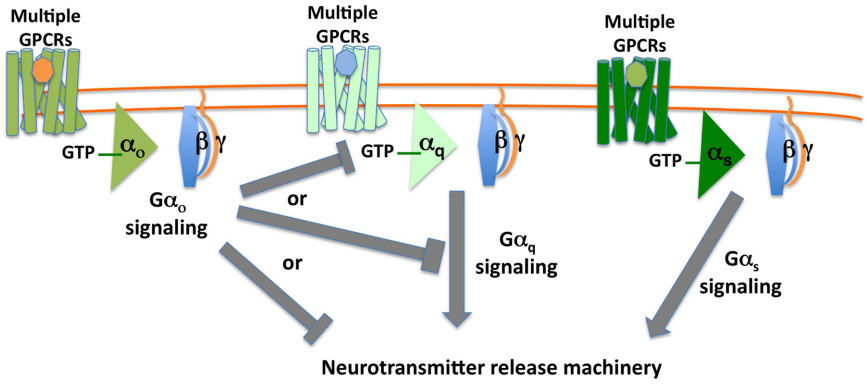


Figure 2. Schematic diagram illustrating how Gαo, Gαq, and Gαs signaling act in a single neuron to together regulate neurotransmitter release. A schematic summary of the effects of signaling by the three Gα proteins on neurotransmitter release, as predicted from genetic studies in *C. elegans*. In such diagrams, an arrow indicates promotion or activation of a target, while a bar denotes an inhibitory effect. The bars extending from Gαo indicate that genetic experiments show Gαo signaling inhibits Gαq signaling, but do not determine whether this inhibition occurs upstream of Gαq, at the level of Gαq, or at some level downstream of Gαo.

Figure 2 is a gross simplification. The neurotransmitter release machinery is complex, with distinct pools of small-clear vesicles present at presynaptic termini, as well as dense-core vesicles at non-synaptic sites, and release can occur tonically or after being evoked by depolarization. Gα signaling may differentially affect the various types of vesicle release (Hu et al., 2015). Further, Gαo, Gαq, and Gαs signaling are known from electrophysiological studies to affect activity of specific ion channels, and from studies in other experimental systems to affect gene expression and synaptic structure. These G proteins are expressed not only in neurons but also in other cell types (e.g., muscles). So, clearly signaling by Gαo, Gαq, and Gαs must have effects other than on the neurotransmitter release machinery. Despite this, studies in *C. elegans* have focused on the effects of G protein signaling on synaptic neurotransmitter release because genetic experiments demonstrate that they actually affect behavior.

There are multiple lines of evidence that Gαo, Gαq, and Gαs signaling affect neurotransmitter release in *C. elegans*, with the most extensive evidence coming from studies of acetylcholine release by ventral chord motor neurons that control locomotion behavior. The four major lines of evidence are: 1) mutations in the G proteins and/or their signaling pathways alter locomotion behavior; 2) mutations in the G proteins and/or their signaling pathways alter response to aldicarb, an inhibitor of the acetylcholinesterase enzyme that clears released acetylcholine from synapses; 3) mutations in the G proteins and/or their signaling pathways alter the localization of GFP-tagged presynaptic proteins at cholinergic synapses; and 4) mutations in the G proteins and/or their signaling pathways cause changes in acetylcholine release that can be measured with electrophysiological methods. The specific studies detailing these lines of evidence for Gαo, Gαq, and Gαs are described and cited below in the remainder of Section 3. As these details will show, all four lines of experimental evidence have established the effects of Gαo and Gαq signaling on acetylcholine release in *C. elegans*, while the effects of Gαs signaling on acetylcholine release from *C. elegans* ventral cord motor neurons rest on just the first two lines of evidence. However additional lines of evidence for the effects of Gαs signaling on neurotransmitter release come from studies of other cell types and species. For example studies in *C. elegans* ALA neurons show that the Gαs signaling pathway affects dense-core vesicle release (Zhou et al., 2007), and electrophysiological studies in mammalian and *Drosophila* neurons have also established effects of Gαs signaling on release of small clear neurotransmitter vesicles (Trudeau et al., 1996; Chen and Regehr, 1997; Kuromi and Kidokoro, 2000).

3.2.2. Isolation of Gαo, Gαq, and Gαs signaling mutants

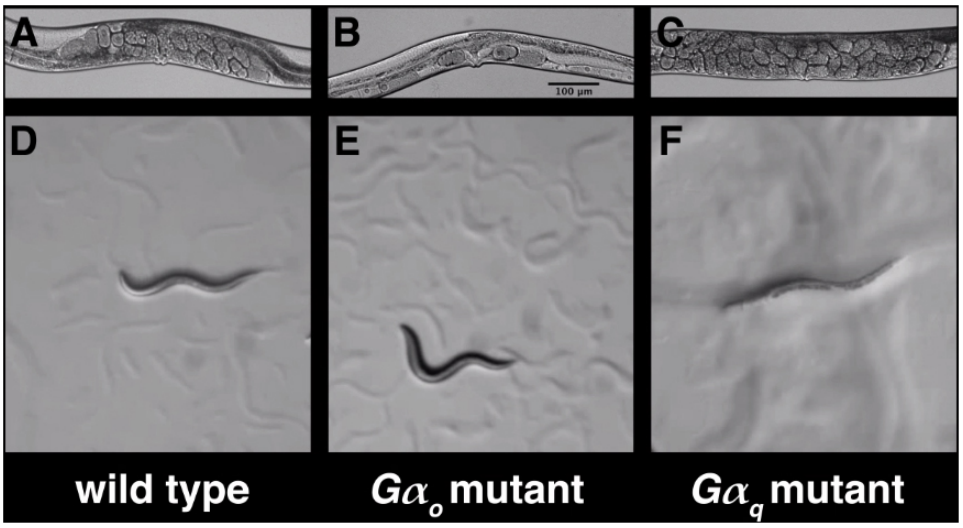
Studies of *C. elegans* neural G protein signaling have been carried out in parallel through both forward and reverse genetics. In the reverse genetic approach, *C. elegans* homologs of mammalian signaling proteins are knocked out by gene-targeting technologies. Thus, for example, the entire sets of G protein subunit genes and RGS

genes have been knocked out and analyzed (Jansen et al., 1999; Hess et al., 2004). The special power of the *C. elegans* system, however, is its capacity for large-scale forward genetic screens in which the genome is randomly mutagenized and vast numbers of animals are screened for specific phenotypes associated with neural G protein signaling defects. The initial screens for mutants with general neural G protein signaling defects took advantage of the fact that heterotrimeric G proteins regulate neurotransmitter release from the egg-laying motor neurons and from the cholinergic motor neurons that control locomotion. Thus these screens looked for mutants that fail to lay eggs (Trent et al., 1983; Desai and Horvitz, 1989), that lay eggs too frequently (Bany et al., 2003), that alter acetylcholine release (Miller et al., 1996; Miller et al., 1999; Miller et al., 2000; Sieburth et al., 2005; Vashlishan et al., 2008), that carry suppressors of previously-isolated G protein signaling mutations (Miller et al., 1999; Schade et al., 2005; Charlie et al., 2006b; Williams et al., 2007), or that have hyperactive locomotion (Schade et al., 2005). Additional screens were for mutants that fail to respond to specific neurotransmitters that signal through GPCRs, including dopamine (Chase et al., 2004:) and serotonin (Gürel et al., 2012).

3.2.3. Phenotypes of $G\alpha_o$, $G\alpha_q$, and $G\alpha_s$ signaling mutants

All genetic studies of neural G protein signaling in *C. elegans* involve assaying mutant phenotypes that arise from G protein signaling defects. Here I describe some of the mutant phenotypes used for this work.

$G\alpha_o$ null and partial loss-of-function mutants have a "hyperactive" phenotype (Mendel et al., 1995; Ségalat et al., 1995), which, as described below, appears to arise from increased neurotransmitter release from neurons throughout the animal. This phenotype can easily be recognized by observing animals growing on a standard laboratory petri dish (Movie 1) and includes body bends that are deeper and more frequent than in the wild type. This defect arises at least in part from increased release of acetylcholine from the ventral cord motor neurons that control locomotion (Vashlishan et al., 2008). The hyperactive phenotype also includes an increased frequency of egg-laying behavior, such that animals lay almost all their eggs as soon as they are produced, so that whereas a wild-type animal might carry ~12 unlaid eggs on average, a hyperactive mutant might carry only one or two (Movie 1). This defect arises at least in part from increased neurotransmitter release from the HSN motor neurons that stimulate egg laying (Tanis et al., 2008). Specific serotonin and dopamine receptors signal through $G\alpha_o$ to inhibit locomotion, such that treating worms with sufficiently high concentrations of dopamine or serotonin paralyzes wild-type worms, but hyperactive $G\alpha_o$ mutants are resistant to paralysis by serotonin or dopamine (Ségalat et al., 1995; Chase et al., 2004; Gürel et al., 2012). As might be expected, since $G\alpha_o$ is expressed in many neurons and non-neuronal cells, $G\alpha_o$ mutants have a number of additional phenotypic defects, including defects in mitotic spindle movements in early embryonic cells (Miller and Rand, 2000; Gotta and Ahringer, 2001) and defects in meiotic maturation of germ cells (Govindan et al., 2006).



Movie 1. The opposite effects of $G\alpha_o$ and $G\alpha_q$ loss-of-function mutations on behavior. A-C, still images of adult mid-body regions of wild type, *goa-1(n1134)* $G\alpha_o$ partial loss-of-function mutant, and *egl-30(n686)* $G\alpha_q$ partial loss-of-function mutant animals, respectively. Unlaid eggs are visible as oval objects inside the body. While wild-type animals average ~12-16 unlaid eggs, $G\alpha_o$ mutants lay almost all their eggs and thus accumulate only 2-3 unlaid eggs, while $G\alpha_q$ mutants fail to lay eggs and accumulate <40 unlaid eggs. D-F, videos of locomotion by larvae of the same genotypes. The locomotion defects seen are most obvious in larvae but adults show qualitatively similar defects. $G\alpha_o$ mutants show hyperactive locomotion, with more frequent and deeper body bends, and more frequent reversals. $G\alpha_q$ mutants show sluggish locomotion, with infrequent, shallow body bends. Magnification is the same in all images, so the scale bar in B applies to all panels.

$G\alpha_q$ null mutants undergo developmental arrest as young larvae (Reynolds et al., 2005), but partial loss-of-function mutants develop to adulthood and show a "sluggish" phenotype that appears to arise from decreased neurotransmitter release (Lackner et al., 1999; Miller et al., 1999) from neurons throughout the animal (Bastiani et al., 2003). Sluggish animals have less frequent and shallower body bends than in the wild type, and strong loss-of-function or null $G\alpha_q$ mutants are virtually paralyzed (Movie 1) (Reynolds et al., 2005; Williams et al., 2007). This defect results at least in part from decreased acetylcholine release from ventral cord motor neurons (Lackner et al., 1999; Hu et al., 2015). Sluggish animals rarely engage in egg-laying behavior, such that adult animals can become bloated with up to ~50 unlaid eggs (Movie 1), and this defect arises at least in part from decreased neurotransmitter release from the HSN motor neurons (Tanis et al., 2008).

The $G\alpha_o$ and $G\alpha_q$ loss-of-function phenotypes are in many respects precisely the opposite of each other. This interpretation is reinforced by the fact that mutations and transgenes that increase $G\alpha_o$ signaling cause a sluggish phenotype essentially indistinguishable from that seen in $G\alpha_q$ loss-of-function mutants (Mendel et al., 1995; Ségalat et al., 1995; Koelle and Horvitz, 1996), while transgenes and mutations that increase $G\alpha_q$ signaling cause a hyperactive phenotype virtually indistinguishable from that seen in $G\alpha_o$ loss-of-function mutants (Bastiani et al., 2003; Hajdu-Cronin et al., 1999; Schade et al., 2005; Reynolds et al., 2005; Charlie et al., 2006a; Williams et al., 2007; Matsuki et al., 2006).

$G\alpha_s$ null mutations are lethal (Korswagen et al., 1997), but mutations that decrease $G\alpha_s$ signaling cause a sluggish, near-paralyzed locomotion phenotype similar to that of $G\alpha_q$ loss-of-function mutants (Moorman and Plasterk, 2002; Reynolds et al., 2005). Mutations and transgenes that increase $G\alpha_s$ signaling induce a smoothly sinusoidal hyperactive phenotype, distinctly different from the deep body bends seen in $G\alpha_o$ loss-of-function or $G\alpha_q$ gain-of-function mutants (Reynolds et al., 2005; Schade et al., 2005; Charlie et al., 2006a).

3.2.4. The neuromuscular junction and the egg-laying synapse are often used to study neural G protein signaling in *C. elegans*

While $G\alpha_o$, $G\alpha_q$, and $G\alpha_s$ signaling affect many *C. elegans* behaviors, locomotion and egg-laying behaviors are two readouts of neural G protein signaling that have been frequently used to carry out genetic studies of the mechanism of neural G protein signaling due to the unique advantages of each.

Egg-laying behavior depends on release of serotonin and other neurotransmitters from the HSN motor neuron. $G\alpha_o$ and $G\alpha_q$ have opposing effects on HSN neurotransmitter release (Tanis et al., 2008), and mutations in their signaling pathways result in easily scored and quantitated defects in egg laying (Chase and Koelle, 2004). Thus it has been possible to carry out genetic screens for mutants that are defective or hyperactive for egg-laying behavior to isolate mutants with defects in $G\alpha_o$ and $G\alpha_q$ signaling (Trent et al., 1983; Desai and Horvitz, 1989; Bany et al., 2003). Such mutants were used to originally discover and subsequently characterize the Regulators of G protein Signaling (RGS proteins) that inhibit most neural G protein signaling in *C. elegans* and in mammals (Koelle and Horvitz, 1996; Hajdu-Cronin et al., 1999). The neural circuit that controls egg laying is particularly simple and well-characterized, and tools to express transgenes in any cell of the circuit, to monitor activity of the circuit in freely-behaving animals with the fluorescent Ca^{2+} indicator GCaMP, and to optogenetically manipulate the circuit have all been developed (Schafer, 2006; Emtage et al., 2012; Collins and Koelle, 2013).

C. elegans locomotion behavior involves release of acetylcholine from ventral cord motor neurons onto body wall muscles, and $G\alpha_o$, $G\alpha_q$, and $G\alpha_s$ all affect acetylcholine release at this neuromuscular junction. The drug aldicarb paralyzes worms by preventing acetylcholine released at this neuromuscular junction from being degraded, and a powerful genetic screen for mutants resistant to aldicarb paralysis has been used to isolate mutants with decreased acetylcholine release that lie in the $G\alpha_o$ and $G\alpha_q$ signaling pathways (Miller et al., 1996; Miller et al., 1999; Miller et al., 2000). Screens for suppressors of aldicarb-resistant mutants and screens for mutants with hyperactive locomotion were used to isolate mutants with increased signaling in the $G\alpha_s$ and $G\alpha_q$ pathways (Miller et al., 1999; Schade et al., 2005; Charlie et al., 2006b). Mutations that affect acetylcholine release were used to discover the RIC-8 protein that promotes signaling by heterotrimeric G proteins in *C. elegans* and in mammals (Miller et al., 2000). The cholinergic neuromuscular junctions in the ventral cord have been used for important experiments visualizing the effects of G protein signaling mutations on the localization of synaptic vesicle release proteins (Lackner et al., 1999; Nurrish et al., 1999; Chan et al., 2012). These neuromuscular junctions are also the most accessible synapses for electrophysiological studies in *C. elegans*, and have allowed studies of the fine details of G protein signaling mutations on neurotransmitter release (Hu et al., 2015).

While mutations in the $G\alpha_o$, $G\alpha_q$ and $G\alpha_s$ signaling pathways have powerful effects on egg-laying behavior by affecting neurotransmitter release from the HSN neurons and on locomotion by affecting acetylcholine release from ventral cord motor neurons, it has been hard to identify the GPCRs that activate the G proteins in these neurons. This difficulty is in line with the understanding, described in Section 2, that an individual neuron appears to express many different GPCRs so that mutations in a single GPCR are expected to have very weak effects compared to mutations in a G protein. In the HSN neuron, the EGL-6 neuropeptide receptor signals through $G\alpha_o$ to inhibit egg laying, but this effect is only obvious in gain-of-function EGL-6 mutants, whereas EGL-6 null mutants do not have detectable defects. In the ventral cord motor neurons, the neuropeptide receptor CKR-2 and the G protein coupled acetylcholine receptor GAR-3 both appear to signal through $G\alpha_q$ to increase acetylcholine release (Hu et al., 2011; Chan J.P. et al., 2013), while a different G protein coupled acetylcholine receptor GAR-2, the heterodimeric G protein coupled $GABA_B$ receptor GBB-1/GBB-2, and the dopamine receptor DOP-3 all appear to signal through $G\alpha_o$ to decrease acetylcholine release (Chase et al., 2004; Dittman and Kaplan, 2008). Mutations in these ventral cord motor neuron GPCRs have detectable effects on locomotion (Chase et al., 2004; Dittman and Kaplan, 2008; Hu et al., 2011; Chan J.P. et al., 2013), but knocking out a single one of these GPCRs, as expected, has much weaker effects than does knocking out its downstream G protein (Mendel et al., 1995; Ségalat et al., 1995; Brundage et al., 1996).

3.3. The mechanism of $G\alpha_q$ signaling

In mammalian cells, active $G\alpha_q$-GTP is classically known to signal by directly binding to and activating its effector, the transmembrane enzyme phospholipase Cβ (PLCβ), which hydrolyzes the membrane lipid phosphatidylinositol 4,5 bisphosphate (PIP_2) to generate the soluble molecule inositol 1,4,5 trisphosphate (IP_3) and the membrane lipid diacylglycerol (DAG). IP_3 can go on to increase intracellular Ca^{2+}, which along with DAG can bind and activate protein kinase C (Figure 3; reviewed by Sánchez-Fernández et al., 2014). Prior to studies of $G\alpha_q$ signaling in *C. elegans*, the only confirmed direct $G\alpha_q$ effector was PLCβ. Genetic analysis of $G\alpha_q$ signaling in *C. elegans* (previously reviewed by Perez-Mansilla and Nurrish, 2009) supported the idea that PLCβ (EGL-8 in *C. elegans*) is a physiologically important effector (Lackner et al., 1999, Miller et al., 1999; Bastiani et al., 2003). However, these same studies also suggested that there had to be additional $G\alpha_q$ effector(s) since the phenotypes of PLCβ null mutants were much less severe than those of $G\alpha_q$ null mutants.

The missing $G\alpha_q$ effector was identified as the Trio RhoGEF through genetic screens for suppressors of the slow growth and hyperactive locomotion phenotypes caused by excessive $G\alpha_q$ pathway activity (Williams et al., 2007). Analysis using loss- and gain-of-function mutants in various double-mutant combinations showed that for control of growth, locomotion, and egg laying, $G\alpha_q$ signals through both PLCβ and Trio RhoGEF. Knocking out either effector alone only partially blocks $G\alpha_q$ signaling, while knocking out both appears to completely block $G\alpha_q$ signaling (Williams et al., 2007). Trio RhoGEF proved to be as or more important than the classical PLCβ effector pathway for multiple effects of $G\alpha_q$ signaling, including effects on growth, locomotion, and egg laying. Concomitant with the discovery of Trio RhoGEF as a $G\alpha_q$ effector in *C. elegans*, biochemical and structural studies demonstrated that mammalian $G\alpha_q$ binds and activates the orthologous mammalian RhoGEF proteins (Lutz et al., 2007; Rojas et al., 2007).

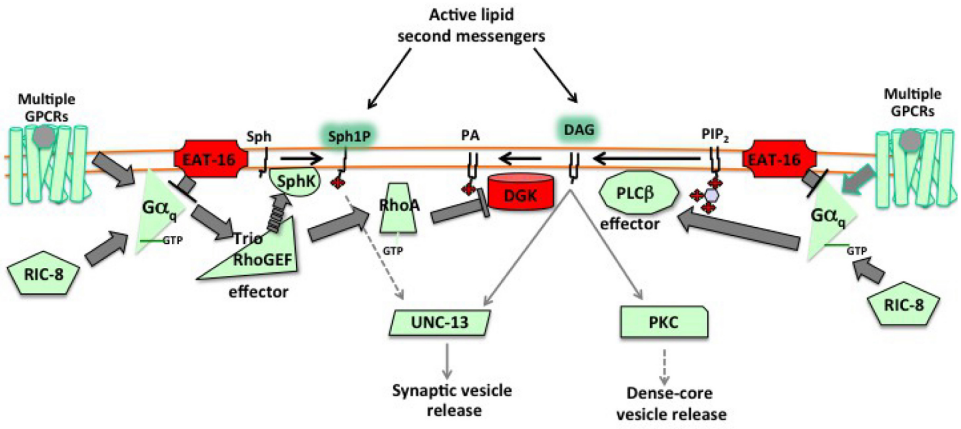


Figure 3. Mechanism of Gαq signaling. Gαq is induced to bind GTP to generate its active form by GPCRs, and induced to hydrolyze GTP to generate its inactive form by the RGS protein EAT-16. RIC-8 promotes Gαq signaling by acting as a chaperone to stabilize Gαq protein and/or by acting as a non-receptor activator to promote GTP binding by Gαq. These proteins are drawn at both left and right to facilitate showing the two Gαq effector pathways at center. Left pathway: Gαq-GTP activates Trio RhoGEF, which in turn activates sphingosine kinase (SphK) to generate the lipid second messenger sphingosine 1-phosphate (Sph1P). Trio RhoGEF also increases levels of another lipid second messenger diacylglycerol (DAG) by activating RhoA to prevent diacylglycerol kinase (DGK) from converting DAG to phosphatidic acid (PA). Right pathway: Gαq-GTP activates phospholipase Cβ (PLCβ) to generate the second messengers DAG and (not shown) IP_3. Sph1P and DAG each help UNC-13 to relocalize to synaptic membranes and to promote synaptic vesicle release. DAG also activates protein kinase C (PKC) to promote dense-core vesicle release. Solid grey arrows (or bars), activation (or inhibition) via direct physical interactions. Dashed arrows or bars, indirect or poorly understood effects. Light green, proteins that promote Gαq signaling; red, proteins that inhibit Gαq signaling.

Two mechanisms for signaling downstream of the Trio RhoGEF effector of Gαq have been identified based on *C. elegans* genetics (Figure 3). First, while PLCβ enzyme activity directly produces DAG, Trio RhoGEF can also increase DAG levels by an indirect mechanism. Trio RhoGEF activates the RhoA protein RHO-1, allowing it to directly bind and inhibit the diacylglycerol kinase DGK-1, blocking the first step in metabolic clearance of DAG, its phosphorylation to produce phosphatidic acid (PA) (McMullan et al., 2006). Thus both effectors for Gαq collaborate to increase DAG levels. Although Rho activity in mammals is principally known to regulate other processes, such as actin cytoskeleton dynamics and transcription, *C. elegans* genetics demonstrates that in its role mediating neurotransmitter signaling downstream of Gαq, a major physiologically significant role of Rho seems to be increasing DAG levels.

A second downstream mediator of the effects of Gαq/RhoGEF/RhoA signaling is the sphingosine kinase SphK (SPHK-1 in *C. elegans*). SphK catalyzes the phosphorylation of the membrane lipid sphingosine (Sph) to sphingosine-1-phosphate (Sph1P), a signaling lipid that among other functions promotes neurotransmitter release (Okada et al., 2009). In ventral cord cholinergic neurons, Gαq signaling via the Trio RhoGEF UNC-73 causes an increase in the perisynaptic localization of SphK, and this in turn appears to increase acetylcholine release (Chan et al., 2012). In a separate study, it was found that Gαq signaling recruits SphK to synapses via calcium influx using a mechanism involving the calcium binding protein CIB (CALM-1 in *C. elegans*) (Chan et al., 2012). The ability of SphK to increase neurotransmitter release depends on both its localization to perisynaptic regions and its ability to generate sphingosine 1-phosphate (Chan et al., 2012). At this point, the details of the mechanism by which Trio RhoGEF leads to recruitment of SphK to perisynaptic regions, and specifically the relationship between the calcium and Trio RhoGEF effects on Sphk localization, remain to be determined.

How does the second messenger DAG produced by Gαq signaling lead to the ultimate effect of Gαq signaling, an increase in neurotransmitter release? Two DAG- binding proteins appear to mediate independent effects. One of these, UNC-13, is a protein essential for synaptic vesicle priming that facilitates neurotransmitter release by binding to the SNARE protein complexes that mediate vesicle fusion (James and Martin, 2013). Binding of UNC-13 to DAG in the membrane may help recruit it to synaptic membranes. The evidence for Gαq acting through UNC-13 includes the observations that in ventral cord motor neurons, Gαq activation of acetylcholine release depends on UNC-13, and that Gαq activation or addition of DAG analogs induces overexpressed UNC-13S::GFP to relocalize from a diffuse pattern in axons to punctate structures at synapses (Lackner et al., 1999). These effects can be abrogated by a mutation that blocks the ability of overexpressed UNC-13S::GFP to bind DAG. Recent electrophysiological studies provide perhaps the strongest evidence that UNC-13 functions in Gαq signaling to promote acetylcholine release from ventral cord motor neurons, with both the long and short isoforms of UNC-13 differentially affecting tonic and evoked release (Hu et al., 2015). Nevertheless, the extent to which UNC-13 mediates the behavioral effects of Gαq signaling has remained unclear. The recent development of methods for

precise genome engineering using CRISPR/Cas9 provide an opportunity to mutate the DAG binding site of native UNC-13 and potentially resolve this issue.

A second DAG binding protein that mediates effects of $G\alpha_q$ signaling in ventral cord motor neurons is protein kinase C (PKC) (Sieburth et al., 2007). PKC appears to affect release of neuropeptide-containing dense-core vesicles, although the targets via which PKC does this remain unknown. PKC does not appear to directly affect release of acetylcholine from small-clear synaptic vesicles. UNC-13 appears to affect small-clear vesicles, and there are differences of opinion as to whether it directly affects dense-core vesicles release (Sieburth et al., 2007; Speese et al., 2007). Thus the current model is that $G\alpha_q$ signaling produces DAG to act via two different DAG-binding proteins, PKC and UNC-13, that together activate release of dense-core and synaptic vesicles (Figure 3).

A key observation about the role of DAG in $G\alpha_q$ signaling is that treating $G\alpha_q$ mutant worms with the DAG analogs known as phorbol esters rescues the paralysis of $G\alpha_q$ null and strong reduction-of-function mutants to wild-type levels of coordinated locomotion (Reynolds et al., 2005; Williams et al., 2007). The effectiveness of such non-localized phorbol ester treatment suggests that DAG is a "licensing factor" that promotes neurotransmitter release, i.e., that DAG need not be produced focally at synapses. Further, the fact that $G\alpha_q$ mutants can be rescued by phorbol ester treatment demonstrates that developmental defects do not contribute to the strong paralysis of $G\alpha_q$ strong loss-of-function or null mutants.

How does the other second messenger produced by $G\alpha_q$ signaling, sphingosine 1-phosphate, increase neurotransmitter release? An initial study suggested the effect of $G\alpha_q$ on neurotransmitter release via SphK appeared to be entirely independent and parallel to the ability of $G\alpha_q$ signaling to increase neurotransmitter release via increasing DAG levels (Chan et al., 2012). However, a subsequent study suggested SphK is essential for $G\alpha_q$ signaling to relocalize UNC-13S::GFP to synapses (Chan and Sieburth, 2012), and UNC-13S::GFP relocalization also appears to depend on DAG (Lackner et al., 1999). So, more work remains to clarify the relationship of DAG and SphK signaling downstream of $G\alpha$ in regulating neurotransmitter release.

Two upstream regulators of $G\alpha_q$ were identified through *C. elegans* genetic analysis. The Regulator of G protein signaling (RGS) protein EAT-16 behaves as a specific inhibitor of $G\alpha_q$ signaling (Hajdu-Cronin et al., 1999). The RIC-8 protein promotes $G\alpha_q$ signaling (Miller et al., 2000). I will elaborate on studies of RGS proteins and RIC-8 in Section 3.6 and Section 3.7.

3.4. The mechanism of $G\alpha_s$ signaling

The $G\alpha_s$ signaling pathway (Figure 4) has been intensively studied for decades in mammalian cells using biochemical approaches (Godinho et al., 2015). $G\alpha_s$ activated by GPCRs binds and activates its effector, the transmembrane enzyme adenylyl cyclase, which generates the second messenger cyclic AMP (cAMP). cAMP binds the regulatory subunits of protein kinase A (PKA), causing them to dissociate from and thus activate the catalytic subunit, which can then phosphorylate various target proteins. Signaling is terminated in part through the action of the enzyme phosphodiesterase to hydrolyze cAMP. $G\alpha_s$ is unique among $G\alpha$ isoforms in that no RGS protein is known to act on $G\alpha_s$ to help it hydrolyze GTP, so the slow intrinsic GTPase activity of $G\alpha_s$ is apparently used to terminate $G\alpha_s$ activity.

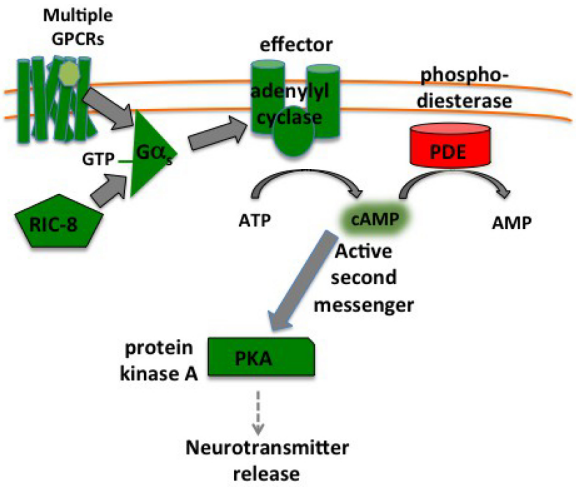


Figure 4. Mechanism of Gαs signaling. Gαs is induced to bind GTP to generate its active form by GPCRs. RIC-8 promotes signaling, either via its Gα chaperone function or as a non-receptor activator. Gαs-GTP inactivates itself (not shown) via its slow intrinsic GTPase activity. Gαs-GTP activates the transmembrane enzyme adenylyl cyclase to generate the second messenger cyclic AMP (cAMP), which ultimately is hydrolyzed by phosphodiesterase. cAMP binds the regulatory subunits of protein kinase A (PKA), causing them to release the active subunit. PKA phosphorylation of unknown target proteins activates release of both synaptic and dense-core vesicles to increase neurotransmitter release. Solid grey arrows indicate direct physical interactions. Dashed arrow indicates a poorly understood effect. Green, proteins that promote Gαs signaling; red, proteins that inhibit Gαs signaling.

Genetic studies show that the same pathway delineated by biochemical studies in mammals also operates in *C. elegans* motor neurons (Figure 4; Schade et al., 2005). Initial studies showed that transgenic overexpression of constitutively-active Gαs kills neurons through a cAMP-dependent mechanism, as the killing could be suppressed with mutations in the adenylyl cyclase ACY-1 (Korswagen et al., 1997; Berger et al., 1998; Moorman and Plasterk, 2002). Gαs null mutants are lethal (Korswagen et al., 1997), but mutants with increased Gαs signaling are viable, and have provided important tools for genetic analysis of Gαs signaling. Mutations that increase Gαs signaling have been identified in Gαs itself (GSA-1 in *C. elegans*), adenylyl cyclase (ACY-1), protein kinase A (loss of the regulatory subunit KIN-2 causes constitutive activity of the catalytic subunit KIN-1), and in phosphodiesterase (PDE-4) (Schade et al., 2005; Charlie et al., 2006b). Increased Gαs signaling results in smoothly hyperactive sinusoidal locomotion, which is distinct from the hyperactive locomotion with abnormally deep body bends induced by loss of Gαo signaling or increased Gαq signaling (Schade et al., 2005). Epistasis analysis using Gαs signaling pathway mutants supports the idea that Gαs in *C. elegans* functions in a pathway analogous to the mammalian Gαs signaling pathway that had been characterized biochemically (Schade et al., 2005; Reynolds et al., 2005).

Gαs pathway activity requires the guanine nucleotide exchange factor RIC-8, (Reynolds et al., 2005), although it remains unclear if this is due to the function of RIC-8 as chaperone or due to RIC-8 acting as a non-receptor nucleotide exchange protein.

The ultimate effect of Gαs signaling in neurons appears to be to promote neurotransmitter release. Schade et al. (2015) showed that activating Gαs signaling in both neurons and muscles using gain-of-function mutations appears to induce acetylcholine release from motor neurons, although Reynolds et al. (2005) showed that loss of *acy-1* just in neurons did not seem to affect neurotransmitter release. Separate studies in ALA neurons show that PKA activation can induce dense-core vesicle release (Zhou et al., 2007), so Gαs signaling may be able to activate neurotransmitter release via both synaptic and dense-core vesicles. The downstream targets of phosphorylation by PKA that increase vesicle release remain unknown.

Gαs signaling may operate in muscles, in addition to neurons, to control *C. elegans* behavior. Null mutants for the adenylyl cyclase ACY-1 show two separable defects: larval arrest and near-paralysis. In an *acy-1* null mutant, re-expression of *acy-1* in either muscles or neurons can rescue the larval arrest, but neither is sufficient to rescue paralysis, indicating that *acy-1* may be required in both neurons and muscles for proper locomotion (Reynolds et al., 2005). Rescue experiments also indicate that Gαs signaling is required for adult neural function, but is not required for neural development, since induced expression of *acy-1* in paralyzed animals lacking neural *acy-1* can restore locomotion behavior in these animals (Reynolds et al., 2005).

Genetic studies initiated to study Gαs signaling have led to a broader genetic analysis of the *in vivo* functions of cAMP in *C. elegans*. While the adenylyl cyclase ACY-1 mediates all known effects of Gαs signaling (Schade et al., 2005), there are other adenylyl cyclase isoforms in *C. elegans* that appear to generate a Gαs-independent pool of

cAMP that also affect locomotion and larval growth (Charlie et al., 2006b). cAMP is an important negative regulator of sleep-like states throughout the animal kingdom (Zimmerman et al., 2008), and $G\alpha_s$ pathway activating mutants are being used to study sleep-like states in *C. elegans* (Belfer et al., 2013). Ghosh-Roy et al., (2010) has also used $G\alpha_s$ pathway mutants to study the role of cAMP in axon regeneration.

3.5. The mechanism of $G\alpha_o$ signaling

The mechanism of $G\alpha_o$ signaling (Figure 5) is poorly understood and remains an area ripe for further investigation using the *C. elegans* system. Biochemical studies in mammals have shown that $G\alpha_o$ is expressed throughout the nervous system and is by orders of magnitude the most abundant $G\alpha$ protein in neurons, constituting ~1.5% of membrane protein in the brain (Sternweis and Robishaw, 1984). Despite efforts to identify $G\alpha_o$ binding proteins that might function as effectors for $G\alpha_o$ or its paralogs (Chen et al., 1999; Takesono et al., 1999; Cuppen et al., 2003), no such protein has been validated as a bona fide effector of $G\alpha_o$. Activation of $G\alpha_o$ releases $G\beta\gamma$ subunits, allowing them to activate specific K^+ channels and inhibit specific Ca^{2+} channels, resulting in inhibition of neural activity (Reuveny et al., 1994; Herlitze et al., 1996).

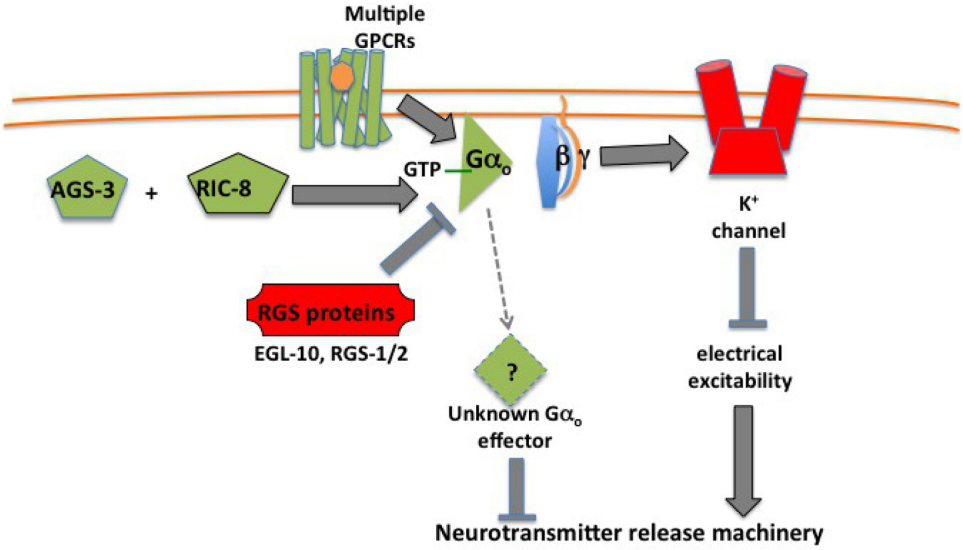


Figure 5. Mechanism of $G\alpha_o$ signaling. $G\alpha_o$ is induced to bind GTP to generate its active form by GPCRs, or by the non-receptor activators AGS-3 and RIC-8. Alternatively, or in addition, RIC-8 may function as a chaperone for $G\alpha_o$. $G\alpha_o$-GTP may signal to inhibit the neurotransmitter release machinery by an unknown effector protein. The $G\beta\gamma$ subunits released from $G\alpha_o$ can activated specific K^+ channels to reduce electrical excitability, which in turn facilitates neurotransmitter release. Green, proteins that promote $G\alpha_o$ signaling; red, proteins that inhibit $G\alpha_o$ signaling.

The ultimate effect of $G\alpha_o$ signaling in *C. elegans* neurons is to inhibit neurotransmitter release, and this has been studied in detail in ventral cord motor neurons, where $G\alpha_o$ signaling inhibits acetylcholine release (Nurrish et al., 1999; Miller et al., 1999; Charlie et al., 2006a; Vashlishan et al., 2008), and in HSN neurons, where $G\alpha_o$ signaling inhibits release of serotonin and other neurotransmitters that stimulate egg laying (Tanis et al., 2008). $G\alpha_q$ and $G\alpha_o$ signaling have the opposite effects on locomotion, egg laying, and a variety of other behaviors (Movie 1), suggesting that in general, while $G\alpha_q$ signaling promotes neurotransmitter release (see Section 3.2), $G\alpha_o$ has an opposing effect of inhibiting neurotransmitter release.

Activation of $G\alpha_q$ signaling in ventral cord motor neurons appears to increase the localization of the synaptic vesicle priming protein UNC-13S to presynaptic termini, as seen by visualizing a fusion of UNC-13S to the green fluorescent protein (Lackner et al., 1999). A $G\alpha_o$ loss-of-function mutation also causes an increase in localization of this UNC-13S::GFP protein to presynaptic termini (Nurrish et al., 1999). This result suggests that that $G\alpha_q$ and $G\alpha_o$ oppose each other at a mechanistic level. The effects of $G\alpha_q$ and $G\alpha_o$ on UNC-13S::GFP localization both depend on the diacylglycerol (DAG) binding site of UNC-13 (Lackner et al., 1999; Nurrish et al., 1999). Thus it is assumed that the increase in DAG levels produced by $G\alpha_q$ signaling cause the observed changes in UNC-13S::GFP localization. $G\alpha_o$ signaling could affect UNC-13::GFP and inhibit neurotransmitter release by decreasing DAG levels (Nurrish et al., 1999). However, no biochemical experiments have demonstrated any effect of $G\alpha_o$ on DAG or on the enzymes that generate or degrade DAG (Jose and Koelle, 2005; Perez-Mansilla and Nurrish, 2009).

While genetic studies in *C. elegans* have not clarified the downstream mechanism of $G\alpha_o$ signaling, they have identified novel upstream regulators of $G\alpha_o$. The RGS protein EGL-10 is expressed in all neurons, and EGL-10 null

mutants show sluggish locomotion and egg-laying defects due to increased Gαo signaling (Koelle and Horvitz, 1996). There are other RGS proteins (RGS-1, RGS-2, RGS-7) that additionally inhibit Gαo in specific cell types or under specific circumstances (see Section 3.7). Gαo signaling is promoted by RIC-8, working in combination with a GPR-domain containing protein (Section 3.6). As in the case of RIC-8's effect on Gαs signaling (Section 3.3), it is unclear whether RIC-8's effect on Gαo signaling reflects its nucleotide exchange activity, its Gα chaperone function (Gabay et al., 2011; Chan P. et al., 2013), or both.

3.5.1. Does Gαo have an effector?

One view is that Gαo has no effector, and that Gαo signaling occurs simply by release of Gβγ subunits that would then be entirely responsible for further downstream signaling by regulating K^+ and Ca^{2+} channels. The plausibility of such a scheme is bolstered by careful genetic studies of heterotrimeric G protein signaling in yeast, in which mating pheromone signaling through a heterotrimeric G protein is indeed carried out by activation of a Gα protein whose principal function is simply to release Gβγ so that Gβγ can induce further signaling events (Dohlman and Thorner, 2001).

Genetic studies in *C. elegans* strongly suggest that Gαo signaling does not proceed purely through Gβγ effectors, and that Gαo must also signal directly through its own yet-to-be-discovered effectors. The only study of Gβγ effectors in *C. elegans* showed that when the EGL-6 neuropeptide receptor activates Gαo in the HSN neurons, the resulting inhibition of egg laying requires IRK-1, a homolog of mammalian Gβγ-activated K^+ channels, and that no other such K^+ channel is required (Emtage et al., 2012). However, this same study also demonstrated that IRK-1 does not mediate inhibition of egg laying when other methods are used to activate Gαo, suggesting that other effectors besides K^+ channels must mediate Gαo signaling. This study leaves open the possibility that these other effectors could be additional Gβγ effectors such as Ca^{2+} channels. However, other genetic results argue in favor of effectors for Gαo itself.

First, Gαq and Gαo release the identical Gβγ subunits, as there is only one Gβγ isoform generally expressed in *C. elegans* neurons, yet activation of Gαq and Gαo, acting in the same neurons, have exactly opposite effects on neurotransmitter release and behavior (Lackner et al., 1999; Nurrish et al., 1999; Tanis et al., 2008). To argue that Gαo signaling principally results from released Gβγ inhibiting neural activity, one needs to explain how Gαq signaling does not give the same result. This discrepancy might result from the far greater abundance of Gαo, or by supposing that Gαo and Gαq reside in different membrane microdomains, but so far there is no actual data to support either idea.

Second, the genetics of Gαo signaling in *C. elegans* does not match the genetics of Gβγ-mediated pheromone signaling pathway in yeast. In the yeast pheromone signaling pathway, a null mutant for the Gα subunit results in constitutive signaling, as Gα is no longer present to sequester Gβγ and inhibit its ability to signal (Whiteway et al., 1989). Conversely, transgenic overexpression of yeast Gα inhibits pheromone signaling, apparently by increasing the pool of Gα available to associate with and inhibit Gβγ (Cole et al., 1990). The analogous experiments in *C. elegans* with Gαo give precisely the opposite results. Gαo null mutations appear to result in a loss of signaling, not constitutive signaling (Mendel et al., 1995; Ségalat et al., 1995), and overexpression of wild-type Gαo appears to result in constitutive signaling, not loss of signaling (Mendel et al., 1995). These results are exactly analogous to those obtained from genetic studies of Gαq in *C. elegans* (Brundage et al., 1996), a case in which it is clear that downstream signaling is due to Gα effectors, not Gβγ effectors.

Could it be that Gαo signals by directly binding to and inhibiting adenylyl cyclase? Adenylyl cyclase is the effector directly activated by Gαs (see Section 3.3.), and it can also be directly bound and inhibited by members of the Gαi/o family of Gα proteins, at least in cultured cells. Indeed, Gαo overexpressed in cultured cells has been shown to inhibit one isoform of adenylyl cyclase (Näsman et al., 2002). While this hypothesis is intriguing, there is currently no genetic evidence that inhibition of adenylyl cyclase is a physiologically relevant mechanism for Gαo signaling. If Gαo signaled by inhibiting adenylyl cyclase, we might expect that Gαo and Gαs signaling would show precisely opposite effects in *C. elegans* genetic experiments. However, this is not the case (see Section 3.3), and Gαo signaling rather shows far more precise opposition to the effects of Gαq signaling.

Could it be that Gαo signals by directly binding to and regulating one of the components of the Gαq signaling pathway, thus neatly solving the problem of identifying the Gαo effector and explaining how Gαo signaling opposes Gαq signaling (Section 3.5)? Two specific Gαq signaling pathway proteins (Figure 3) have been proposed as Gαo effectors: DGK and EAT-16. First, all genetic studies are consistent with the possibility that Gαo could signal by activating diacylglycerol kinase (DGK), the enzyme that terminates Gαq signaling by destroying the Gαq second messenger diacylglycerol. However, all efforts to demonstrate a biochemical effect of Gαo on DGK activity have

proven negative (Jose and Koelle, 2005; Perez-Mansilla and Nurrish, 2009). The second hypothesis is that the effector for Gαo could be the Gαq-specific RGS protein EAT-16. An elegant model based on both genetic analysis and structural features of the EAT-16 protein complex proposes that inactive Gαo sequesters EAT-16, and activation of Gαo releases EAT-16 to inhibit Gαq (Robatzek et al., 2001). However, no biochemical studies of either the *C. elegans* proteins nor their mammalian equivalents has produced any support for this model.

If Gαo indeed signals directly through its own effectors, why have these effectors not yet been identified? Known effectors for other G proteins are often multi-subunit membrane protein complexes, and the cDNA expression library screening methods that have been employed in attempts to identify Gαo effectors (Chen et al., 1999; Takesono et al., 1999; Cuppen et al., 2003) are not well suited for finding such proteins. Genetic screens in *C. elegans* for Gαo signaling proteins would have failed to identify Gαo effectors if there are multiple redundant effectors, such that mutating a single effector gene causes little reduction in Gαo signaling. In addition, if a Gαo effector is essential for viability or reproduction, mutating such an effector would not produce animals that could be detected and recovered in a genetic screen. Because there are perfectly reasonable explanations why a Gαo effector may have eluded discovery, the absence of such a discovery at this point should not be taken as evidence that Gαo effectors do not exist.

3.6. The relationship between Gαq, Gαs, and Gαo signaling

Gαq, Gαs, and Gαo signaling all operate at the same time in the same neurons, and Figure 2 presents a model showing them acting in parallel to each other until they intersect downstream by ultimately all regulating neurotransmitter release. However, genetic epistasis experiments have been used to examine the relationship between the three G protein signaling pathways, and other relationships between the G protein signaling pathways are also consistent with these genetic results.

Double mutants for Gαq and Gαo resemble Gαq single mutants, and this and results from other double-mutant combinations are consistent with the interpretation that Gαo signaling acts to inhibit Gαq signaling (Hajdu-Cronin et al., 1999; Miller et al., 1999; Charlie et al., 2006a). More specifically, this means that Gαo signaling could inhibit the Gαq pathway at any level, upstream of Gαq, at the level of Gαq, or at any level downstream of Gαq. Only molecular experiments will ultimately be able to distinguish between these possibilities.

The relationship between Gαs and Gαq signaling has been investigated by combining mutations that increase signaling in one pathway with mutations that block signaling in the other pathway (Reynolds et al., 2005; Charlie et al., 2006a). This was done in both directions (i.e., a Gαs pathway gain-of-function mutation combined with a Gαq null mutation, and also a Gαq gain-of-function mutation combined with a Gαs pathway null mutation). The results of these experiments do not yield a simple epistatic relationship between the two signaling pathways, but do yield several important insights. First, the Gαs pathway is virtually completely dependent on the Gαq pathway to exert its effects on locomotion (Reynolds et al., 2005). Second, the Gαq pathway can still promote locomotion in the absence of a functional Gαs pathway, but the resulting locomotion is uncoordinated (Reynolds et al., 2005). Third, phorbol esters (analogs of the DAG ultimately produced by the Gαq pathway) can also promote uncoordinated locomotion in the absence of a functional Gαs pathway, suggesting Gαs signaling regulates locomotion downstream of DAG production (Reynolds et al., 2005). Fourth, the Gαq and Gαs pathways have distinct effects in driving locomotion, one line of evidence for which is that loss of Gαq signaling appears to strongly reduce acetylcholine release from motor neurons while loss of Gαs signaling does not (Charlie et al., 2006a).

3.7. Receptor-independent activation of heterotrimeric G proteins

An important contribution of the *C. elegans* system was the discovery of the receptor-independent Gα chaperone and activator RIC-8, which was first identified in a genetic screen for mutants with reduced acetylcholine release from ventral cord motor neurons (Miller et al., 1996). Genetic studies showed that reduction of function mutations in RIC-8 and Gαq have similar phenotypes and were consistent with RIC-8 acting upstream of Gαq to promote Gαq signaling (Miller et al., 2000). Subsequent genetic studies demonstrated that RIC-8 also appears to promote Gαs signaling (Reynolds et al., 2005) and Gαo signaling (Miller and Rand, 2000; Hofler and Koelle, 2011).

Biochemical studies of mammalian RIC-8 have identified two mechanisms by which RIC-8 can promote Gα signaling. First, RIC-8 can act as a nucleotide exchange factor to convert inactive Gα-GDP to active Gα-GTP (Tall et al., 2003; Chan et al., 2011). Unlike GPCRs, which are transmembrane proteins that act as nucleotide exchange factors to activate Gαβγ heterotrimers, RIC-8 is a soluble protein and will not act on the heterotrimer, but rather can only activate free Gα-GDP. This leads to the model depicted in Figure 1 in which a Gαβγ heterotrimer would need

to initially be activated by a GPCR to generate Gα-GTP, but after GTP hydrolysis the inactive Gα-GDP produced might be reactivated by the nucleotide exchange activity of RIC-8, thus prolonging signaling.

The second mechanism by which RIC-8 can activate Gα signaling is by simply stabilizing Gα proteins. Knocking out RIC-8 in mammalian cells causes dramatic reductions in the levels of Gα proteins (Gabay et al., 2011). Biochemical studies show RIC-8 acts as a chaperone to help fold nascent Gα proteins (Chan P. et al., 2013). It has not yet been determined if RIC-8 mutations in *C. elegans* similarly cause reductions of Gα protein levels. The genetics of RIC-8 are consistent with RIC-8 promoting Gα signaling either by simply stabilizing Gα proteins, by acting as a nucleotide exchange factor, or by both mechanisms.

Another type of receptor-independent activator for $G\alpha_o$ are proteins containing a short motif that binds specifically to Gα proteins of the $G\alpha_{i/o}$ subfamily and that is known either as the GPR (G protein regulatory) or GoLoco motif (Siderovski et al., 1999; Peterson et al., 2000). *C. elegans* has three such proteins. The two very similar and functionally redundant GPR-1 and GPR-2 proteins act in early embryonic cells with $G\alpha_o$ to regulate spindle positioning during asymmetric cell divisions (Colombo et al., 2003; Gotta et al., 2003; Srinivasan et al., 2003). The AGS-3 protein acts with $G\alpha_o$ to regulate function of adult neurons (Hofler and Koelle, 2011). Biochemical studies of mammalian GPR/GoLoco proteins emphasize the fact that when these proteins bind Gα-GDP, they inhibit nucleotide exchange, which would suggest that they prevent formation of active Gα-GTP and thus inhibit signaling (Siderovski et al., 1999; Peterson et al., 2000). However, *C. elegans* genetic studies clearly demonstrate that precisely the opposite is true, since GPR-1/2 promote $G\alpha_o$ activity in embryonic cell divisions and AGS-3 also promotes $G\alpha_o$ signaling in adult neurons (Colombo et al., 2003; Gotta et al., 2003; Srinivasan et al., 2003; Hofler and Koelle, 2011). The apparent conflict between these results can be resolved by the findings that all known functions of the GPR/GoLoco proteins in *C. elegans* also require RIC-8, along with the biochemical result that RIC-8 can act as a nucleotide exchange factor on the complex between $G\alpha_o$-GDP and a GPR/GoLoco protein to produce active $G\alpha_o$-GTP (Thomas et al., 2008). Thus an attractive model is that when active $G\alpha_o$-GTP hydrolyzes GTP to become $G\alpha_o$-GDP, the function of the GPR/GoLoco protein is to bind $G\alpha_o$-GDP, preventing it from reassociating with Gβγ, which would have terminated signaling, and rather to present $G\alpha_o$-GDP to RIC-8 for nucleotide exchange to convert it back to active $G\alpha_o$-GTP (Figure 1). The net result would be to prolong signaling that had been initiated earlier by a GPCR.

The *C. elegans* GPR/GoLoco protein AGS-3 is of particular interest as it has a close mammalian brain ortholog, AGS3, and a role in mediating behavioral response to food deprivation. AGS-3 is expressed in most or all *C. elegans* neurons and is required for several different behavioral changes that normally occur in response to short-term food deprivation (Hofler and Koelle, 2011). Biochemical studies show that over the first few hours of food deprivation, AGS-3 protein from *C. elegans* lysates changes from a detergent-insoluble form to a detergent-solubilizable form, indicating that a physical change in the protein occurs. These results lead to a model in which food deprivation leads to a physical change in AGS-3, allowing it to activate $G\alpha_o$ signaling to induce behavioral changes appropriate for the food-deprived state of the animal. It will be fascinating to see if this role of AGS-3 in mediating response to food deprivation is conserved in mammals (Hofler and Koelle, 2012).

3.8. Regulators of G protein signaling inhibit $G\alpha_o$ and $G\alpha_q$ signaling

Another important contribution of the *C. elegans* system was the discovery and characterization of the family of Regulator of G protein Signaling (RGS) proteins. The first RGS protein, Sst2p, was identified in yeast (Dietzel and Kurjan, 1987), but it was not clear that it had any homologs in higher eukaryotes. Genetic studies identified the *C. elegans* RGS protein EGL-10 as an inhibitor of $G\alpha_o$ signaling, and comparison of Sst2p and EGL-10 allowed the discovery of an "RGS domain" conserved between the two and that is also found in a large family of proteins in *C. elegans* and mammals (Koelle and Horvitz, 1996). Biochemical studies showed that the RGS domain binds directly to Gα proteins, and by stabilizing the transition state for GTP hydrolysis, inactivates signaling by driving the conversion of active Gα-GTP to the inactive species Gα-GDP (Berman et al., 1996; Tesmer et al., 1997). GTPase activators were previously known for the small ras-like G proteins, which have structural similarity to the GTPase domain of Gα proteins but lack a catalytic arginine residue that Gα proteins use for GTP hydrolysis. Thus small G proteins are unable to convert GTP to GDP at a meaningful rate without first binding a GTPase activating protein that inserts an arginine residue into their active site (Li and Zhang, 2004). In contrast, Gα subunits of heterotrimeric G proteins were initially thought to not require help from any GTPase activating proteins to convert to their inactive GDP-bound state, since they have an intrinsic GTPase activity orders of magnitude faster than that of small GTPases. However, genetic studies in *C. elegans* show that *in vivo*, the $G\alpha_o$ and $G\alpha_q$ proteins require RGS proteins to appropriately terminate signaling (Koelle and Horvitz, 1996; Hajdu-Cronin et al., 1999). In contrast, neither genetic studies in worms nor biochemical studies of mammalian proteins have identified a GTPase activator for $G\alpha_s$. Thus $G\alpha_s$ may indeed simply use its slow intrinsic GTPase activity to terminate signaling.

The two most intensively studied RGS proteins in *C. elegans* are EGL-10 and EAT-16, which serve as specific inhibitors of $G\alpha_o$ and $G\alpha_q$ signaling, respectively (Koelle and Horvitz, 1996; Hajdu-Cronin et al., 1999). They are members of the R7 family of RGS proteins, of which there are four members in mammals that are expressed in the brain (Anderson et al., 2009). *In vitro*, these mammalian R7 proteins all preferentially inactivate $G\alpha_o$ rather than $G\alpha_q$, and it is unclear if any of them might actually be $G\alpha_q$ specific *in vivo*, like *C. elegans* EAT-16. Analysis of chimeras between EGL-10 and EAT-16 shows that the $G\alpha_o$ versus $G\alpha_q$ specificity of these proteins lies in an N-terminal domain that remains of unknown biochemical function (Patikoglou and Koelle, 2002). The *C. elegans* R7 RGS proteins, like their mammalian homologs, contain a Gγ-like domain that is constitutively bound to a Gβ-like protein known as $G\beta_5$ in mammals and GPB-2 in *C. elegans* (Chase et al., 2001; Robatzek et al., 2001; van der Linden et al., 2001). Thus the R7 RGS/Gβ complex in many respects resembles a Gβγ complex, but there is as yet no understanding of what this means or how it might be used. EAT-16, like the mammalian R7 RGS proteins, is anchored to the plasma membrane via a lipid-modified membrane anchoring subunit (Porter and Koelle, 2009). There is no known membrane anchor for EGL-10.

There are at least 13 RGS proteins in *C. elegans*, and the genes for all have been knocked out and studied (reviewed by Porter and Koelle, 2009). While the R7 family RGS proteins EGL-10 and EAT-16 are widely expressed, and mutants cause severe disruption of G protein signaling and behavior, the other RGS proteins appear to have much more specific functions. For example, RGS-3 is expressed only in a subset of sensory neurons, where it dampens signaling in response to strong stimuli, increasing the dynamic range for sensing these stimuli (Ferkey et al., 2007). RGS-1, RGS-2, and RGS-7 are all inhibitors of $G\alpha_o$, but have much more limited functions than does the main $G\alpha_o$ inhibitor EGL-10. RGS-7 is expressed in early embryos where it regulates the function of $G\alpha_o$ in controlling asymmetric cell divisions (Hess et al., 2004). RGS-1 and RGS-2 are very similar to each other and are functionally redundant (Dong et al., 2000). Knocking out both disrupts a specific behavioral response that occurs when starved animals are re-fed. Knockouts for a number of additional RGS genes so far have shown no detectable defects, suggesting that these RGS genes, like RGS-1 and RGS-2, may have redundant and/or very specific functions that will be difficult to define.

3.9. Reconciling studies of neural Gα signaling in *C. elegans* with those in more complex model organisms

The studies in *C. elegans* reviewed above show that the molecular details of the $G\alpha_q$, $G\alpha_s$, and $G\alpha_o$ signaling pathways, to the extent they are known, are the same in *C. elegans* versus the other invertebrate and vertebrate systems in which they have been studied, until we get down to the very most downstream outputs of these signaling pathways. The *C. elegans* work has been focused largely on the effects of the G proteins in ventral cord motor neurons on locomotion behavior and in the HSN neurons on egg-laying behavior. Using these two behaviors as readouts, the relevant outputs of signaling that have been identified are changes in the efficiency of the neurotransmitter release machinery. All the proteins involved in these changes are highly conserved across evolution, and functional studies in mammalian neurons support the idea that the very same mechanisms are used in mammalian brain to allow G protein signaling to modulate neurotransmitter release (Rhee et al., 2002; Wierda et al., 2007).

Studies of neural G protein signaling in more complex model organisms have focused on readouts of signaling very different from those used in *C. elegans*. When electrophysiological measurements have been used to measure signaling outcomes, the focus has been on the regulation of specific ion channels by Gβγ subunits (Reuveny et al., 1994; Herlitze et al., 1996), and indeed some of the effects of neural G protein signaling in *C. elegans* are mediated by such ion channels (Emtage et al., 2012). When effects on long-term memories have been the readout of signaling, the focus has been on the lasting effects of neural G protein signaling on synaptic structure or on transcription (Kandel, 2004). It is not yet fully clear whether neural G protein signaling in *C. elegans* results in these sorts of lasting changes. We do know that $G\alpha_q$ signaling in worms, as in mammals, results in activation of the Rho GTPase, whose best-studied function is as a regulator of the actin cytoskeleton, but it remains to be seen if $G\alpha_q$ signaling through Rho leads to functionally significant and lasting structural changes in *C. elegans* synapses. In *Aplysia* and mammals, $G\alpha_s$ signaling produces cAMP that activates the transcription factor CREB to induce changes in transcription that may underlie long-term memories (Kida et al., 2002; Kandel, 2012), although the relevance of CREB is less clear in *Drosophila* memory (Perazzona et al., 2004). *C. elegans* also has a CREB homolog that was shown to be essential for worms to form a long-term memory of a food-odorant association (Kauffman et al., 2010) and that mediates G protein regulation of gene expression (Suo et al,. 2006). Thus it seems likely that the mechanisms of neural G protein signaling are deeply conserved across evolution, but that the different types of behavioral readouts used to study such signaling in different model organisms tends to focus attention on different downstream effects of the signaling pathways on neural function.

4. Concluding remarks and future directions

Finally, I return to the simplified model presented in Figure 2 to illustrate an overview of what has been learned from studies of neurotransmitter signaling through heterotrimeric G proteins in *C. elegans*. As described in Section 2, a typical neuron expresses many GPCRs, and these receptors sense the levels of neurotransmitters that are released, not just from presynaptic partners to our typical neuron, but also from distant neurons and are thus acting extrasynaptically. Each neurotransmitter and its receptor are expressed in very specific and small sets of neurons, and the extrasynaptic communication between these cells is a crucial type of functional connection that goes beyond the physical synaptic connections between neurons and that is essential for understanding neural circuits. As described in Section 3, three different Gα proteins, $G\alpha_q$, $G\alpha_s$, and $G\alpha_o$, integrate signaling by the many GPCRs that may be active on a neuron at any one time. The major outcomes of neural G protein signaling studied in *C. elegans* are the opposing effects that $G\alpha_q$ and $G\alpha_o$ have to activate and inhibit the synaptic vesicle release machinery, respectively. $G\alpha_s$ signaling collaborates with the $G\alpha_q$ pathway to promote synaptic output. Other outcomes of Gα activation, such as regulation of K^+ and Ca^{2+} channels, or effects on neural structure or transcription, also seem to occur but are not responsible for the most obvious effects of the G proteins on *C. elegans* behavior. The neurotransmitters, receptors, G proteins, downstream signaling molecules, and mechanisms used in *C. elegans* are all conserved in mammals. The contribution of *C. elegans* studies has been to focus attention on the ubiquity of extrasynaptic signaling through GPCRs, the effects of neural G proteins on the neurotransmitter release machinery, and on new signaling molecules such as RIC-8, TrioRhoGEF, and RGS proteins that were discovered in *C. elegans* using the power of forward genetic screens.

Opportunities for future progress using *C. elegans* include the chance to grapple with the huge family of GPCRs that activate neural G proteins. Perhaps the entire set of GPCRs expressed on some model neurons, such as the ventral cord motor neurons or the HSN egg-laying neurons, can be defined and used to study how these receptors act together to regulate activity of these neurons. More work remains to define the mechanisms by which the three major neural G proteins affect neurotransmitter release, and at what level they intersect. In particular, the effector for $G\alpha_o$, if it exists, still remains to be identified. Finally, it remains to be seen whether *C. elegans* can be used to successfully study the types of changes in synaptic structure and transcription that occur downstream of G proteins in neurons of more complex organisms.

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6. Table 1 and Table 2

Table 1. G protein coupling and pharmacological profiles of *C. elegans* G protein coupled small molecule neurotransmitter receptors

Serotonin receptors	Experimental evidence for ligand assignment	G protein coupling	References G protein coupling	References binding studies	Agonists (EC_{50}) in heterologous cells	References heterologous cell studies
SER-1	Binding studies, heterologous cell signaling, mutant resistant to serotonin	$G\alpha_q$ in heterologous cells	Hamdan et al., 1999	Hamdan et al., 1999	Serotonin (0.72 and 0.8 µM), lisuride (partial, 0.2 and 2.4 µM), DOI (partial, >100 µM). No activity with dopamine or octopamine.	Hamdan et al., 1999

SER-4	Binding studies, heterologous cell signaling, mutant resistant to serotonin	$G\alpha_{i/o}$ in heterologous cells	Olde and McCombie, 1997	Olde and McCombie, 1997	Serotonin (~0.1 µM), tyramine	Olde and McCombie, 1997; Petrascheck et al., 2007
SER-5	Mutant resistant to serotonin	$G\alpha_s$ predicted from sequence analysis	Harris et al., 2009			
SER-7	Binding studies, heterologous cell signaling, mutant resistant to serotonin	$G\alpha_s$ in heterologous cells, $G\alpha_s$ in MC neurons, $G\alpha_{12}$ in M4 neuron	Hobson et al., 2003; Hobson et al., 2006; Song and Avery, 2012	Hobson et al., 2003; Hobson et al., 2006	Serotonin (30 nM), tryptamine (4.7 µM)	Hobson et al., 2003; Hobson et al., 2006
Dopamine receptors	**Experimental evidence for ligand assignment**	**G protein coupling**	**References G protein coupling**	**References binding studies**	**Agonists (EC_{50}) in heterologous cells**	**References heterologous cell studies**
DOP-1	Binding studies, mutation affects dopamine response	$G\alpha_q$ in ventral cord motor neurons and touch response neurons	Chase et al., 2004; Kindt et al., 2007	Suo et al., 2002		
DOP-2	Binding studies, mutant resistant to dopamine, heterologous cell signaling	$G\alpha_{i/o}$ in heterologous cells, GOA-1 in SIA neurons, GPA-14 in yeast interaction assay and ADE neurons. GOA-1 and GPA-7 in male mating.	Suo et al., 2003; Suo et al., 2009; Correa et al., 2012; Pandey and Harbinder, 2012; Mersha et al., 2013	Suo et al., 2003	dopamine (75 nM)	Suo et al., 2003
DOP-3	Mutant resistant to dopamine, heterologous cell signaling	$G\alpha_o$ in ventral cord motor neurons, $G\alpha_{i/o}$ in heterologous cells	Chase et al., 2004; Sugiura et al., 2005		dopamine (27 nM), tyramine (500 nM), octopamine (250 µM)	Sugiura et al., 2005
DOP-4	Mutant resistant to dopamine, heterologous cell signaling	$G\alpha_s$ in heterologous cells	Sugiura et al., 2005		dopamine (9.3 µM), >1 mM for dopamine, octopamine, tyramine and serotonin	Sugiura et al., 2005

Octopamine receptors	**Experimental evidence for ligand assignment**	**G protein coupling**	**References G protein coupling**	**References binding studies**	**Agonists (EC_{50}) in heterologous cells**	**References heterologous cell studies**
OCTR-1	Binding studies, heterologous cell signaling, mutant resistant to octopamine	$G\alpha_o$ in ASH neurons, $G\alpha_o$ in heterologous cells	Harris et al., 2010	Wragg et al., 2007	octopamine (0.39 µM in *Xenopus* oocytes, but binding studies suggest higher affinity)	Mills et al., 2012
SER-3	Mutant resistant to octopamine, heterologous cell signaling	$G\alpha_q$ in ASH neurons, $G\alpha_q$ in heterologous cells	Suo et al., 2006		octopamine (24 nM in mammalian cells; 0.33 µM in *Xenopus* oocytes), tyramine (26 µM in mammalian cells)	Petrascheck et al., 2007; Mills et al., 2012
SER-6	Mutant resistant to octopamine	$G\alpha_s$ in ADL neurons	Mills et al., 2012;		octopamine (2 µM in *Xenopus* oocytes) > tyramine. No response to dopamine or serotonin.	Mills et al., 2012;
Tyramine receptors	**Experimental evidence for ligand assignment**	**G protein coupling**	**References G protein coupling**	**References binding studies**	**Agonists (EC_{50}) in heterologous cells**	**References heterologous cell studies**
SER-2	heterologous cell signaling, mutant resistant to tyramine	$G\alpha_{i/o}$ in heterologous cells, $G\alpha_o$ in VD neurons	Rex and Komuniecki, 2002; Donelly et al., 2013	Rex and Komuniecki, 2002	Tyramine 360 nM, 403 nM)	Rex and Komuniecki, 2002; Rex et al., 2004
TYRA-2	Binding studies, heterologous cell signaling	$G\alpha_{i/o}$ in heterologous cells	Rex et al., 2005	Rex et al., 2005	Tyramine (50 nM), octopamine and dopamine had little/no effect	Rex et al., 2005
TYRA-3	Binding studies, mutant resistant to tyramine	$G\alpha_q$ in heterologous cells	Hapiak et al., 2013	Wragg et al., 2007	tyramine (70.8 nM)	Hapiak et al., 2013

Acetylcholine receptors	**Experimental evidence for ligand assignment**	**G protein coupling**	**References G protein coupling**	**References binding studies**	**Agonists (EC_{50}) in heterologous cells**	**References heterologous cell studies**
GAR-1	heterologous cell signaling	$G\alpha_i$ in *Xenopus* oocytes	Park et al., 2000		Acetylcholine (~100 nM), carbachol, no effect of oxotremorine	Park et al., 2000
GAR-2	heterologous cell signailng	$G\alpha_{i/o}$ in *Xenopus* oocytes, $G\alpha_o$ in ventral chord cholinergic neurons	Lee et al., 2000; Dittman and Kaplan, 2008		acetylcholine (~35 nM), carbachol, no effect of oxotremorine	Lee et al., 2000; Suh et al., 2001
GAR-3	heterologous cell signaling, mutant resistant to acetylcholine agonist arecoline	$G\alpha_q$ and $G\alpha_{i/o}$ in heterologous cells, $G\alpha_q$ in pharyngeal muscles, $G\alpha_q$ in male spicule protraction	Min et al., 2000; Park et al., 2003; Steger and Avery 2004; Park et al., 2006; Liu et al., 2007		carbachol (13.0 and 11.5 µM), oxotremorine	Hwang et al., 1999; Min et al., 2000; Park et al., 2003
GABA receptors	**Experimental evidence for ligand assignment**	**G protein coupling**	**References G protein coupling**	**References binding studies**	**Agonists (EC_{50}) in heterologous cells**	**References heterologous cell studies**
GBB-1	Mutant insensitive to optogenetically released GABA	$G\alpha_o$ in cholinergic motor neurons	Dittman and Kaplan, 2008			
GBB-2	Mutant insensitive to optogenet-ically released GABA	$G\alpha_o$ in cholinergic motor neurons	Dittman and Kaplan, 2008			
Glutamate receptor homologs	**Experimental evidence for ligand assignment**	**G protein coupling**	**References G protein coupling**	**References binding studies**	**Agonists (EC_{50}) in heterologous cells**	**References heterologous cell studies**
MGL-1	Mutant resistant to mGluR agonists *trans*-ACPD and LCCG-I					
MGL-2	heterologous cell signaling	$G\alpha_q$ in heterologous cells	Tharmalingam et al., 2012		glutamate (8.61 µM), quisqualate (169 µM), DHPG (partial agonist),	

MGL-3	None—inferred to be a glutamate receptor from sequence homology					
Poorly-studied putative small-molecule GPCRs	**Experimental evidence for ligand assignment**	**G protein coupling**	**References G protein coupling**	**References binding studies**	**Agonists (EC_{50}) in heterologous cells**	**References heterologous cell studies**
T02E9.3						
C24A8.1						
C24A8.6						
F35H10.10						
F59D12.1						
T21B4.4						

Table 2. Genetically-established functions and expression patterns of *C. elegans* G protein coupled small molecule neurotransmitter receptors

Serotonin receptors	Functions revealed in mutants	References mutant phenotype	Expression pattern	References expression pattern
SER-1	Exogenous serotonin-stimulated egg laying, feeding, and slowing of locomotion, α-methyl-5-HT stimulated egg laying, male ventral tail curling, food-induced slowing, food modulation of aversive response, heat shock response, longevity	Carnell et al., 2005; Dempsey et al., 2005; Xiao et al., 2006; Dernovici et al., 2007; Murakami and Murakami, 2007; Srinivasan et al., 2008; Harris et al., 2011	Pharyngeal muscle (pm3, pm4, pm5, pm6, pm7, pm8), CEP, RMG, RMH, RMF, RMD, RIA, RIC, URY, additional head neurons, vulval muscle and epithelial cells, tail neurons (PVT, PVQ, possibly DVC) ventral nerve cord, excretory cell, uterine cells, ray sensory neurons in male	Cho et al., 2000; Tsalik et al., 2003; Carnell et al., 2005; Dempsey et al., 2005; Xiao et al., 2006; Dernovici et al., 2007
SER-4	Egg laying induced by imipramine but not by fluoxetine or serotonin, inhibition of locomotion by serotonin, effects of ethanol on gustatory plasticity and locomotion, thermotaxis memory behavior	Cho et al., 2000; Dempsey et al., 2005; Murakami and Murakami, 2007; Wang et al., 2011; Gürel et al., 2012; Li et al., 2013	NSM, RIB or AIB, RIS, pharyngeal neuron, pair of sublateral interneurons or motorneurons, retrovesicular ganglion, PVT tail neuron, vm2 vulval muscles, DVA or DVC tail interneuron	Tsalik et al., 2003; Gürel et al., 2012

SER-5	Exogenous serotonin-stimulated egg laying, food and serotonin-dependent increase in sensitivity of ASH neurons to octanol and decrease in sensitivity to Cu^{2+}. Reduced sensitivity to serotonin and fluoxetine induced paralysis.	Hapiak et al., 2009; Kullyev et al., 2010; Harris et al., 2011; Guo et al., 2015	Neurons including AWB and ASH, body wall muscles, vulval muscles	Hapiak et al., 2009
SER-7	Exogenous serotonin-stimulated egg laying, pharyngeal pumping, and food intake. Regulation of pharyngeal pumping. Egg laying. Hypoxia regulation of gustatory and effects of ethanol on gustatory plasticity, thermotaxis memory behavior	Hobson et al., 2006; Donohoe et al., 2009; Hapiak et al., 2009; Pocock and Hobert, 2010; Wang et al., 2011; Song and Avery, 2012; Song et al., 2013; Li et al., 2013; Gomez-Amaro et al., 2015; Leiser et al., 2015	Pharyngeal neurons MC, M4, I2, I3, M5, M3, I4, I6, and M2, vulval muscles	Hobson et al., 2006
Dopamine receptors	**Functions revealed in mutants**	**References mutant phenotype**	**Expression pattern**	**References expression pattern**
DOP-1	Antagonizes DOP-3 to control locomotion, a behavioral decision-making paradigm, and acetylcholine release. Regulates swim-to-crawl transition, fat reservoirs, local food search behavior. Delays habituation to mechanical stimulation. Promotes nicotine approach behavior.	Chase et al., 2004; Sanyal et al., 2004; Kindt et al., 2007; Allen et al., 2011; Vidal-Gadea et al., 2011; Sellings et al., 2013; Barros et al., 2014; Bhattacharya et al., 2014; Wang et al., 2014	DVA, PLM, PHC, ALN, ALM, AVM, PLN, PVQ, AUA, RIB, RIM, RIS, unidentified head neurons, head muscles, labial and amphid sheath/socket cells, excretory gland cells, cholinergic ventral cord motor neurons	Tsalik et al., 2003; Sanyal et al., 2004; Chase et al., 2004; Bhattacharya et al., 2014

DOP-2	Antagonizes octopamine signaling, inhibits CRE-mediated gene expression in SIA. Mediates swimming-induced paralysis. Inhibits unproductive male mating behavior. Promotes nicotine approach behavior. Regulates touch habituation and chemosensory associative learning. Affects a decision-making paradigm.	Suo et al., 2009; Carvelli et al., 2010; Correa et al., 2012; Sellings et al., 2013; Mersha et al. 2013; Wang et al., 2014; Correa et al., 2015	All dopaminergic neurons of hermaphrodite (CEP, ADE, PDE) and probably of male (R5A, R7A, R9A). RIA, SIA, SIB, RID, PDA, HOA.	Suo et al., 2003; Tsalik et al., 2003; Suo et al., 2009; Correa et al., 2015
DOP-3	Antagonizes DOP-1 to control locomotion, a behavioral decision-making paradigm, and acetylcholine release. Mediates swimming induced paralysis. Inhibits unproductive male mating behavior. Antagonizes octopamine signaling and inhibits CRE-mediated gene expression in SIA. Regulates avoidance of octanol and 2-nonanone.	Chase et al., 2004; McDonald et al., 2007; Suo et al., 2009; Ezak and Ferkey, 2010; Carvelli et al., 2010; Kimura et al., 2010; Omura et al., 2012; Correa et al., 2012; Wang et al., 2014	Ventral cord GABAergic and cholinergic neurons, ASK, PVD, SIA, RIC, head neurons, tail neurons, body wall muscles, male tail cells	Chase et al., 2004; Suo et al., 2009; Ezak and Ferkey, 2010; Correa et al., 2012;
DOP-4	Mediates swimming induced paralysis, swim/crawl transition, food enhancement of ASH response to repellents, alcohol-induced disinhibition of crawling.	Carvelli et al., 2010; Ezcurra et al., 2011; Topper et al., 2014; Vidal-Gadea et al., 2011	I1, I2, ASG, AVL, CAN, PQR, vulva, intestine, rectal glands, rectal epithelial cells, male ray 8, additional head neurons, ASH (inferred indirectly).	Sugiura et al., 2005; Ezcurra et al., 2011
Octopamine receptors	**Functions revealed in mutants**	**References mutant phenotype**	**Expression pattern**	**References expression pattern**
OCTR-1	Mediates effect of exogenous octopamine on response to octanol. Controls innate immunity by regulating the unfolded protein response.	Wragg et al., 2007; Harris et al., 2010; Sun et al., 2011; Mills et al., 2012; Sun et al., 2012	ASI, ASH, AIY, ADE, CEP, spermatheca, uterine toroid cells, head and tail neurons	Wragg et al., 2007

SER-3	Mediates octopamine- and starvation-induced expression of a CREB-dependent reporter gene in SIA neurons. Antagonizes OCTR-1 in the control of octanol response by exogenous octopamine. Mediates inhibition of ASI sensory neuron by ASH sensory neuron via a circuit involving octopamine released from RIC.	Suo et al., 2006; Mills et al., 2012; Guo et al., 2015	ASH, ASG, ASI, SIA, PHA, PHB, PVQ, neurons in the head and tail, head muscles, intestine, phasmid socket cells, spermatheca	Suo et al., 2006; Mills et al., 2012; Guo et al., 2015
SER-6	Mediates octopamine- and starvation-induced expression of a CREB-dependent reporter gene in SIA neurons. Mediates exogenous serotonin-stimulated fat reduction. Mediates exogenous octopamine mediated inhibition of response to 100% octanol.	Srinivasan et al., 2008; Mills et al., 2012; Yoshida et al., 2014	SIA, RIC, AWB, ADL, ASI, head neurons, tail neurons, posterior ventral cord neurons, intestine	Srinivasan et al., 2008; Yoshida et al., 2014
Tyramine receptors	**Functions revealed in mutants**	**References mutant phenotype**	**Expression pattern**	**References expression pattern**
SER-2	Mediates paralysis and suppression of pharyngeal pumping by exogenous tyramine. Suppresses head movements during backing. Facilitates execution of omega turns.	Rex et al., 2004; Donelly et al., 2013	Neurons: AIY, AVH, AUA, RIC, SAB, RID, RIA, SDQ, CAN, DA9, LUA, ALN, PVC, NSM, AIZ, DVA, BDU, SIA, RME, PVT, OLL, PVD, VD. Excretory gland cells. Muscles: pm1, pm6, head muscles, male posterior body wall muscles and diagonal muscles. Possibly male CP neurons. Uterine toroid cells ut1 and ut2.	Tsalik et al., 2003; Rex et al., 2004; Donelly et al., 2013
TYRA-2			MCL, NSM, ASE, ASG, ASH, ASI, PVD, CAN, ALM	Rex et al., 2005

TYRA-3	Mediates effect of exogenous tyramine on response to octanol; affects decision to leave bacterial lawn. Antagonizes the effect of serotonin to sensitize aversive responses.	Wragg et al., 2007; Bendesky et al., 2011; Hapiak et a., 2013;	ADE, CEP, ASK, ADL, AIM, AUA, BAG, DEP, OLQ, SDQL, AFD, AWC, RIC, ASI, spermatheca, distal tip cell, vulval muscles, additional head and tail neurons	Carre-Pierrat et al., 2006; Unpublished results cited in Wragg et al., 2007; Bendesky et al., 2011
Acetylcholine receptors	**Functions revealed in mutants**	**References mutant phenotype**	**Expression pattern**	**References expression pattern**
GAR-1			head neurons, PVM	Lee et al., 2000
GAR-2	Mediates inhibition of egg laying. Mediates feedback inhibition of cholinergic ventral cord motor neurons to regulate locomotion.	Bany et al., 2003; Dittman and Kaplan, 2008	head neurons, ventral cord GABAergic and cholinergic, neurons, cell near vulva	Lee et al., 2000; Dittman and Kaplan, 2008
GAR-3	Regulates multiple calcium-dependent processes in pharyngeal muscle. Sensitizes nicotinic signaling in male spicule neurons and muscles to facilitate mating. Regulates head movements by compartmentalizing axon activity in the RIA interneuron. Promotes acetylcholine release from cholinergic motor neurons.	Steger and Avery, 2004; Liu et al., 2007; Hendricks et al., 2012; Chan J.P. et al., 2013	I3, pharyngeal muscle, body wall muscles, anal depressor muscle, cholinergic ventral cord neurons, VD, DD, male diagonal muscles, male ray 8 neuron, SPC, PCB, and PCA neurons and the spicule protractor muscles	Steger and Avery, 2004; Liu et al., 2007; Chan J.P. et al., 2013
GABA receptors	**Functions revealed in mutants**	**References mutant phenotype**	**Expression pattern**	**References expression pattern**
GBB-1	With GBB-2, decreases acetylcholine release from ventral cord motor neurons to regulate locomotion. Affects lifespan.	Dittman and Kaplan, 2008; Schultheis et al., 2011; Chun et al., 2015	Expression in ventral cord cholinergic neurons in inferred indirectly.	Dittman and Kaplan, 2008; Schultheis et al., 2011
GBB-2	With GBB-1, decreases acetylcholine release from ventral cord motor neurons to regulate locomotion.	Dittman and Kaplan 2008; Schultheis et al., 2011	Expression in ventral cord cholinergic neurons in inferred indirectly.	Dittman and Kaplan 2008; Schultheis et al., 2011

Glutamate receptor homologs	Functions revealed in mutants	References mutant phenotype	Expression pattern	References expression pattern
MGL-1	Regulates starvation response, perhaps by directly sensing amino acids from food. Pharmacological activation of MGL-1 inhibits pharyngeal pumping. Mediates reduction in pharyngeal pumping when animals are removed from food.	Kang and Avery, 2009; Dillon et al,. 2015	Expression in AIY and AIB is inferred indirectly. Head neurons, tail neurons, pharyngeal neurons including NSM.	Kang and Avery, 2009; Dillon et al,. 2015
MGL-2	Regulates starvation response, perhaps by directly sensing amino acids from food.	Kang and Avery, 2009	Expression in AIY and AIB is inferred indirectly. Head neurons, tail neurons, pharyngeal neurons including NSM, I5, intestine, pharyngeal-intestinal valve, pharyngeal muscle.	Kang and Avery, 2009; Dillon et al,. 2015
MGL-3			Head neurons, pharyngeal neurons including NSM.	Dillon et al., 2015

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