Published in final edited form as:

Prog Neurobiol. 2012 March ; 96(3): 304–321. doi:10.1016/j.pneurobio.2012.01.009.

GPCR Mediated Regulation of Synaptic Transmission

Katherine M. Betke, Christopher A. Wells, and Heidi E. Hamm*

Vanderbilt University Medical Center, 442 Robinson Research Building, 23rd Ave. South @
Pierce, Nashville, Tennessee 37232-6600

Katherine M. Betke: katherine.m.betke@Vanderbilt.Edu; Christopher A. Wells: christopher.wells@Vanderbilt.Edu

Abstract

Synaptic transmission is a finely regulated mechanism of neuronal communication. The release of neurotransmitter at the synapse is not only the reflection of membrane depolarization events, but rather, is the summation of interactions between ion channels, G protein coupled receptors, second messengers, and the exocytotic machinery itself which exposes the components within a synaptic vesicle to the synaptic cleft. The focus of this review is to explore the role of G protein signaling as it relates to neurotransmission, as well as to discuss the recently determined inhibitory mechanism of G $\beta\gamma$ dimers acting directly on the exocytotic machinery proteins to inhibit neurotransmitter release.

Keywords

presynaptic regulation; exocytosis; SNARE proteins; heterotrimeric G proteins

1 Introduction

Efficient communication between neurons in the brain is crucial to the normal functioning of the nervous system as it ensures integration of both external and internal sensory input, and permits the generation of appropriate behaviors to meet the demands of an individual's environment (Goodman et al., 2001). As a result, understanding the process of synaptic transmission is essential to understanding how normal processes are coordinated, but also how they may be disrupted by injury and disease. In its most elementary form, synaptic transmission is simply the communication between one presynaptic neuron and a single postsynaptic cell as well as the processing by the postsynaptic cell of the signal that it receives (Kandel et al., 2000). At chemical synapses, signal transduction is achieved by the rapid conversion of an arriving electrical signal into a chemical one that diffuses between the cells (Schoch and Gundelfinger, 2006; Zhai and Bellen, 2004). Structurally, these complex, asymmetrical cell-cell contact sites are formed from the axon terminal membrane of the presynaptic neuron, juxtaposed with the postsynaptic density on the postsynaptic cell (Dresbach et al., 2001; Schoch and Gundelfinger, 2006).

Membrane depolarization caused by the arrival of presynaptic action potentials induces the opening of voltage-dependent calcium channels (VDCC), with the resulting calcium

© 2012 Elsevier Ltd. All rights reserved.

*Corresponding Author: heidi.hamm@Vanderbilt.Edu, Tel.: 615-343-3533, Fax: 615-343-1084.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

transient stimulating synaptic vesicle exocytosis from the active zone of the presynaptic terminal (Sudhof, 2004). Such regulated exocytosis releases neurotransmitter into the synaptic cleft whereupon it activates receptors or channels on the postsynaptic membrane to ensure continued propagation of the signal (Dresbach et al., 2001; Sudhof, 2004). When these chemical synapses are examined, two distinct types of vesicles are evident: small, clear synaptic vesicles which are filled with classical neurotransmitters such as acetylcholine, glutamate, and the monoamines, and large dense-core vesicles filled with neuropeptides and neurohormones. While these vesicles differ in their morphology, release kinetics, and distribution, they maintain conserved machinery for fusion events, and both exhibit calcium-dependence for exocytosis (Park and Kim, 2009).

Released neurotransmitters bind to ligand-gated ion channels on postsynaptic neurons to mediate voltage changes in the postsynaptic cell. Major modulators of neurotransmitter action are G protein coupled receptors (GPCRs). These seven membrane-spanning α -helical proteins bind specifically to their respective neurotransmitters, causing a change in the structure of the receptor, and resulting in activation of heterotrimeric guanine-nucleotide binding proteins. $G_{i/o}$ -coupled autoreceptors on both pre- and postsynaptic cells guard against overstimulation by release of G protein $\beta\gamma$ subunits which postsynaptically activate G protein-coupled inward rectifier K^+ (GIRK) channels and presynaptically inhibit voltage activated calcium channels (Kajikawa et al., 2001; Takahashi et al., 1996). $G\beta\gamma$ subunits can also directly interact with the vesicle exocytotic machinery, soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins (Blackmer et al., 2001; Delaney et al., 2007; Gerachshenko et al., 2005). $G_{i/o}$ -coupled heteroreceptors mediate circuit-level modulation of neurotransmitter responses.

The goal of this review is to discuss the regulation of exocytosis at the synapse by G protein coupled receptor signaling pathways with a focus on the role and mechanism of $G\beta\gamma$ signaling and the novel direct modulation on the exocytotic machinery.

2 The Exocytotic Machinery

2.1 Regulatory Components Controlling Vesicle Docking, Priming, and Exocytosis at the Presynaptic Membrane

The short latency between calcium influx at a presynaptic terminal and the release of neurotransmitter suggests that a population of vesicles sits poised to fuse with the plasma membrane immediately following calcium entry (Katz, 1969; Weimer and Richmond, 2005). To achieve such temporal precision, vesicles undergo a series of maturation steps at the presynaptic membrane known as docking and priming, in order to become fusion competent (see Figure 1). Docked vesicles were traditionally defined morphologically based on electron micrographs as those without measurable distance between vesicle and plasma membrane (Verhage and Sorensen, 2008). Based on this definition, docked vesicles were often reported to cluster around electron-dense pyramidal densities at the presynaptic terminal, and were suggested to make direct contact with the active zone (Fernandez-Busnadiego et al., 2010; Weimer and Richmond, 2005). Unfortunately, while ultrastructure studies are unmatched in resolution, they cannot easily be used to study vesicle dynamics and preclude identification of different docked states, while potentially overestimating the number of docked vesicles through inclusion of those which are primed and/or being endocytosed (Toonen et al., 2006; Weimer and Richmond, 2005). Further, chemical fixation required for electron microscopy is known to introduce crosslinking artifacts which can alter the distribution of synaptic vesicles at a presynaptic terminal (Fernandez-Busnadiego et al., 2010). More recent studies observing single synaptic vesicles using tools such as total internal reflection fluorescence microscopy show evidence for three distinct docking states by which vesicles approach the plasma membrane for variable lengths of time and contact it

only during fusion events (Fernandez-Busnadiego et al., 2010; Weimer and Richmond, 2005). Such studies suggest that docking may be an easily reversible event prior to priming where stable interactions with the plasma membrane would be established (Fernandez-Busnadiego et al., 2010; Weimer and Richmond, 2005). Despite increasing numbers of perturbation and biophysical studies, however, relatively few docking phenotypes have been associated with genetic mutations compared to secretion phenotypes (Verhage and Sorensen, 2008). Given the spatial fidelity by which synaptic vesicles cluster at an active zone, however, it is likely that specific molecular recognition mechanisms mediate attachment of the vesicles to the target membrane during the docking process.

Comparatively more is known about priming, a vesicle maturation process wherein vesicles transition from a docked but unprimed pool to a releasable pool which can fuse with the plasma membrane immediately upon calcium entry (Becherer and Rettig, 2006; Malsam et al., 2008; Weimer and Richmond, 2005). While the fidelity of vesicle priming and subsequent exocytosis has been shown to depend on the coordinated actions of a large number of proteins, the core machinery underlying this process revolves around members of the SNARE complex. Further regulation is provided by a series of ancillary proteins such as the Sec-1/Munc-18 (SM) proteins Munc-18 and Munc-13, and the calcium sensor, synaptotagmin (Parpura and Mohideen, 2008). The SM proteins have been shown to have important interactions with syntaxin1A that regulate the docking of vesicles to the synaptic membrane by influencing the open and closed state of syntaxin1A (de Wit, 2010; Gerber et al., 2008; Misura et al., 2000; Rizo et al., 2006; Rizo and Rosenmund, 2008; Sudhof, 2004; Sudhof and Rothman, 2009; Toonen et al., 2006; Wojcik and Brose, 2007). In contrast, synaptotagmin1 plays a role in both priming and docking by its ability to interact with t-SNARE, the complex of SNAP25 and syntaxin1A at the synaptic membrane, to stabilize it for further interaction with VAMP2 on the synaptic vesicle (Weninger et al., 2008).

2.2 Soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins

The protein machinery that drives and regulates exocytosis has been shown to be remarkably conserved, with SNARE proteins ubiquitously used for membrane fusion in organisms ranging from yeast to humans (Jahn and Scheller, 2006; Parpura and Mohideen, 2008). A mechanistic model has subsequently emerged termed the 'SNARE Hypothesis' whereby membrane fusion is thought to occur through formation of a four-helix bundle which draws opposing membranes into close proximity and releases enough free energy to drive fusion events (Jahn and Scheller, 2006; Sollner et al., 1993a; Sollner et al., 1993b; Weber et al., 1998). Central to this hypothesis are the three proteins which make up the SNARE complex. The representative major isoforms in brain of t-SNAREs are syntaxin 1A and synaptosome-associated protein of 25 kDa (SNAP-25), and of the v-SNARE is synaptobrevin/VAMP (vesicle-associated membrane protein)-2. When the structure of syntaxin 1A is examined, three conserved domains are apparent: a carboxy-terminal transmembrane domain anchored to the presynaptic membrane, and two conserved α -helical structures - a SNARE domain single α -helix and an amino-terminal triple α -helix H_{abc} domain - which play a role in regulating synaptic transmission. The SNARE domain mediates interaction of syntaxin with other members of the SNARE complex to form the exocytotic machinery. By folding back on itself to cover the SNARE domain, the H_{abc} domain places syntaxin in a 'closed' conformation and prevents the formation of the core fusion complex through further interaction with chaperone proteins such as Munc-18 (Sudhof, 2004; Teng et al., 2001).

Similarly, synaptobrevin is also a transmembrane protein whose cytoplasmic domain forms an α -helical SNARE domain. While *in vitro* studies have suggested synaptobrevin may interact with other synaptic proteins such as voltage-dependent calcium and sodium channels (Sampo et al., 2000), the primary role for these proteins at this time appears to be

mediating vesicle endo- and exocytosis (Hong, 2005; Seagar et al., 1999). The final member of the SNARE complex, SNAP-25, is unique in that while it is found localized to the presynaptic membrane, it lacks a transmembrane domain. Rather, it is anchored via post-translational palmitoylation of four cysteine residues located near the central region of the molecule between two α -helical SNARE domains (Matteoli et al., 2009). Two other members of this family, SNAP-23 and SNAP-29 have also been shown to be important for regulating exocytotic events, but despite these isoforms co-localizing to regions such as the cerebellar cortex, SNAP-25 appears to be the major homolog mediating regulated exocytosis in the brain (Matteoli et al., 2009).

The predominant view of SNARE complex formation is that initial assembly of the intermediate binary complex between syntaxin and SNAP-25 (t-SNARE) is followed by formation of the ternary complex through interactions with synaptobrevin on the secretory vesicle as the vesicle is drawn to the presynaptic membrane during priming. As vesicles approach the presynaptic membrane, the SNARE motifs interact in a process referred to as “zippering”, forming a stable, four-helical bundle which brings the vesicle progressively closer to the presynaptic membrane to allow fusion to occur.

2.3 Sec-1/Munc-18 (SM) Proteins

While formation of the SNARE complex has been shown to be sufficient to drive exocytosis, a number of studies examining fusion kinetics have suggested that additional proteins may be required. One group of such proteins includes the cytosolic SM proteins, Munc-18 and Munc-13 (Sudhof, 2004). All members of the SM family are composed of a conserved ≈ 600 amino acid sequence which folds into an arch-shaped structure important for protein-protein interactions (Sudhof and Rothman, 2009). Munc-18 is thought to serve multiple roles in exocytosis, the first being as an inhibitory protein which prevents assembly of the SNARE complex at inopportune times and which plays an important role in mediating vesicle docking (de Wit, 2010; Dulubova et al., 1999; Toonen et al., 2006; Voets et al., 2001; Wojcik and Brose, 2007; Yang et al., 2000). Evidence for a second, post-docking role for this SM protein, however, comes from analysis of Munc-18-1 knockout mice which exhibit a total block of neurotransmitter release (Burgoyne and Morgan, 2011; Verhage et al., 2000). Recent evidence suggests that rather than dissociate completely, Munc-18 actually remains anchored to syntaxin by its N-terminal lobe, allowing it to take on an additional regulatory role. As ternary SNARE formation proceeds, Munc-18 is believed to fold back over the four helix SNARE bundle and in doing so, restrict the diffusion of the SNARE proteins in the membrane such that they are maintained in the correct orientation for fusion to occur, as well as allow energy transduction to the membranes (Rizo and Rosenmund, 2008; Rizo, 2009; Sudhof and Rothman, 2009).

A second SM protein, Munc-13, has also been shown to play a prominent role in vesicle exocytosis, although as opposed to Munc-18, Munc-13 primarily regulates the priming process to increase the number of primed vesicles. Munc-13 null mice demonstrate total abolishment of both evoked and spontaneous neurotransmitter release while docking remains unaffected (Augustin et al., 1999), and this phenotype is also found in knockouts of homologs in both *C. elegans* (Richmond et al., 1999) and *Drosophila* (Aravamudan et al., 1999). Its actions appear to be downstream of Munc-18 as overexpression of Munc-13 does not affect the number of docked vesicles, while open forms of syntaxin 1A are able to at least partially rescue the *unc-13* null phenotype in worms (Weimer et al., 2006) but not that of *unc-18* (Hammarlund et al., 2007; Verhage and Sorensen, 2008). This role in priming is thought to be mediated through both its C-terminal C2 domain (C2C) as well as the Munc homology domains (MHD) located in the C-terminal third of the protein. These MHDs allow binding to syntaxin 1A after it forms a heterodimer with SNAP-25 or the fully formed SNARE complex (Becherer and Rettig, 2006; Rizo and Rosenmund, 2008; Stevens et al.,

2005). In doing so, Munc-13 may help transform syntaxin to an open conformation to facilitate SNARE complex formation, or stabilize the exocytotic machinery to promote vesicle fusion once the complex is fully formed.

2.4 Synaptotagmins

In addition to SM proteins, synaptotagmin has also been shown to be essential for vesicle fusion to occur (Brose et al., 1992). Synaptotagmins are a family of calcium binding proteins (Fukuda, 2003a; b; Schiavo et al., 1998; Südhof, 2002). Genetic ablation of synaptotagmin-1 results in abrogation of fast, synchronous neurotransmitter release without affecting asynchronous events, leading to the suggestion that this protein acts as a calcium sensor for calcium-dependent vesicle fusion (Geppert et al., 1994; Maximov and Südhof, 2005; Rizo et al., 2006; Tang et al., 2006). Gene mutation in mice of the calcium-binding site of synaptotagmin, that disrupts calcium binding but no other structural features of synaptotagmin, results in a decrease in calcium sensitivity of neurotransmitter release, but has no effect on the size or spontaneous release of the ready releasable pool of synaptic vesicles (Fernandez-Chacon et al., 2001). Synaptotagmin-1 is localized to both synaptic and large dense core vesicle membranes and is characterized by two tandem, cytoplasmic, PKC-like C₂ domains: C₂A and C₂B respectively. Two of the most important binding partners for this protein are SNARE proteins and the lipid membrane, and it's believed that through cooperative interactions with each of these, the C₂ domains play an important role in exocytosis. Specifically, calcium influx has been shown to induce binding of synaptotagmin-1 to both syntaxin and the C-terminal of SNAP-25, as well as t-SNARE heterodimers and fully formed SNARE complexes (Dai et al., 2007; Gerona et al., 2000; Rizo et al., 2006; Zhang et al., 2002), a process which may be mediated at least in part through interactions between acidic residues on SNAP-25 and basic residues in the second loop of the C₂A domain (Lynch et al., 2007), or perhaps with the polybasic region of the C₂B domain (Rizo and Rosenmund, 2008).

Anionic phospholipids are a second important effector for synaptotagmin-1 (Chapman and Jahn, 1994; Chapman and Davis, 1998; Fernandez et al., 2001) and mutations which reduce calcium-dependent synaptotagmin binding to the lipid membrane exhibit a decrease in synaptic release probability (Zhang et al., 2002) suggesting that this function is essential for its role in exocytosis. While calcium-dependent binding of synaptotagmin to both SNARE proteins and lipid membranes is essential for fast exocytosis, debate still exists as to how exactly this occurs. One possibility is that synaptotagmin interacts with SNARE complexes prior to calcium influx, bringing the vesicle and plasma membranes into close apposition. This would be expected to promote vesicle fusion as it would enable tighter coiling of the SNARE complex, while subsequent interactions with the lipid bilayer upon calcium entry may accelerate membrane fusion through electrostatic interactions acting to perturb the lipid bilayer and overcome the energy barrier to fusion (Rizo and Rosenmund, 2008). Another unanswered question is how synaptotagmin interacts on a molecular level with the SNARE complex. Fluorescence resonance energy transfer-based studies detected an interaction of the SNARE complex on the opposing surfaces of the C₂ domains of synaptotagmin (Choi et al., 2010). Further, NMR data demonstrated a primary interaction interface with SNARE may be through a polybasic region on the C₂B domain (Dai et al., 2007). Most recently, electron paramagnetic resonance spectroscopy data reveal a structurally heterogeneous interaction maintaining the polybasic and C₂B domain interactions, but also a site near loop 2 of the calcium binding site in C₂A; an interaction not seen in the other two studies mentioned (Lai et al., 2011). These authors suggest that this new binding mode allows for simultaneous interaction with the SNARE complex as well as two membrane surfaces in apposition such as the cell membrane and the docked synaptic vesicle. Regardless of how exactly this process functions, however, synaptotagmin clearly plays a major role in

regulating synchronous release with both SNARE interactions and phospholipid binding playing a crucial part. Further, synaptotagmins or other calcium sensors may play an important role in regulating asynchronous release events which are unrelated to action potentials. Studies examining synaptotagmin 1 and 2 knockout mice both demonstrate increases in the frequency of spontaneous release, suggesting an important role for these proteins in regulating this process (Kochubey and Schneggenburger, 2011; Sun et al., 2007). Similarly, doc2, a protein that is related to synaptotagmins in that it also has two homologous calcium-binding domains, has been shown to act as the calcium sensor for asynchronous release in cerebellar slices (Groffen et al., 2010) and cultured hippocampal neurons (Groffen et al., 2010; Yao et al., 2011), highlighting the importance of these sensors in many aspects of vesicle exocytosis.

2.5 Complexin, tomosyn, and heterotrimeric G proteins

Taken together, the SNARE proteins, as well as ancillary players such as SM proteins and synaptotagmin, function as the classical exocytotic machinery required for fusion events to occur. Studies in which individual components have been removed or inhibited, display severe disruption of synaptic transmission. Further exploration of processes which modulate their function has provided useful information about how exocytosis is regulated. Several additional proteins such as complexin, tomosyn, and heterotrimeric G proteins have also been shown to interact with either the proteins individually, the t-SNARE dimer, or the full ternary SNARE. Complexin is a regulatory protein thought to play a role in calcium-sensitive fusion (Chicka and Chapman, 2009; Tang et al., 2006) as well as vesicle priming (Hobson et al., 2011; McMahon et al., 1995; Tang et al., 2006). X-ray crystallography studies demonstrate that it interacts with the ternary SNARE complex by binding as a fifth α -helix to the SNARE bundle of ternary SNARE (Bracher et al., 2002; Chen et al., 2002). More recent x-ray crystallography and FRET data suggest that complexins can simultaneously bind to two neighboring incompletely “zippered” SNARE complexes stabilizing a structural state that is incompatible with fusion. After full incorporation of VAMP2 into the ternary SNARE bundle, complexin remains bound to the SNARE complex, but loses its interaction with the neighboring SNARE complex (Kümmel et al., 2011). Tomosyn is a protein that binds to the t-SNARE complex (Fujita et al., 1998) and prevents the association of VAMP2 (Hatsuzawa et al., 2003). Tomosyn has two domains, an N-terminal domain of two sets of 7 WD 40 repeats that form two tandem 7-bladed β -propellers, and a C-terminal domain with a SNARE binding motif. Both domains are needed to regulate exocytosis *in vivo* (Burdina et al., 2011). The C-terminal SNARE motif binds to t-SNARE in a way similar to that of VAMP2 binding to t-SNARE (Pobbati et al., 2004). Finally, the G-protein dimer, $G\beta\gamma$, interacts with ternary SNARE as well as the individual SNARE proteins to mediate inhibition of exocytosis by GPCRs (Blackmer et al., 2001; Blackmer et al., 2005; Gerachshenko et al., 2005; Photowala et al., 2006; Yoon et al., 2007; Yoon et al., 2008). This novel interaction and its role in the context of GPCR regulation of synaptic transmission is the subject of the remainder of this manuscript.

3 GPCR Mediated Regulation of Synaptic Transmission

3.1 G protein Coupled Receptors

GPCRs are a large superfamily of proteins which convey the majority of signal transduction across cell membranes and mediate a vast array of cellular responses necessary for the normal physiology of the body (Eglen and Reisine, 2009; Millar and Newton, 2010; Oldham and Hamm, 2008). Encoded by nearly 800 different genes in humans, these proteins are activated by a wide variety of ligands ranging from single photons, odorants, and amino acids to hormones, neurotransmitters, and proteolytic enzymes as well as many others (Millar and Newton, 2010; Oldham and Hamm, 2008). As depicted in Figure 2A, while all

GPCRs share a common architecture consisting of seven transmembrane-spanning α -helices, an extracellular N-terminus, an intracellular C-terminus, and three interhelical loops on each side of the membrane (Oldham and Hamm, 2007; 2008), they exhibit unique combinations of signal transduction pathways as well as complex regulatory processes controlling their activity and expression (Rosenbaum et al., 2009). At present, these receptors are phylogenetically divided into five main families: Glutamate, Rhodopsin, Adhesion, Frizzled-Taste-2, and Secretin, based on sequence and structural similarities (Schiöth and Fredriksson, 2005) with each family exhibiting unique ligand binding properties.

GPCRs are known to mediate their effects through coupling to heterotrimeric G proteins in order to modulate downstream effectors, but it is only recently that combinations of biochemical, crystallographic, and biophysical studies have begun to elucidate the structural basis by which this is accomplished (Rosenbaum et al., 2009). These studies demonstrate a complex picture by which specific ligands stabilize unique active state receptor conformations for interaction with particular effector molecules (Rosenbaum et al., 2009). The results of these conformational changes is an increase the receptor's affinity for the G protein heterotrimer, resulting in the formation of a transient ligand-GPCR-G protein ternary complex necessary for G protein activation (Buranda et al., 2007).

3.2 Heterotrimeric G proteins

Despite the diversity of the GPCR superfamily, interestingly enough, they mediate their effects through relatively few heterotrimeric G proteins. G proteins consist of α , β , and γ subunits, and in humans, 16 genes and 11 splice variants account for 27 $G\alpha$ isoforms (Wilkie et al., 1992; Yokoyama and Starmer, 1992), while 5 and 12 genes encode 5β and 13γ protein subunits, respectively (Downes and Gautam, 1999; Hildebrandt, 1997). Heterotrimers are generally classified into four families, G_s , $G_{i/o}$, G_q , and $G_{12/13}$, based on the functional similarity of the $G\alpha$ subunit (Simon et al., 1991), but all $G\alpha$ subunits share a similar tertiary structure composed of two domains: a GTPase domain and a helical domain (Oldham and Hamm, 2007). Crystal structures demonstrate that the GTPase domain is conserved among all members of the G protein superfamily, including small G proteins and elongation factors (Oldham and Hamm, 2008). This domain contains the site of GTP hydrolysis as well as the sites for binding to the $G\beta\gamma$ dimer, receptors, and a wide variety of effectors. In contrast, the helical domain is unique to the heterotrimeric $G\alpha$ subunit and functions as a lid to cover the nucleotide-binding pocket such that it is retained deep within the core of the protein (see Figure 2B). Further, post-translational modifications at the N-terminus of all $G\alpha$ subunits help regulate membrane localization and protein-protein interactions (Oldham and Hamm, 2008).

G protein β subunits are made up of two structurally distinct regions, an amino terminal segment which is an α helix of approximately 20 amino acids, and a propeller-like structure comprising seven blades of four anti-parallel β strands known as a WD repeat (Clapham and Neer, 1997; Gautam et al., 1998; Smrcka, 2008) (see Figure 2B). In contrast, the $G\gamma$ subunit is much smaller, composed of only two α -helices, a carboxy-terminal helix which extends across the surface of blade 5 on $G\beta$ as well as a small section of the N-terminal region, and an amino terminus helix which forms a coiled-coil with the amino terminal non-WD repeat region of the β subunit (Clapham and Neer, 1997) (see Figure 2B). All $G\gamma$ subunits are post-translationally modified at the C-terminus with either a farnesyl or geranylgeranyl moiety which aids in membrane localization and may play a role in receptor interactions (Oldham and Hamm, 2007; Smrcka, 2008). Together, $G\beta$ and $G\gamma$ form a functional monomer as subunits cannot be dissociated except with denaturants (Smrcka, 2008) and neither subunit can signal on its own.

3.3 Specificity of Gβγ Interactions

Sequence comparisons show that Gβ₁₋₄ share up to 90% sequence identity compared to 50% identity for Gβ₅ while Gγ subunits are much more divergent, with isoforms sharing only 30–70% sequence identity (Betty et al., 1998; Smrcka, 2008). Given the high sequence identity shared among the isoforms, it might be expected that functional dimerization of all isoforms would be possible. While most Gβ and Gγ subunits have been shown to form dimer pairs *in vitro*, studies have shown that subtypes do not randomly associate, but rather show distinct affinities for one another, and even, that certain combinations are unable to pair. As evidence has shown with Gβγ dimerization, it is increasingly becoming evident that unique Gβγ combinations play specific roles in mediating interactions with receptors and effectors (Albert and Robillard, 2002; Betty et al., 1998; Lim et al., 2001; Lindorfer et al., 1998). Indeed, while early biochemical studies found few clear functional differences in the ability of isoform combinations to regulate effectors *in vitro*, subsequent studies using anti-sense oligonucleotides, site directed ribozymes, and genetic deletion have suggested very specific roles for Gβ and Gγ isoforms in signaling in intact cells (Smrcka, 2008). For example, Gγ₇ knockout mice exhibit reduced expression of Gα_{olf} and reduced adenylyl cyclase activity (Schwindinger et al., 2003), while Gγ₃ knockout mice show increase susceptibility to seizures, reduced body weight, and decreased adiposity (Schwindinger et al., 2004). It is possible even, that isoforms may show tissue-dependent specificity for an individual effector (Robishaw and Berlot, 2004). Given that most cell types express multiple Gα, Gβ, and Gγ subtypes, a greater understanding of Gβγ-effector regulation is necessary to fully elucidate how specificity is achieved.

3.4 Gβγ Interaction with Effectors

Initially, Gβγ was thought to play a passive, inhibitory role by facilitating the completion of intracellular information transfer by merely returning Gα to a heterotrimeric state while also preventing Gα-mediated signaling in the absence of receptor activation (Cabrera-Vera et al., 2003). An independent role in cellular signaling was initially discovered, however, in 1987 when Logothetis *et al.* showed that Gβγ subunits could activate potassium-selective ion channels in cardiac atrial cells (Logothetis et al., 1987). Today, Gβγ subunits are known to regulate a wide variety of effectors including phospholipase Cβ, adenylyl cyclase, calmodulin, and components of the mitogen-activated protein kinase cascade (among others), as well as many effectors which are involved in mediating synaptic transmission such as calcium and potassium channels and members of the exocytotic machinery (Blackmer et al., 2005; Cabrera-Vera et al., 2003; Gerachshenko et al., 2005; Smrcka, 2008) (see Figure 3).

Although Gβγ-effector interaction sites were initially hypothesized to be localized to the Gα interface, this is not the only site important for effector regulation. Mutagenesis studies have since identified binding regions within the blades of the Gβ propeller and along its N-terminal coiled-coil, as well as areas outside of the Gα subunit interface, and regions on Gγ subunits (Clapham and Neer, 1997; Myung et al., 2006; Panchenko et al., 1998; Peng et al., 2003; Smrcka, 2008). An example of this is the interaction between Gβγ and the GIRK channels. Zhao *et al.* (2003) demonstrated that mutation of residues Thr-86, Thr-87, and Gly-131 on Gβ₁, which are outside of the Gα binding site, resulted in decreased GIRK activity (Zhao et al., 2003). As such, while the Gα interface likely represents a core site for effector binding being that it is primarily exposed when a GPCR has activated the Gα subunit, other regions may also play important roles in facilitating downstream signaling for Gβγ subunits.

3.5 Mechanisms of Presynaptic Regulation By Heterotrimeric G proteins

GPCRs have been shown to play an important role in controlling synaptic transmission, as activation of presynaptic $G_{i/o}$ -coupled auto- and heteroreceptors such as 5HT₁ serotonin receptors, D₂ dopamine receptors, M₄ muscarinic receptors, α_{2A} adrenergic receptors, opioid receptors, as well as many others is known to inhibit evoked transmitter release (Stephens and Mochida, 2005; Stephens, 2009). Such regulation is attributed to the actions of both the α and $\beta\gamma$ subunits of heterotrimeric G proteins. Activation of $G\alpha$ subunits leads to diffusible second messengers that can have effects at a distance from the site of activation. The $G\alpha$ subunits of G_s and G_q have facilitatory roles on exocytosis through activation of adenylyl cyclase and phospholipase C leading to second messengers that activate downstream kinases (protein kinase A, by increasing cyclic adenosine monophosphate levels by $G\alpha_s$ activation of adenylyl cyclase, and protein kinase C, by $G\alpha_q$ activation of phospholipase C β). The phosphorylation of proteins involved in vesicle recruitment, docking, and fusion can cause both inhibition or facilitation of synaptic transmission (Brown and Sihra, 2008). Activation of presynaptic $G\alpha_q$ has been described to play a role in slow voltage-independent inhibition (Delmas et al., 1998; Haley et al., 2000) through activation of phospholipase C and reduction of the second messenger phosphatidylinositol-4, 5-bisphosphate (PIP₂) (Gamper et al., 2004). Other regulatory pathways downstream of GPCRs include arrestin signaling, which can also affect second messenger systems (DeFea, 2011). Arrestin signaling processes can affect, for example, scaffolding of phosphodiesterase close to G_s -coupled receptors (Lynch et al., 2005).

The activation of presynaptic GPCRs also leads to dissociation of $G\beta\gamma$ subunits whose actions remain local as they are membrane-associated via $G\gamma$ C-terminal prenylation. Autoreceptors sensing neurotransmitter released from the activated synapse, or heteroreceptors sensing neurotransmitters from neighboring nerve terminals, activate $G_{i/o}$ receptors, and release $G\beta\gamma$. Obviously, the effects of neurotransmitters depend not only on the pharmacology of the receptors, but also on the spatial arrangement in relation to the sites of transmitter release. Some presynaptic GPCRs are found distant from the active zone (Shigemoto et al., 1997), and thus are not likely to affect active zone processes, but most probably work by traditional mechanisms of second messenger generation. Those presynaptic $G_{i/o}$ -coupled GPCRs that are localized to the active zone or peri-active zone region (Corti et al., 2002) are likely to generate $G\beta\gamma$ subunits that can diffuse to the exocytotic machinery and mediate fast regulation of exocytosis (Blackmer et al., 2005; Gerachshenko et al., 2005). The detailed localization of GPCRs must be done by electron microscopic immunocytochemistry, and though there are perhaps hundreds of presynaptic receptors, fine localization is limited to a very few subtypes, (see Table I). Further work will be needed to determine subcellular localization of other $G_{i/o}$ -coupled presynaptic receptors. On the other hand, there is much more information on the ability of presynaptic GPCRs to influence postsynaptic responses in paired recordings. Whether they work by modifying voltage-gated Ca²⁺ channels, or downstream of Ca²⁺ entry directly on the exocytotic machinery is known in some cases via studies of the effects of blocking various voltage-dependent calcium channels or inhibition of “minis” (see Table 1).

Presynaptic $G\beta\gamma$ modulation of synaptic transmission occurs mainly through three mechanisms: inhibition of voltage-dependent calcium channels at presynaptic nerve terminals, regulation downstream of calcium entry via direct interaction with one or more of the components of the exocytotic machinery (Miller, 1998), or regulation of GIRK channels that affect the shape of the invading action potential (Fernandez-Alacid et al., 2009). Such diversity highlights the physiological importance of G proteins in regulating synaptic transmission but also raises numerous questions about which mechanism functions at particular synapses, the relevance of each mechanism *in vivo*, and the contribution of each mechanism to modulation. While we attempt to highlight some of the key findings for each

mechanism below, further work is needed to clarify these questions and assess the importance of each at a systems level.

3.5.1 G $\beta\gamma$ Regulation of Presynaptic Voltage-Dependent Calcium Channels—

As mentioned, GPCR mediated regulation of synaptic transmission has been shown to occur through multiple mechanisms, the best characterized of which is through direct inhibition of VDCC. These channels play an essential role in regulating vesicle exocytosis as they control calcium influx into the cell. As the presynaptic membrane is depolarized, VDCC become activated resulting in an influx of calcium into the cell which can then bind to synaptotagmin to trigger exocytotic events. At present, five classes of VDCC have been identified in mammals based on their pharmacological and electrophysiological properties, with further delineation of subtypes into low-voltage (T-type) and high-voltage (N-, P/Q-, and R-type) activated channels based on the strength of depolarization necessary to trigger them (Catterall et al., 2005). The core of the calcium channel complex which forms the channel is the α_1 subunit. This subunit has 4 transmembrane subdomains and is believed to organize and form the pore. Other subunits that have been determined to associate with and modulate the calcium channel are an intracellular β subunit and a $\alpha_2\delta$ subunit complex. Lastly, there is a γ subunit with variable tissue expression that also is part of some calcium channels (Catterall et al., 2005). G $\beta\gamma$ can directly bind to N- and P/Q-type calcium channels (Currie, 2010; Herlitze et al., 1996; Ikeda, 1996; Tedford et al., 2006; Tedford and Zamponi, 2006). Examination of the structural determinants which underlie this modulation have identified regions within the N-terminal loop (Canti et al., 1999), the I–II loop (Herlitze et al., 1997; Zamponi et al., 1997), and the C-terminal loop (Furukawa et al., 1998; Li et al., 2004; Qin et al., 1997) of the pore-forming α_1 -subunit of the calcium channels which are important for G $\beta\gamma$ modulation. Within the I–II loop, there are two regions that have been implicated in binding to the G $\beta\gamma$ dimer. The first overlaps with the α -interaction domain (AID) and contains the consensus sequence QXXER (De Waard et al., 1997; Herlitze et al., 1997; Zamponi et al., 1997). A second domain within the I–II is located C-terminal to the AID, and is termed the G protein interaction domain (Zamponi et al., 1997). Although still subject to a great deal of study, it's believed that G $\beta\gamma$ binding actually stabilizes the channel in a conformational state which permits only reluctant opening, thus preventing vesicle exocytosis (Bean, 1989; Boland and Bean, 1993; Elmslie, 1992; Golard and Siegelbaum, 1993; Jarvis et al., 2000; Kasai and Aosaki, 1989).

G protein inhibition of inward calcium currents was initially demonstrated by Dunlap and Fischbach (1978) when they were able to show that the contribution of calcium channels to action potentials in chick dorsal root ganglion neurons was reduced following activation of gamma-aminobutyric acid (GABA) $_B$, serotonin, or adrenergic receptors (Dunlap and Fischbach, 1978). Subsequent studies over the next 20 years have implicated primarily G $_{i/o}$ -coupled GPCRs in this regulation as muscarinic, opioid, dopamine, and many other G $_{i/o}$ -coupled receptors all have the propensity to exert negative control over calcium currents and exhibit sensitivity to pertussis toxin (Bean, 1989; Boland and Bean, 1993; Elmslie, 1992; Golard and Siegelbaum, 1993; Kasai and Aosaki, 1989). However, it should be noted that modulation of VDCC may not occur solely through the actions of G $_{i/o}$ -coupled GPCRs as activation of G $_s$ and G $_z$ -coupled G proteins has also been shown to mediate voltage-dependent inhibition of N-type calcium channels (Zhu and Ikeda, 1994). Whether this reflects a ubiquitous mechanism of regulation remains unclear, however, it is apparent that inhibition of calcium channels by GPCRs is widespread and an important regulatory factor mediating control of neurotransmitter release.

In addition to G $\beta\gamma$ regulation of VDCC, there is evidence of syntaxin1A regulation of the channels themselves, as well as an intersection between VDCC, syntaxin, and G $\beta\gamma$. Syntaxin1A was initially found to be associated with N-type calcium channels in a pre-

fusion complex (Bennett et al., 1992; Leveque et al., 1994; O'Connor et al., 1993), however, later studies confirmed the molecular and functional interactions between these proteins (Bezprozvanny et al., 2000; Sheng et al., 1994). Further, in addition to the previously mentioned studies of G $\beta\gamma$ regulation of VDCC above, there is evidence of syntaxin1A promoting inhibition of VDCC by G $\beta\gamma$ (Jarvis et al., 2000; Jarvis and Zamponi, 2001) and that this may occur through G $\beta\gamma$ and VDCC binding to separate domains of syntaxin1A (Jarvis et al., 2002).

3.5.2 G $\beta\gamma$ Regulation of G protein-Activated Inward Rectifying (GIRK) Potassium Channels—

GIRK channels are largely believed to be expressed somatodendritically (Fernandez-Alacid et al., 2009; Kulik et al., 2006; Luscher et al., 1997) where they act to hyperpolarize the cell membrane, thereby making it harder to reach the threshold for initiation of an action potential (Luscher et al., 1997; Luscher and Slesinger, 2010). They are members of the inwardly rectifying potassium channel (K_{ir}) superfamily of proteins and are abundantly distributed in atrial cells and neurons where they function to regulate heart rate and neuronal excitability (Clapham and Neer, 1997; Ikeda et al., 2003; Yokogawa et al., 2011). Upon activation, they facilitate efflux of potassium ions out of the cell, hyperpolarizing the cell membrane and dampening electrical activity by making it more difficult to elicit an action potential (Finley et al., 2004). To date, four mammalian GIRK subunits (GIRK 1–4) have been identified which assemble to form functional channels although studies have shown that generally only heterotetramers composed of GIRK 1/2 and to a lesser extent GIRK 2/3 as well as GIRK 2 homotetramers are expressed in the brain (Dascal, 1997; Finley et al., 2004; Peng et al., 2003; Sadjia and Reuveny, 2009).

Unique to the K_{ir} family, however, the activity of postsynaptic GIRK channels is regulated through activation of G_{i/o}-coupled GPCRs such as opioid, M₂ muscarinic, α_2 adrenergic, 5-HT_{1A} serotonin, and GABA_B receptors (Ikeda et al., 2003; Ponce et al., 1996; Sadjia et al., 2003), with activation occurring as a result of direct interactions of G $\beta\gamma$ subunits with the N and C-termini (Huang et al., 1995; Logothetis et al., 1987; Sadjia and Reuveny, 2009). As with many G $\beta\gamma$ effectors, regulation of GIRK channels was initially proposed to occur at sites along the G α -G $\beta\gamma$ interface. Studies by Ford *et al.* (Ford et al., 1998) and Albsoul-Younes *et al.* (Albsoul-Younes et al., 2001) demonstrated that eight residues falling within this region (Leu55, Lys78, Ile80, Lys89, Trp99, Asp228, Asp246 and Trp332) were important for channel activation. Subsequent work went on to identify additional sites of interaction with Absoul-Younes *et al.* first showing that mutation of residues along or near the loops connecting blades 1 and 2 of the G β torus was sufficient to disrupt GIRK channel activation. Subsequent work by Mirshahi *et al.* (Mirshahi et al., 2002) and Zhao *et al.* (Zhao et al., 2003) narrowed this to five specific residues within these loops (Ser67, Thr86, Thr87, Thr128, and Gly131) as playing a crucial role in GIRK activation, while Li *et al.* (Li et al., 2005) recently reported the ability of the C-terminal region of G β_2 (residues 271–305) to induce GIRK channel activation. It should be noted, however, that in addition to G β , G γ has been shown to be essential for G protein activation of GIRK channels. A recent study by Peng *et al.* demonstrated that residues 35–71 of G γ_2 are required for full stimulation of GIRK4 currents by G $\beta_1\gamma_2$ (Peng et al., 2003). Further, within this region, ten residues form an intricate network of interactions with G β to determine the stimulatory effects on the channel while Asp36 and Asp48 maintain the conformation of the G $\beta_1\gamma_2$ subunits to allow full stimulation of GIRK4. Together, these results highlight the fact that G $\beta\gamma$ regulation of GIRK channels may be much more complicated than originally believed and illustrate the need for further research in this area.

Studies examining specificity of GIRK channel regulation have further shown that only G $\beta\gamma$ dimers containing G β_{1-4} could enhance GIRK channel activity while those containing G β_5 subunits actually suppress it (Lei et al., 2000; Lei et al., 2001; Reuveny et al., 1994; Takao et

al., 1994; Wickman et al., 1994; Yamada et al., 1994). While the exact reason for this discrepancy is still being elucidated, a plausible explanation presented by Lei *et al.* is that Gβ₅ containing dimers actually displace activating Gβγ pairs by competitively binding to the same regions on the cytoplasmic domains of the GIRK channels (Lei et al., 2003). Although no hypothesis was put forth to explain how this would prevent channel opening, given that Gβ₅ is the most divergent of the Gβ subunits, it is possible that while sufficient sequence identity exists between the isoforms to permit binding to cytoplasmic domains, critical residues necessary for channel activation may be missing. As such, by competitively displacing activating Gβγ dimers, Gβ₅ pairs could occupy binding sites and prevent channel activation. Conversely, however, Xie *et al.* (2010) recently showed that Gβ₅ subunits in complex with members of the Regulator of G protein Signaling (RGS) 7 family bound to the C-terminal cytoplasmic domains of GIRK channels in order to facilitate their coupling to GABA_B receptors (Xie et al., 2010). In doing so, they found that the Gβ₅/RGS complex acted to influence the sensitivity of the inhibitory signaling pathway in hippocampus and that further, the absence of the Gβ₅ subunits disrupted the temporal fidelity of the GIRK response. Such discrepancies highlight that the role of Gβ₅ subunits in regulating potassium currents merits further investigation.

While a postsynaptic role for GIRK channels has been recognized in many studies, consistent presynaptic labeling of these channels via immunostaining and electron microscopy in the proximity of the active zone also hints for a role in modulating exocytotic release (Fernandez-Alacid et al., 2009; Ladera et al., 2008; Lujan et al., 2009; Michaeli and Yaka, 2010; Ponce et al., 1996). At least in the case of GABA signaling, there is evidence to support this assumption as recent reports from Ladera *et al.* (2008) as well as Michaeli and Yaka (2010) suggest new mechanisms in which presynaptic GIRK channels play a significant role. For example, Ladera *et al.* (2008) demonstrated via electron microscopy that GIRK channels and GABA_B receptors were co-expressed in a restricted subset of cerebrocortical nerve terminals and that further, these GIRK channels mediated the inhibition of glutamate release. Similarly, Michaeli and Yaka (2010) recently proposed a new mechanism for dopamine-induced inhibition of GABA_A neurotransmission whereby activation of D₂-like receptors is thought to lead to activation of presynaptic GIRK channels in ventral tegmental neurons (Michaeli and Yaka, 2010). As opposed to postsynaptic GIRK regulation, however, such effects may not be mediated through Gβγ subunits. In case of the GABA_B receptor-GIRK pathway in cerebrocortical nerve terminals, responses exhibited resistance to pertussis toxin, whereas in the case of GABA_A currents in the ventral tegmental area, the authors propose regulation through protein kinase A substrates given attenuation of effects with protein kinase A inhibitors. It remains to be elucidated whether this represents regulation unique to presynaptic GIRK channels in addition to the well characterized regulation by Gβγ subunits.

Although a great deal of work has been done to decipher the mechanism by which Gβγ subunits regulate activation of GIRK channels and thus synaptic transmission, many questions remain unanswered. Given the diversity of binding sites on both the channel and the G protein, it is possible that multiple dimers bind to the channel at the same time to elicit the rotation and expansion of the N- and C-termini required for GIRK channel activation (Sadja and Reuveny, 2009). How this may occur and how it would affect synaptic regulation remains to be seen but such a possibility certainly highlights that a great deal of work still remains.

3.6 Modulation of Synaptic Transmission Downstream of Ion Channels: Gβγ Modulation of SNARE Proteins

While G protein modulation of calcium and potassium entry is well established, more recently, modulation of synaptic transmission distal to calcium entry has been demonstrated.

G protein modulation of vesicle release in a manner independent of calcium entry via calcium channels was first noted by Silinsky in 1984, who observed that evoked acetylcholine release at the frog neuromuscular junction could be reduced following activation of presynaptic adenosine A1 receptors (Silinsky, 1984). He suggested that this inhibition was exerted at an intracellular site associated with the exocytotic process, such as the synaptic vesicles or the active zone, as miniature end plate potentials could still be reduced when the normal route of calcium entry was bypassed (Miller, 1998; Silinsky, 1984). These observations have been corroborated over the years as studies investigating receptor function in the hippocampus and other brain regions have indicated that activation of $G_{i/o}$ -coupled presynaptic receptors such as GABA_B, muscarinic, metabotropic glutamate (mGluR), serotonergic, and opioid receptors all result in a reduction in the frequency of miniature excitatory post-synaptic currents (Miller, 1998). Similarly, the use of agents such as ionomycin and α -latrotoxin which enhanced secretion while bypassing calcium channels lent further support to this conclusion as GPCR-mediated inhibition was maintained in permeabilised cells (Blackmer et al., 2005; Meldolesi et al., 1984). Taken together, these results suggested the presence of a novel mechanism controlling vesicle release which needed to be characterized.

Given that receptor-mediated inhibition can be mimicked by treatment with non-hydrolyzable GTP analogues (Ahnert-Hilger et al., 1987; Knight and Baker, 1985; Luini and De Matteis, 1990), a great deal of work went into identifying the G protein responsible for mediating the effect. This was initially challenging due to an inability of exogenous peptides to access presynaptic terminals but was overcome by direct injection of G $\beta\gamma$ subunits into the Calyx of Held (Kajikawa et al., 2001) and lamprey reticulospinal-motor neuron synapses (Blackmer et al., 2001). In the latter case, G $\beta\gamma$ subunits not only were able to inhibit evoked transmitter release, but did so without an effect on presynaptic calcium entry (Blackmer et al., 2001) suggesting that these subunits were responsible for the observed inhibition. Subsequent injection of a G $\beta\gamma$ scavenger, G protein-coupled receptor kinase 2 (GRK2) (Blackmer et al., 2001) or phosducin (Blackmer et al., 2005) supported this, as the inhibitory actions of G $\beta\gamma$ were completely alleviated. Investigations into the mechanism by which G $\beta\gamma$ inhibited vesicle exocytosis initially focused on known effectors in G $\beta\gamma$ signaling cascades. However, as blockade of classical effectors did little to attenuate G $\beta\gamma$ -mediated inhibition (Blackmer et al., 2001) and the onset of inhibition due to laser uncaging of 5HT was too rapid to affect docking and priming mechanisms (Gerachshenko et al., 2005), it was proposed that G $\beta\gamma$ may act directly upon the already-primed exocytotic machinery. In fact, inhibition was already initiated by 20 ms after uncaging 5HT, suggesting that both the GPCR and the G $\beta\gamma$ were close to the active zone. Interestingly, the ability of G $\beta\gamma$ to modulate the inhibitory effect on exocytosis was abolished by treatment with botulinum toxin A, which cleaves 9 amino acids from the C-terminus of SNAP-25 (Binz et al., 1994; Gerachshenko et al., 2005; Schiavo et al., 1993). The relevance of this C-terminal region of SNAP-25 was additionally demonstrated as the C-terminal 14 amino acid peptide from SNAP-25 itself eliminated the G $\beta\gamma$ -mediated inhibition (Blackmer et al., 2005; Gerachshenko et al., 2005). In addition, removal of the C-terminal residues from SNAP-25 decreased binding to G $\beta\gamma$ *in vitro* (Yoon et al., 2007; Zhao et al., 2010). These findings provided a “fingerprint” for studies of the mechanism of inhibition of exocytosis downstream from modulation of voltage-dependent Ca²⁺ channels directly on the exocytotic fusion apparatus that later studies have taken advantage of (Delaney et al., 2007; Zhang et al., 2011; Zhao et al., 2010).

In vitro binding studies also demonstrated direct interaction between G $\beta\gamma$ subunits and syntaxin 1A, SNAP-25B and VAMP/synaptobrevin that make up the SNARE complex (Blackmer et al., 2001; Blackmer et al., 2005; Yoon et al., 2007) while subsequent electrophysiological studies, fluorescence-based assays, and PC12 “cracked cell”-based

amperometry studies provided evidence of G $\beta\gamma$ binding to the H₃ SNARE domain of syntaxin and confirmed the importance of the carboxy-terminal residues of SNAP-25 (Yoon et al., 2007; Zhao et al., 2010). Additional sites of interaction may be important including the H_{abc} domain (Yoon et al., 2007) or even the C-terminal transmembrane domain of syntaxin which has been suggested to potentially form a binding pocket for anesthetic molecules (Herring et al., 2011). Similarly, mutagenesis studies suggest SNARE binding sites on G $\beta\gamma$ subunits may reside outside the G α binding face, the site of interaction with many G $\beta\gamma$ effectors (Ford et al., 1998). Blackmer *et al.* (2005) demonstrated that mutation of residues that contact G α subunits, as well as numerous other effectors such as adenylyl cyclase, GRK2, and phospholipase C β 2, did not induce a loss of function in the ability of the G $\beta\gamma$ subunit to inhibit calcium-triggered exocytosis. Rather, several mutations demonstrated increased potency for inhibiting secretion, suggesting further studies are necessary to characterize the binding surface required for G $\beta\gamma$ -SNARE interactions.

Regardless of the site at which G $\beta\gamma$ binds to SNARE proteins, in doing so, it is thought to prevent the tight zippering of the SNARE complex necessary to drive membrane fusion, and thus can inhibit vesicle exocytosis. However, the H₃ domain and the C-terminus of SNAP-25 are also known to constitute functionally important calcium-dependent synaptotagmin binding sites, and detailed examination has revealed that G $\beta\gamma$ subunits and synaptotagmin actually compete with one another for binding to the SNARE proteins as calcium levels increase; at low intracellular calcium, G $\beta\gamma$ binding predominates, while after increasing intracellular calcium, synaptotagmin binding actually competes with G $\beta\gamma$ (Yoon et al., 2007). Although the significance of such competition is as yet unknown, the current working hypothesis is that G $\beta\gamma$ inhibits exocytosis by competing with synaptotagmin for binding to the SNARE proteins. Increasing intracellular calcium due to repetitive stimulation of neurons may serve to attenuate this by increasing synaptotagmin's affinity for the SNARE proteins and allowing it to displace G $\beta\gamma$ subunits from the exocytotic machinery. Thus, depending on presynaptic G_{i/o}-coupled GPCR activation and calcium influx in response to synaptic activity, it is possible that G $\beta\gamma$ -mediated regulation of synaptic transmission could occur independently through more than one mechanism, or potentially even be additive or synergistic at a particular synapse (see Figure 4). It should be noted, however, that regardless of whether G protein modulation occurs via calcium currents or not, intact SNARE proteins have been shown in each instance to be required for the effect to be seen (Searl and Silinsky, 2005; Silinsky, 2008; Stanley and Mirotznik, 1997). Furthermore, synaptotagmin or another calcium sensor in addition to intact SNARE was important for G protein modulation of calcium dependent miniature endplate potentials, but not calcium independent miniature endplate potentials (Searl and Silinsky, 2005).

While the implications for such a graded physiological mechanism are profound, currently little is known about the occurrence or relevance of this mechanism *in vivo*. Studies examining rhythmic motor activities in vertebrate species, have suggested a role for G $\beta\gamma$ interactions in modulating fictive locomotion, which drives the coordinated contraction of muscles necessary for behaviors such as swimming. In their 2005 paper, Schwartz *et al.*, demonstrated that inhibition of glutamate release via activation of 5HT_{1D} receptors prolonged the frequency of ventral root bursting in lamprey reticulospinal neurons by presynaptically inhibiting synaptic transmission (Schwartz et al., 2005). In doing so, 5-HT was shown to slow the rhythm of fictive locomotion (Gabriel et al., 2009; Gerachshenko et al., 2009; Photowala et al., 2006; Schwartz et al., 2005). As extensive evidence previously demonstrated a role for G $\beta\gamma$ /SNARE interactions in the lamprey during serotonergic modulation, the authors suggest that 5-HT mediates its effects through activation of presynaptic receptors to inhibit transmitter release and decrease glutamatergic synaptic drive (Schwartz et al., 2005). This mechanism was elucidated further in 2009, when Gerachshenko *et al.* demonstrated that 5-HT_{1D} receptor-mediated reductions in glutamate

release acted to modulate locomotor rhythms (Gerachshenko et al., 2009). During physiologically relevant trains of activity similar to those that evoke locomotion, the authors were able to show that 5-HT/G $\beta\gamma$ -mediated inhibition is relieved over time, consistent with the idea that rising presynaptic calcium concentrations permit synaptotagmin competition with G $\beta\gamma$ (Gerachshenko et al., 2009). As a result, frequency-dependent increases in synaptic concentrations of glutamate are observed which serve to reestablish synaptic vesicle release (Gerachshenko et al., 2009). Modulation of locomotor patterns and motor outputs by presynaptic GPCRs has further been explored in other vertebrate species including *Xenopus* (Sillar et al., 1992), neonatal rats (Liu and Jordan, 2005), and zebrafish (Gabriel et al., 2009). For example, Gabriel *et al.* (2009), show that 5-HT is endogenously released during fictive locomotion in juvenile and adult zebrafish and acts to potentiate inhibitory synaptic transmission resulting in a decrease in the locomotor frequency, similar to that seen in lamprey (Gabriel et al., 2009). While the exact mechanism is not elucidated in their papers, it is conceivable that much like what is seen in lamprey, release of G $\beta\gamma$ subunits modulates SNARE function to limit frequency and duration of synaptic vesicle release and thus alter locomotor behaviors.

While such effects in lower vertebrate species is interesting, extrapolating these results to mammals is difficult, particularly as very few of the mechanisms by which G $\beta\gamma$ -coupled GPCRs modulate synaptic transmission in humans have been fully explored; further, those that have primarily point to activated G $\beta\gamma$ modifying channel properties. Some recent studies have begun to counteract this, giving evidence for the relevance of the G $\beta\gamma$ /SNARE mechanism in a variety of systems. For example, insulin secretion from pancreatic β cells has been shown to be reduced following activation of GPCRs in a number of ways, the most powerful of which is a distal inhibitory effect occurring downstream of increased intracellular calcium (Komatsu et al., 1995). Zhao *et al.*, demonstrated in 2010 that the ability of noradrenaline to mediate this inhibition was due to a reduction in the number of exocytotic events without changes in vesicle size or fusion pore properties (Zhao et al., 2010). Further efforts attributed these effects to G $\beta\gamma$ subunits as the $\beta\gamma$ -activating peptide, mSIRK, inhibited exocytosis to a similar extent as noradrenaline, while anti-G β antibodies were able to eliminate it (Zhao et al., 2010). Further, the authors went on to suggest that such modulation occurs via G $\beta\gamma$ binding directly to SNARE proteins, as pretreatment with botulinum toxin A, which cleaves the C-terminal 9 amino acids of SNAP-25, a functionally important G $\beta\gamma$ binding site (Yoon et al., 2007), was able to prevent noradrenaline from inhibiting exocytosis. Finally when the duration of calcium influx was extended via longer depolarizing pulses, the authors were able to show that both noradrenaline-induced and mSIRK-mediated inhibition was abolished, consistent with the idea that synaptotagmin is able to successfully compete with G $\beta\gamma$ for binding to SNAP-25 (Blackmer et al., 2005; Gerachshenko et al., 2005; Zhao et al., 2010). While the relative importance of this mechanism *in vivo* has yet to be fully elucidated, it is conceivable that it may play an important modulatory role which is disrupted in diabetic patients. As such, further efforts into understanding its role may provide insight into disease progression and highlight new avenues of treatment.

Such actions also appear to exist in the central nervous system as Delaney *et al.* (2007) recently provided evidence to support the idea that the nociceptive actions of noradrenaline may be mediated through G $\beta\gamma$ /SNARE interactions in the central nucleus of the amygdala (Delaney et al., 2007). This region receives dense ascending noradrenergic projections and exhibits high levels of α_{2a} adrenergic receptors, stimulation of which modulates nociceptive information into the amygdala via a presynaptic mechanism (Delaney et al., 2007). Using whole-cell recording techniques, the authors were able to show that both noradrenaline and clonidine dose-dependently inhibited parabrachial excitatory input into the central amygdala, an effect which was reversed through application of the selective α_2 -adrenoceptor

antagonist, yohimbine (Delaney et al., 2007). Using strontium, they went on to demonstrate that the effect occurred via a presynaptic mechanism while application of pertussis toxin or the sulphhydryl alkylating agent N-ethylmaleimide both abolished inhibition, confirming a $G_{i/o}$ -mediated effect (Delaney et al., 2007). Such actions were further attributed to $G\beta\gamma$ subunits as application of a $G\beta\gamma$ binding peptide blocked the effect of noradrenaline, while examination of changes in the action potential-driven rise in terminal free calcium levels confirmed the effect was downstream of calcium entry (Delaney et al., 2007). Finally, direct interactions with SNARE proteins were confirmed through incubation with botulinum toxin A, yielding similar results to previously published reports (Blackmer et al., 2005; Gerachshenko et al., 2005; Yoon et al., 2007) whereby noradrenergic inhibition was reduced with increasing incubation times (Delaney et al., 2007). Similarly, Iremonger and Bains (2009) demonstrated that dynorphin released from dendritic vesicles in response to postsynaptic activity acted in a retrograde manner to inhibit excitatory neurotransmission through a presynaptic mechanism downstream of calcium entry in the hypothalamus. Using whole-cell patch recordings from magnocellular neurosecretory cells in the paraventricular nuclei of the hypothalamus, the authors showed that dynorphin produced a robust inhibition of glutamate release onto vasopressin-expressing magnocellular neurosecretory cells via activation of presynaptic κ -opioid receptors. Further, they showed that this effect did not require inhibition of the adenylyl cyclase/protein kinase A pathway, and that it persisted despite inhibition of N- or P/Q-type calcium channels. Rather, the authors suggest that activation of presynaptic κ -opioid receptors directly modulates glutamate release through direct interaction of the $G\beta\gamma$ subunits with the exocytotic machinery, given that such inhibition could be alleviated through rising intracellular calcium, consistent with competition for binding to SNARE proteins by synaptotagmin (Iremonger and Bains, 2009).

While these examples support the idea that $G\beta\gamma$ /SNARE interactions play important roles in transient presynaptic inhibition, the question remains as to whether they play a role in long-term alterations of presynaptic signals associated with long-term potentiation (LTP) or long-term depression (LTD). Activity dependent, long-term changes in synaptic strength associated with LTP and LTD play important roles in CNS functions such as learning and memory, development of neural networks, and fine-tuning of synaptic connections (Zhang et al., 2011). GPCRs have been shown to be central to induction of LTD, but until recently it was believed that this process was primarily mediated by $G\alpha_i$ subunits. This was recently questioned as Zhang *et al.* sought to investigate whether $G\beta\gamma$ released along with $G\alpha_i$ may also be necessary for the transition from transient inhibition to presynaptic LTD in the hippocampus (Zhang et al., 2011). Using botulinum toxin-A, the authors showed that the C-terminus of SNAP-25 is critical for LTD but not LTP at Schaffer collateral-CA1 synapses. Cleavage of residues 198–206 on SNAP-25 completely prevented the induction of stimulus-evoked LTD, but alterations in extracellular calcium concentrations demonstrated that this reduction by botulinum toxin-A was not simply due to a reduction in vesicle release probabilities (Zhang et al., 2011). To examine the possibility that binding of $G\beta\gamma$ to SNAP-25 was a necessary step in mediating the presynaptic component of LTD, the authors then went on to electroporate a 14 amino acid peptide of the C-terminus of SNAP-25 into presynaptic CA3 pyramidal neurons as this peptide had previously been shown to scavenge free $G\beta\gamma$ (Gerachshenko et al., 2005). Compared to a scrambled peptide, infusion of the SNAP-25 peptide significantly reduced mGluR-mediated presynaptic depression and stimulus-evoked LTD. Further, infusion of a $G\beta\gamma$ binding peptide, mSIRK, which similarly blocked induction of LTD, suggested that scavenging of $G\beta\gamma$ at Schaffer collateral release sites could reverse GPCR mediated reductions in transmitter release (Zhang et al., 2011). Finally, imaging of calcium concentrations showed that presynaptic calcium influx was not significantly altered following cleavage of SNAP-25, suggesting that presynaptic LTD is not a result of long-term reductions in calcium concentrations (Zhang et al., 2011). Taken together, such data led the authors to conclude that $G\beta\gamma$ released along with $G\alpha_i$ may play an

important role in mediating long term changes at presynaptic release sites and postulate that a greater understanding of such molecular mechanisms will be critical to evaluating the role that presynaptic long-term plasticity plays in persistent changes associated with learning and memory (Zhang et al., 2011).

4 Implications of G protein Modulation of Synaptic Transmission for Disease

As many $G_{i/o}$ -coupled GPCRs act as feedback regulators for transmitter release from presynaptic terminals, a greater understanding of the mechanisms by which they operate will be important for understanding normal neural processing, but also because dysregulation of this process can have serious health consequences. For example, mutations in the promoter of the 5HT_{1a} serotonin receptor results in abnormally high autoreceptor expression, decreased serotonin release, and increased susceptibility to depression (Le François et al., 2008). Patients known to carry a risk-associated α_{2a} -adrenergic receptor (*ADRA2A*) polymorphism have been shown to express increased levels of presynaptic α_{2A} -adrenergic receptors and reduced glucose-stimulated insulin release, resulting in higher incidences of diabetes (Rosengren et al., 2010). This effect was also seen in mice with restricted overexpression of *ADRA2A* in pancreatic islet cells (Devedjian et al., 2000). In Alzheimer's patients, activation of M₂ autoreceptors during anticholinesterase treatment has been suggested to actually contribute to disease progression by reducing acetylcholine release (Albrecht et al., 1999) while continuous D₂ autoreceptor-mediated attenuation of dopamine release during dopamine agonist treatment is thought to contribute to the deterioration of cognitive function in Parkinson's patients (Arnsten et al., 1994; Breitenstein et al., 2006). As such, these receptors represent major targets in drug development, with agonists such as risperidone (Janssen et al., 1988), buspirone (Taylor et al., 1985), and guanfacine (Hunt et al., 1995) targeting the D₂ dopamine, 5HT_{1a} serotonin, and α_{2a} adrenergic receptors for the treatment of schizophrenia, anxiety disorders, and attention deficit hyperactivity disorder, respectively. In addition, a number of drugs at various stages in the development pipeline target this class of receptors, such as M₄ muscarinic receptors for schizophrenia, Parkinson's disease, psychosis and dystonia, H₃ histamine receptors for cognitive deficits, sleep-wake disorders, and attention-deficit disorder, mGluR2 and 3 metabotropic glutamate receptors for schizophrenia and anxiety, and mGluR4 receptors for Parkinson's disease and dystonia. Unfortunately, many current therapies exhibit significant side effects which limit their efficacy. For instance, risperidone is often associated with severe weight gain while guanfacine can induce orthostatic hypotension in patients. In some cases, these side effects may be due to off-target effects such as seen with risperidone where weight gain is thought to be mainly associated with an affinity of the molecule for serotonin 5HT_{2a} receptors; in others, the effects may be because these receptors modulate differing functions in various brain regions. Regardless, these examples highlight the fact that targeting the GPCRs directly may mediate both the therapeutically desirable effect but also untoward side effects. As such, a better understanding of the downstream mechanisms by which presynaptic inhibitory GPCRs modulate exocytosis, such as through G $\beta\gamma$ /SNARE interactions, may reveal more disease-selective targets for therapeutic intervention.

5 Open Questions

A number of open questions remain regarding the role G $\beta\gamma$ -SNARE interactions play in regulating exocytosis. For example, what is the role of individual G $\beta\gamma$ isoforms in this process? Despite the 5 G β and 13 G γ subunits sharing high levels of sequence identity (Downes and Gautam, 1999; Hildebrandt, 1997), it is known that pairs can exhibit functional selectivity in their interactions with both receptors and effectors (Dingus et al., 2005; Kleuss et al., 1993; Richardson and Robishaw, 1999). Unfortunately, little is known

about the subcellular localization of particular isoforms to specific presynaptic terminals, nor which isoforms are activated by a particular receptor. Given that most cell types express multiple $G\alpha$, $G\beta$, and $G\gamma$ subtypes, how is specificity for one mechanism over another achieved? Does modulation of ion channels or SNARE proteins at a given synapse occur through activation of distinct $G\beta\gamma$ subunits or is regulation specified by a particular ligand or receptor? Further, do conditions exist which would allow switching between mechanisms for a particular synapse, and what will the functional consequences of such a situation be? In any case, a greater understanding of cellular distributions as well as $G\beta\gamma$ -receptor and $G\beta\gamma$ -effector interactions will be necessary to fully elucidate this process.

Similarly, the question arises as to how applicable $G\beta\gamma$ regulation of release is in other cell types which don't involve the classical neuronal SNAREs of syntaxin 1a, SNAP-25, and synaptobrevin. Multiple isoforms of all three proteins exist, with SNAP-23 and SNAP-29 both known to be expressed in the brain and be important for exocytosis. Further, secretion is a common physiological event in many other cell types besides neurons. Some such as insulin secretion from pancreatic β cells is through the same SNARE proteins as neurons, so the extension is straightforward from neurons to β cells. Other systems such as platelets (Chen et al., 2000; Graham et al., 2009; Jardin et al., 2007; Polgar et al., 2002), endothelial cells (Predescu et al., 2005; Pulido et al., 2011; Yamakuchi et al., 2008), and immune cells such as neutrophils, eosinophils, macrophages, and mast cells (Stow et al., 2006) primarily use SNAP-23 instead of SNAP-25, as well as a variety of different syntaxin and VAMP isoforms as well. While there is a high degree of sequence similarity between the SNARE isoforms, and a preponderance of GPCR signaling in their regulation and activation, little is known at this point about whether $G\beta\gamma$ or activation of $G_{i/o}$ -coupled receptors have a similar role in these systems as is seen in the brain.

Another unanswered question is what the full extent of $G\beta\gamma$ regulation on vesicle release is beyond competition with synaptotagmin 1. As reviewed above, there are other critical members of the vesicle docking/priming/fusion process that interact with the exocytotic machinery which could be affected by $G\beta\gamma$ interaction with SNARE proteins. $G\beta\gamma$ interaction with syntaxin 1A is not limited to the extreme C-terminus where synaptotagmin 1 binds (Chapman et al., 1995; Yoon et al., 2007). An interaction with the N-terminal H_{abc} domain may have implications with Munc18-syntaxin 1A interactions for example and thereby influence vesicle docking and/or priming. The ability of $G\beta\gamma$ to bind directly to other members of the exocytotic process has not been reported. Another facet of this question is a stoichiometric one. Does the interaction of one $G\beta\gamma$ dimer with a docked vesicle inhibit its fusion, or does each SNARE complex require a bound $G\beta\gamma$? Furthermore, is there a gradation depending on the stoichiometry of SNARE complexes surrounding a given fusion pore of a vesicle? Based on the three-dimensional structure of a toroid shape of the $G\beta\gamma$ dimer and the rod like coiled-coil of four α -helices of the ternary SNARE complex, does a $G\beta\gamma$ dimer interact simultaneously with two juxtaposed SNARE complexes at a fusion pore? The interaction of $G\beta\gamma$ with the SNARE complexes may provide a unique ability to address these questions and to answer fundamental unknown mechanisms of exocytosis.

There are many proteins that have varied roles in exocytosis, and the implications of $G\beta\gamma$ regulation in their function is not fully understood. Dynamin, for example, is a GTPase protein that is important in both endocytosis, playing a role in forming synaptic endosomes, as well as exocytosis by its ability to regulate quantal size and to have a dual function in both kiss-and-run and full fusion modes in chromaffin cells (Chan et al., 2010). Further, dynamin has been shown to interact with $G\beta\gamma$ *in vitro* (Lin and Gilman, 1996) and in cultured neuroendocrine cells (Liu et al., 1997), and this interaction can inhibit its GTPase activity. Would $G\beta\gamma$ control quantal size by directly modulating the activity of dynamin at

the exocytotic pore in addition to competing with synaptotagmin for binding to SNARE proteins? Another interesting protein is the cysteine string protein (CSP), a member of the heat shock protein 40 family that has implications in preventing presynaptic neurodegeneration (Schmitz and Fernández-Chacón, 2009), and recently has been shown to be a chaperone for SNAP-25 (Sharma et al., 2011). CSP can bind both $G\beta\gamma$ and $G\alpha$, and it acts as a nucleotide exchange factor for $G\alpha$ exchanging GDP for GTP (Natochin et al., 2005). The binding of CSP by $G\beta\gamma$ has been shown to facilitate the ability of $G\beta\gamma$ to inhibit calcium channels (Miller et al., 2003). Do $G\beta\gamma$ -SNARE and $G\beta\gamma$ -CSP interactions intersect at the synapse to provide cross-regulation of exocytosis and its preservation through chaperoning SNAP-25? Additionally, two proteins already mentioned in this review that bind SNARE proteins are tomosyn and complexin. What role does $G\beta\gamma$ binding of SNARE proteins play in their function, or is there competition or synergy of their interactions? Lastly, this manuscript focused on the competition between synaptotagmin1 and $G\beta\gamma$ for SNARE protein binding, but could $G\beta\gamma$ interfere with other isoforms of synaptotagmins, or perhaps with doc2's role in asynchronous release of synaptic vesicles?

Finally, the question remains as to the physiological relevance of $G\beta\gamma$ regulation to synaptic function. While it is known for example, that $G\beta\gamma$ can modulate both SNARE proteins and VDCC, it is as yet unclear what the relative contribution of either mechanism is to normal synaptic functions. The differential calcium requirement for these mechanisms provides for differential calcium-sensitivity which may complete a feedback loop, because $G\beta\gamma$ may control both Ca^{2+} influx through VDCC and access of the Ca^{2+} effector synaptotagmin to its site on the SNARE. Thus, depending on presynaptic $G_{i/o}$ -coupled GPCR activation and Ca^{2+} influx in response to synaptic activity, a range of efficiencies of exocytosis potentially exists. Does such dynamic regulation exist, and if so, what role does it play in presynaptic integration and plasticity? Further, what are the implications for human health and disease if this process is disrupted?

6 Conclusion

GPCR mediated regulation of synaptic transmission is complex and transcends many different pathways. While a great deal has been learned to date, numerous questions still remain as to the mechanisms underlying this process in the brain as well as other systems. While interactions between $G\beta\gamma$ subunits and classical effectors is well established, newer mechanisms such as $G\beta\gamma$ -SNARE interactions highlight levels of intricacy as yet unknown. Further research into this novel protein-protein interaction will be essential for elucidating its role in regulating synaptic transmission, as well as its integration with other effectors. Only in doing so will it be possible to accurately describe normal synaptic functioning as well as develop strategies to correct situations where it has gone awry.

Highlights

- > This review focuses on the presynaptic regulation of neurotransmission.
- > Heterotrimeric G protein signaling is an important part of that regulation.
- > $G\beta\gamma$ has a novel means of regulating synaptic neurotransmission.
- > This regulation may have a large impact on disease pathology and treatment.
- > There are many open questions relating to G protein signaling and neurotransmission.

Acknowledgments

Research in the authors' laboratory was supported by a grant from the National Institute of Health, R01 EY010291.

References

- Ahnert-Hilger G, Brautigam M, Gratzl M. Ca²⁺-stimulated catecholamine release from alpha-toxin-permeabilized PC12 cells: biochemical evidence for exocytosis and its modulation by protein kinase C and G proteins. *Biochemistry*. 1987; 26(24):7842–7848. [PubMed: 3322408]
- Albert PR, Robillard L. G protein specificity: traffic direction required. *Cell. Signal*. 2002; 14(5):407–418. [PubMed: 11882385]
- Albrecht C, Bloss HG, Jackisch R, Feuerstein TJ. Evaluation of autoreceptor-mediated control of [3H]acetylcholine release in rat and human neocortex. *Exp. Brain Res*. 1999; 128(3):383–389. [PubMed: 10501810]
- Albsoul-Younes AM, Sternweis PM, Zhao P, Nakata H, Nakajima S, Nakajima Y, Kozasa T. Interaction sites of the G protein beta subunit with brain G protein-coupled inward rectifier K⁺ channel. *J. Biol. Chem*. 2001; 276(16):12712–12717. [PubMed: 11278861]
- Allen TG, Brown DA. M2 muscarinic receptor-mediated inhibition of the Ca²⁺ current in rat magnocellular cholinergic basal forebrain neurones. *J. Physiol*. 1993; 466:173–189. [PubMed: 8410690]
- Aravamudan B, Fergestad T, Davis WS, Rodesch CK, Broadie K. Drosophila Unc-13 is essential for synaptic transmission. *Nat. Neurosci*. 1999; 2(11):965–971. [PubMed: 10526334]
- Arnsten A, Cai J, Murphy B, Goldman-Rakic P. Dopamine D1 receptor mechanisms in the cognitive performance of young adult and aged monkeys. *Psychopharmacology*. 1994; 116(2):143–151. [PubMed: 7862943]
- Augustin I, Rosenmund C, Sudhof TC, Brose N. Munc13-1 is essential for fusion competence of glutamatergic synaptic vesicles. *Nature*. 1999; 400(6743):457–461. [PubMed: 10440375]
- Bean BP. Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. *Nature*. 1989; 340(6229):153–156. [PubMed: 2567963]
- Becherer U, Rettig J. Vesicle pools, docking, priming, and release. *Cell Tissue Res*. 2006; 326(2):393–407. [PubMed: 16819626]
- Bellingham MC, Berger AJ. Presynaptic depression of excitatory synaptic inputs to rat hypoglossal motoneurons by muscarinic M2 receptors. *J. Neurophysiol*. 1996; 76(6):3758–3770. [PubMed: 8985874]
- Bennett MK, Calakos N, Scheller RH. Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. *Science*. 1992; 257(5067):255–259. [PubMed: 1321498]
- Betty M, Harnish SW, Rhodes KJ, Cockett MI. Distribution of heterotrimeric G-protein beta and gamma subunits in the rat brain. *Neuroscience*. 1998; 85(2):475–486. [PubMed: 9622245]
- Bezprozvanny I, Zhong P, Scheller RH, Tsien RW. Molecular determinants of the functional interaction between syntaxin and N-type Ca²⁺ channel gating. *Proc. Natl. Acad. Sci. USA*. 2000; 97(25):13943–13948. [PubMed: 11087812]
- Binz T, Blasi J, Yamasaki S, Baumeister A, Link E, Sudhof TC, Jahn R, Niemann H. Proteolysis of SNAP-25 by types E and A botulinum neurotoxins. *J. Biol. Chem*. 1994; 269(3):1617–1620. [PubMed: 8294407]
- Blackmer T, Larsen EC, Takahashi M, Martin TF, Alford S, Hamm HE. G protein betagamma subunit-mediated presynaptic inhibition: regulation of exocytotic fusion downstream of Ca²⁺ entry. *Science*. 2001; 292(5515):293–297. [PubMed: 11303105]
- Blackmer T, Larsen EC, Bartleson C, Kowalchuk JA, Yoon EJ, Preiner AM, Alford S, Hamm HE, Martin TF. G protein betagamma directly regulates SNARE protein fusion machinery for secretory granule exocytosis. *Nat. Neurosci*. 2005; 8(4):421–425. [PubMed: 15778713]
- Boehm S. Presynaptic alpha2-adrenoceptors control excitatory, but not inhibitory, transmission at rat hippocampal synapses. *J. Physiol*. 1999; 519(Pt 2):439–449. [PubMed: 10457061]

- Boland L, Bean B. Modulation of N-type calcium channels in bullfrog sympathetic neurons by luteinizing hormone-releasing hormone: kinetics and voltage dependence. *J. Neurosci.* 1993; 13(2):516–533. [PubMed: 7678856]
- Bracher A, Kadlec J, Betz H, Weissenhorn W. X-ray Structure of a Neuronal Complexin-SNARE Complex from Squid. *J. Biol. Chem.* 2002; 277(29):26517–26523. [PubMed: 12004067]
- Breitenstein C, Korsukewitz C, Floel A, Kretzschmar T, Diederich K, Knecht S. Tonic Dopaminergic Stimulation Impairs Associative Learning in Healthy Subjects. *Neuropsychopharmacology.* 2006; 31(11):2552–2564. [PubMed: 16880771]
- Brose N, Petrenko AG, Sudhof TC, Jahn R. Synaptotagmin - A Calcium Sensor On The Synaptic Vesicle Surface. *Science.* 1992; 256(5059):1021–1025. [PubMed: 1589771]
- Brown DA, Sihra TS. Presynaptic signaling by heterotrimeric G-proteins. *Handb. Exp. Pharmacol.* 2008; (184):207–260. [PubMed: 18064416]
- Brown SP, Safo PK, Regehr WG. Endocannabinoids inhibit transmission at granule cell to Purkinje cell synapses by modulating three types of presynaptic calcium channels. *J. Neurosci.* 2004; 24(24):5623–5631. [PubMed: 15201335]
- Buranda, T.; Waller, A.; Wu, Y.; Simons, PC.; Biggs, S.; Prossnitz, ER.; Sklar, LA. Some Mechanistic Insights into GPCR Activation from Detergent-Solubilized Ternary Complexes on Beads. In: Stephen, RS., editor. *Adv. Protein Chem. Academic Press*; 2007. p. 95-135.
- Burdina AO, Klosterman SM, Shtessel L, Ahmed S, Richmond JE. *In Vivo* Analysis of Conserved C. *elegans* Tomosyn Domains. *PLoS One.* 2011; 6(10):e26185. [PubMed: 22022557]
- Burgoyne RD, Morgan A. Chaperoning the SNAREs: a role in preventing neurodegeneration? *Nat. Cell. Biol.* 2011; 13(1):8–9. [PubMed: 21173802]
- Cabrera-Vera TM, Vanhauwe J, Thomas TO, Medkova M, Preininger A, Mazzoni MR, Hamm HE. Insights into G Protein Structure, Function, and Regulation. *Endocr. Rev.* 2003; 24(6):765–781. [PubMed: 14671004]
- Cabrera-Vera TM, Hernandez S, Earls LR, Medkova M, Sundgren-Andersson AK, Surmeier DJ, Hamm HE. RGS9-2 modulates D2 dopamine receptor-mediated Ca²⁺ channel inhibition in rat striatal cholinergic interneurons. *Proc. Natl. Acad. Sci. USA.* 2004; 101(46):16339–16344. [PubMed: 15534226]
- Canti C, Page KM, Stephens GJ, Dolphin AC. Identification of Residues in the N Terminus of $\alpha 1B$ Critical for Inhibition of the Voltage-Dependent Calcium Channel by G $\beta\gamma$. *J. Neurosci.* 1999; 19(16):6855–6864. [PubMed: 10436043]
- Capogna M. Distinct properties of presynaptic group II and III metabotropic glutamate receptor-mediated inhibition of perforant pathway-CA1 EPSCs. *Eur. J. Neurosci.* 2004; 19(10):2847–2858. [PubMed: 15147318]
- Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J. International Union of Pharmacology. XLVIII. Nomenclature and Structure-Function Relationships of Voltage-Gated Calcium Channels. *Pharmacol. Rev.* 2005; 57(4):411–425. [PubMed: 16382099]
- Chan S-A, Doreian B, Smith C. Dynamin and Myosin Regulate Differential Exocytosis from Mouse Adrenal Chromaffin Cells. *Cell. Mol. Neurobiol.* 2010; 30(8):1351–1357. [PubMed: 21061163]
- Chapman ER, Jahn R. Calcium-Dependent Interaction Of The Cytoplasmic Region Of Synaptotagmin With Membranes - Autonomous Function Of A Single C-2-Homologous Domain. *J. Biol. Chem.* 1994; 269(8):5735–5741. [PubMed: 8119912]
- Chapman ER, Hanson PI, An S, Jahn R. Ca²⁺ regulates the interaction between synaptotagmin and syntaxin 1. *J. Biol. Chem.* 1995; 270(40):23667–23671. [PubMed: 7559535]
- Chapman ER, Davis AF. Direct interaction of a Ca²⁺-binding loop of synaptotagmin with lipid bilayers. *J. Biol. Chem.* 1998; 273(22):13995–14001. [PubMed: 9593749]
- Chen D, Bernstein AM, Lemons PP, Whiteheart SW. Molecular mechanisms of platelet exocytosis: role of SNAP-23 and syntaxin 2 in dense core granule release. *Blood.* 2000; 95(3):921–929. [PubMed: 10648404]
- Chen X, Tomchick DR, Kovrigin E, Araç D, Machius M, Südhof TC, Rizo J. Three-Dimensional Structure of the Complexin/SNARE Complex. *Neuron.* 2002; 33(3):397–409. [PubMed: 11832227]

- Chicka MC, Chapman ER. Concurrent binding of complexin and synaptotagmin to liposome-embedded SNARE complexes. *Biochemistry*. 2009; 48(4):657–659. [PubMed: 19128031]
- Choi UB, Strop P, Vrljic M, Chu S, Brunger AT, Weninger KR. Single-molecule FRET-derived model of the synaptotagmin 1-SNARE fusion complex. *Nat. Struct. Mol. Biol.* 2010; 17(3):318–324. [PubMed: 20173763]
- Clapham DE, Neer EJ. G protein beta gamma subunits. *Annu. Rev. Pharmacol. Toxicol.* 1997; 37:167–203. [PubMed: 9131251]
- Clarke OB, Caputo AT, Hill AP, Vandenberg JI, Smith BJ, Gulbis JM. Domain Reorientation and Rotation of an Intracellular Assembly Regulate Conduction in Kir Potassium Channels. *Cell*. 2010; 141(6):1018–1029. [PubMed: 20564790]
- Corti C, Aldegheri L, Somogyi P, Ferraguti F. Distribution and synaptic localisation of the metabotropic glutamate receptor 4 (mGluR4) in the rodent CNS. *Neuroscience*. 2002; 110(3):403–420. [PubMed: 11906782]
- Currie KP. Inhibition of Ca²⁺ channels and adrenal catecholamine release by G protein coupled receptors. *Cell. Mol. Neurobiol.* 2010; 30(8):1201–1208. [PubMed: 21061161]
- Dai H, Shen N, Arac D, Rizo J. A quaternary SNARE-synaptotagmin-Ca²⁺-phospholipid complex in neurotransmitter release. *J. Mol. Biol.* 2007; 367(3):848–863. [PubMed: 17320903]
- Dascal N. Signalling via the G protein-activated K⁺ channels. *Cell. Signal.* 1997; 9(8):551–573. [PubMed: 9429760]
- De Waard M, Liu H, Walker D, Scott VE, Gurnett CA, Campbell KP. Direct binding of G-protein betagamma complex to voltage-dependent calcium channels [see comments]. *Nature*. 1997; 385(6615):446–450. [PubMed: 9009193]
- de Wit H. Molecular mechanism of secretory vesicle docking. *Biochem. Soc. Trans.* 2010; 38:192–198. [PubMed: 20074058]
- DeFea KA. Beta-arrestins as regulators of signal termination and transduction: how do they determine what to scaffold? *Cell. Signal.* 2011; 23(4):621–629. [PubMed: 20946952]
- Delaney AJ, Crane JW, Sah P. Noradrenaline Modulates Transmission at a Central Synapse by a Presynaptic Mechanism. *Neuron*. 2007; 56(5):880–892. [PubMed: 18054863]
- Delmas P, Abogadie FC, Dayrell M, Haley JE, Milligan G, Caulfield MP, Brown DA, Buckley NJ. G-proteins and G-protein subunits mediating cholinergic inhibition of N-type calcium currents in sympathetic neurons. *Eur. J. Neurosci.* 1998; 10(5):1654–1666. [PubMed: 9751138]
- Devedjian JC, Pujol A, Cayla C, George M, Casellas A, Paris H, Bosch F. Transgenic mice overexpressing alpha2A-adrenoceptors in pancreatic beta-cells show altered regulation of glucose homeostasis. *Diabetologia*. 2000; 43(7):899–906. [PubMed: 10952463]
- Dingus J, Wells CA, Campbell L, Cleator JH, Robinson K, Hildebrandt JD. G Protein betagamma dimer formation: Gbeta and Ggamma differentially determine efficiency of in vitro dimer formation. *Biochemistry*. 2005; 44(35):11882–11890. [PubMed: 16128590]
- Dittman JS, Regehr WG. Contributions of calcium-dependent and calcium-independent mechanisms to presynaptic inhibition at a cerebellar synapse. *J. Neurosci.* 1996; 16(5):1623–1633. [PubMed: 8774431]
- Downes GB, Gautam N. The G protein subunit gene families. *Genomics*. 1999; 62(3):544–552. [PubMed: 10644457]
- Dresbach T, Qualmann B, Kessels MM, Garner CC, Gundelfinger ED. The presynaptic cytomatrix of brain synapses. *Cell. Mol. Life Sci.* 2001; 58(1):94–116. [PubMed: 11229820]
- Dulubova I, Sugita S, Hill S, Hosaka M, Fernandez I, Sudhof TC, Rizo J. A conformational switch in syntaxin during exocytosis: role of munc18. *EMBO. J.* 1999; 18(16):4372–4382. [PubMed: 10449403]
- Dunlap K, Fischbach GD. Neurotransmitters decrease the calcium component of sensory neurone action potentials. *Nature*. 1978; 276(5690):837–839. [PubMed: 31570]
- Eglen RM, Reisine T. New insights into GPCR function: implications for HTS. *Methods Mol. Biol.* 2009; 552:1–13. [PubMed: 19513638]
- Elmslie KS. Calcium current modulation in frog sympathetic neurones: multiple neurotransmitters and G proteins. *J. Physiol.* 1992; 451(1):229–246. [PubMed: 1357163]

- Fernandez-Alacid L, Aguado C, Ciruela F, Martin R, Colon J, Cabanero MJ, Gassmann M, Watanabe M, Shigemoto R, Wickman K, Bettler B, Sanchez-Prieto J, Lujan R. Subcellular compartment-specific molecular diversity of pre- and post-synaptic GABA-activated GIRK channels in Purkinje cells. *J. Neurochem.* 2009; 110(4):1363–1376. [PubMed: 19558451]
- Fernandez-Busnadiego R, Zuber B, Maurer UE, Cyrklaff M, Baumeister W, Lucic V. Quantitative analysis of the native presynaptic cytomatrix by cryoelectron tomography. *J. Cell Biol.* 2010; 188(1):145–156. [PubMed: 20065095]
- Fernandez-Chacon R, Konigstorfer A, Gerber SH, Garcia J, Matos MF, Stevens CF, Brose N, Rizo J, Rosenmund C, Sudhof TC. Synaptotagmin I functions as a calcium regulator of release probability. *Nature.* 2001; 410(6824):41–49. [PubMed: 11242035]
- Fernandez I, Arac D, Ubach J, Gerber SH, Shin OH, Gao Y, Anderson RGW, Sudhof TC, Rizo J. Three-dimensional structure of the synaptotagmin 1 C2B-domain: Synaptotagmin 1 as a phospholipid binding machine. *Neuron.* 2001; 32(6):1057–1069. [PubMed: 11754837]
- Finley M, Arrabit C, Fowler C, Suen KF, Slesinger PA. betaL-betaM loop in the C-terminal domain of G protein-activated inwardly rectifying K(+) channels is important for G(betagamma) subunit activation. *J. Physiol.* 2004; 555(Pt 3):643–657. [PubMed: 14724209]
- Ford CE, Skiba NP, Bae H, Daaka Y, Reuveny E, Shekter LR, Rosal R, Weng G, Yang C-S, Iyengar R, Miller RJ, Jan LY, Lefkowitz RJ, Hamm HE. Molecular Basis for Interactions of G Protein β Subunits with Effectors. *Science.* 1998; 280(5367):1271–1274. [PubMed: 9596582]
- Fujita Y, Shirataki H, Sakisaka T, Asakura T, Ohya T, Kotani H, Yokoyama S, Nishioka H, Matsuura Y, Mizoguchi A, Scheller RH, Takai Y. Tomosyn: a syntaxin-1-binding protein that forms a novel complex in the neurotransmitter release process. *Neuron.* 1998; 20(5):905–915. [PubMed: 9620695]
- Fukuda M. Molecular Cloning, Expression, and Characterization of a Novel Class of Synaptotagmin (Syt XIV) Conserved from *Drosophila* to Humans. *J. Biochem.* 2003a; 133(5):641–649. [PubMed: 12801916]
- Fukuda M. Molecular cloning and characterization of human, rat, and mouse synaptotagmin XV. *Biochem. Biophys. Res. Commun.* 2003b; 306(1):64–71. [PubMed: 12788067]
- Furukawa T, Miura R, Mori Y, Strobeck M, Suzuki K, Ogihara Y, Asano T, Morishita R, Hashii M, Higashida H, Yoshii M, Nukada T. Differential Interactions of the C terminus and the Cytoplasmic I–II Loop of Neuronal Ca²⁺ Channels with G-protein α and β Subunits. *J. Biol. Chem.* 1998; 273(28):17595–17603. [PubMed: 9651354]
- Gabriel JP, Mahmood R, Kyriakatos A, Soll I, Hauptmann G, Calabrese RL, El Manira A. Serotonergic modulation of locomotion in zebrafish: endogenous release and synaptic mechanisms. *J. Neurosci.* 2009; 29(33):10387–10395. [PubMed: 19692613]
- Gamper N, Reznikov V, Yamada Y, Yang J, Shapiro MS. Phosphatidylinositol [correction] 4,5-bisphosphate signals underlie receptor-specific Gq/11-mediated modulation of N-type Ca²⁺ channels. *J. Neurosci.* 2004; 24(48):10980–10992. [PubMed: 15574748]
- Gautam N, Downes GB, Yan K, Kisselev O. The G-protein betagamma complex. *Cell. Signal.* 1998; 10(7):447–455. [PubMed: 9754712]
- Geppert M, Goda Y, Hammer RE, Li C, Rosahl TW, Stevens CF, Sudhof TC. Synaptotagmin I: a major Ca²⁺ sensor for transmitter release at a central synapse. *Cell.* 1994; 79(4):717–727. [PubMed: 7954835]
- Gerachshenko T, Blackmer T, Yoon EJ, Bartleson C, Hamm HE, Alford S. Gbetagamma acts at the C terminus of SNAP-25 to mediate presynaptic inhibition. *Nat. Neurosci.* 2005; 8(5):597–605. [PubMed: 15834421]
- Gerachshenko T, Schwartz E, Bleckert A, Photowala H, Seymour A, Alford S. Presynaptic G-protein-coupled receptors dynamically modify vesicle fusion, synaptic cleft glutamate concentrations, and motor behavior. *J. Neurosci.* 2009; 29(33):10221–10233. [PubMed: 19692597]
- Gerber SH, Rah J-C, Min S-W, Liu X, de Wit H, Dulubova I, Meyer AC, Rizo J, Arancillo M, Hammer RE, Verhage M, Rosenmund C, Südhof TC. Conformational Switch of Syntaxin-1 Controls Synaptic Vesicle Fusion. *Science.* 2008; 321(5895):1507–1510. [PubMed: 18703708]

- Gereau RWT, Conn PJ. Multiple presynaptic metabotropic glutamate receptors modulate excitatory and inhibitory synaptic transmission in hippocampal area C1. *J. Neurosci.* 1995; 15:6879–6889. [PubMed: 7472445]
- Gerona RRL, Larsen EC, Kowalchuk JA, Martin TFJ. The C Terminus of SNAP25 Is Essential for Ca²⁺-dependent Binding of Synaptotagmin to SNARE Complexes. *J. Biol. Chem.* 2000; 275(9): 6328–6336. [PubMed: 10692432]
- Giustizieri M, Bernardi G, Mercuri NB, Berretta N. Distinct mechanisms of presynaptic inhibition at GABAergic synapses of the rat substantia nigra pars compacta. *J. Neurophysiol.* 2005; 94(3): 1992–2003. [PubMed: 15944237]
- Glitsch M. Selective inhibition of spontaneous but not Ca²⁺-dependent release machinery by presynaptic group II mGluRs in rat cerebellar slices. *J. Neurophysiol.* 2006; 96(1):86–96. [PubMed: 16611839]
- Golard A, Siegelbaum S. Kinetic basis for the voltage-dependent inhibition of N-type calcium current by somatostatin and norepinephrine in chick sympathetic neurons. *J. Neurosci.* 1993; 13(9):3884–3894. [PubMed: 8103554]
- Goodman, LS.; Hardman, JG.; Limbird, LE.; Gilman, AG. Goodman & Gilman's the pharmacological basis of therapeutics. 10th ed.. New York: McGraw-Hill; 2001.
- Graham GJ, Ren Q, Dilks JR, Blair P, Whiteheart SW, Flaumenhaft R. Endobrevin/VAMP-8-dependent dense granule release mediates thrombus formation in vivo. *Blood.* 2009; 114(5):1083–1090. [PubMed: 19395672]
- Groffen AJ, Martens S, Arazola RD, Cornelisse LN, Lozovaya N, de Jong APH, Goriounova NA, Habets RLP, Takai Y, Borst JG, Brose N, McMahon HT, Verhage M. Doc2b Is a High-Affinity Ca²⁺ Sensor for Spontaneous Neurotransmitter Release. *Science.* 2010; 327(5973):1614–1618. [PubMed: 20150444]
- Haley JE, Delmas P, Offermanns S, Abogadie FC, Simon MI, Buckley NJ, Brown DA. Muscarinic inhibition of calcium current and M current in α q-deficient mice. *J. Neurosci.* 2000; 20(11): 3973–3979. [PubMed: 10818132]
- Hamilton BR, Smith DO. Autoreceptor-mediated purinergic and cholinergic inhibition of motor nerve terminal calcium currents in the rat. *J. Physiol.* 1991; 432:327–341. [PubMed: 1653322]
- Hammarlund M, Palfreyman MT, Watanabe S, Olsen S, Jorgensen EM. Open syntaxin docks synaptic vesicles. *PLoS Biol.* 2007; 5(8):e198. [PubMed: 17645391]
- Harvey VL, Stephens GJ. Mechanism of GABA receptor-mediated inhibition of spontaneous GABA release onto cerebellar Purkinje cells. *Eur. J. Neurosci.* 2004; 20(3):684–700. [PubMed: 15255979]
- Hatsuzawa K, Lang T, Fasshauer D, Bruns D, Jahn R. The R-SNARE motif of tomosyn forms SNARE core complexes with syntaxin 1 and SNAP-25 and down-regulates exocytosis. *J. Biol. Chem.* 2003; 278(33):31159–31166. [PubMed: 12782620]
- Heinke B, Gingl E, Sandkuhler J. Multiple targets of μ -opioid receptor-mediated presynaptic inhibition at primary afferent A δ - and C-fibers. *J. Neurosci.* 2011; 31(4):1313–1322. [PubMed: 21273416]
- Herlitze S, Garcia DE, Mackie K, Hille B, Scheuer T, Catterall WA. Modulation of Ca²⁺ channels by G-protein β γ subunits. *Nature.* 1996; 380(6571):258–262. [PubMed: 8637576]
- Herlitze S, Hockerman GH, Scheuer T, Catterall WA. Molecular determinants of inactivation and G protein modulation in the intracellular loop connecting domains I and II of the calcium channel α 1A subunit. *Proc. Natl. Acad. Sci. USA.* 1997; 94(4):1512–1516. [PubMed: 9037084]
- Herring BE, McMillan K, Pike CM, Marks J, Fox AP, Xie Z. Etomidate and propofol inhibit the neurotransmitter release machinery at different sites. *J. Physiol.* 2011; 589(5):1103–1115. [PubMed: 21173083]
- Higley MJ, Soler-Llavina GJ, Sabatini BL. Cholinergic modulation of multivesicular release regulates striatal synaptic potency and integration. *Nat. Neurosci.* 2009; 12(9):1121–1128. [PubMed: 19668198]
- Higley MJ, Sabatini BL. Competitive regulation of synaptic Ca²⁺ influx by D2 dopamine and A2A adenosine receptors. *Nat. Neurosci.* 2010; 13(8):958–966. [PubMed: 20601948]

- Hildebrandt JD. Role of subunit diversity in signaling by heterotrimeric G proteins. *Biochem. Pharmacol.* 1997; 54(3):325–339. [PubMed: 9278091]
- Hobson RJ, Liu Q, Watanabe S, Jorgensen EM. Complexin Maintains Vesicles in the Primed State in *C. elegans*. *Current Biology.* 2011; 21(2):106–113. [PubMed: 21215631]
- Hong W. SNAREs and traffic. *Biochim. Biophys. Acta.* 2005; 1744(2):120–144. [PubMed: 15893389]
- Huang C-L, Slesinger PA, Casey PJ, Jan YN, Jan LY. Evidence that direct binding of G $\beta\gamma$ to the GIRK1 G protein-gated inwardly rectifying K⁺ channel is important for channel activation. *Neuron.* 1995; 15(5):1133–1143. [PubMed: 7576656]
- Hunt RD, Arnsten AFT, Asbell MD. An Open Trial of Guanfacine in the Treatment of Attention-Deficit Hyperactivity Disorder. *J. Am. Acad. Child Adolesc. Psychiatry.* 1995; 34(1):50–54. [PubMed: 7860456]
- Ikedo K, Yoshii M, Sora I, Kobayashi T. Opioid receptor coupling to GIRK channels. In vitro studies using a *Xenopus* oocyte expression system and in vivo studies on weaver mutant mice. *Methods Mol. Med.* 2003; 84:53–64. [PubMed: 12703317]
- Ikedo SR. Voltage-dependent modulation of N-type calcium channels by G-protein beta gamma subunits. *Nature.* 1996; 380(6571):255–258. [PubMed: 8637575]
- Iremonger KJ, Bains JS. Retrograde opioid signaling regulates glutamatergic transmission in the hypothalamus. *J. Neurosci.* 2009; 29(22):7349–7358. [PubMed: 19494156]
- Isaacson JS. GABAB receptor-mediated modulation of presynaptic currents and excitatory transmission at a fast central synapse. *J. Neurophysiol.* 1998; 80(3):1571–1576. [PubMed: 9744963]
- Jahn R, Scheller RH. SNAREs--engines for membrane fusion. *Nat. Rev. Mol. Cell Biol.* 2006; 7(9): 631–643. [PubMed: 16912714]
- Janssen PA, Niemegeers CJ, Awouters F, Schellekens KH, Megens AA, Meert TF. Pharmacology of risperidone (R 64 766), a new antipsychotic with serotonin-S₂ and dopamine-D₂ antagonistic properties. *J. Pharmacol. Exp. Ther.* 1988; 244(2):685–693. [PubMed: 2450200]
- Jardin I, Ben Amor N, Hernández-Cruz JM, Salido GM, Rosado JA. Involvement of SNARE proteins in thrombin-induced platelet aggregation: Evidence for the relevance of Ca²⁺ entry. *Arch. Biochem. Biophys.* 2007; 465(1):16–25. [PubMed: 17543880]
- Jarvis SE, Magga JM, Beedle AM, Braun JEA, Zamponi GW. G Protein Modulation of Ntype Calcium Channels Is Facilitated by Physical Interactions between Syntaxin 1A and G $\beta\gamma$. *J. Biol. Chem.* 2000; 275(9):6388–6394. [PubMed: 10692440]
- Jarvis SE, Zamponi GW. Distinct molecular determinants govern syntaxin 1A-mediated inactivation and G-protein inhibition of N-type calcium channels. *J. Neurosci.* 2001; 21(9):2939–2948. [PubMed: 11312277]
- Jarvis SE, Barr W, Feng ZP, Hamid J, Zamponi GW. Molecular determinants of syntaxin 1 modulation of N-type calcium channels. *J. Biol. Chem.* 2002; 277(46):44399–44407. [PubMed: 12221094]
- Kajikawa Y, Saitoh N, Takahashi T. GTP-binding protein beta gamma subunits mediate presynaptic calcium current inhibition by GABA(B) receptor. *Proc. Natl. Acad. Sci. U S A.* 2001; 98(14): 8054–8058. [PubMed: 11416164]
- Kandel, ER.; Schwartz, JH.; Jessell, TM. Principles of neural science. 4th ed.. New York: McGraw-Hill, Health Professions Division; 2000.
- Kasai H, Aosaki T. Modulation of Ca-channel current by an adenosine analog mediated by a GTP-binding protein in chick sensory neurons. *Pflugers Arch.* 1989; 414(2):145–149. [PubMed: 2547194]
- Katz, B. The release of Neural Transmitter Substances (The Sherrington Lectures X). Springfield, IL: Charles C. Thomas; 1969.
- Kleuss C, Scherubel H, Hescheler J, Schultz G, Wittig B. Selectivity in signal transduction determined by gamma subunits of heterotrimeric G proteins. *Science.* 1993; 259(5096):832–834. [PubMed: 8094261]
- Knight DE, Baker PF. Guanine nucleotides and Ca-dependent exocytosis. Studies on two adrenal cell preparations. *FEBS Lett.* 1985; 189(2):345–349. [PubMed: 3930291]

- Kochubey O, Schneggenburger R. Synaptotagmin Increases the Dynamic Range of Synapses by Driving Ca^{2+} -Evoked Release and by Clamping a Near-Linear Remaining Ca^{2+} Sensor. *Neuron*. 2011; 69(4):736–748. [PubMed: 21338883]
- Komatsu M, Schermerhorn T, Aizawa T, Sharp GW. Glucose stimulation of insulin release in the absence of extracellular Ca^{2+} and in the absence of any increase in intracellular Ca^{2+} in rat pancreatic islets. *Proc. Natl. Acad. Sci. USA*. 1995; 92(23):10728–10732. [PubMed: 7479873]
- Kulik A, Vida I, Fukazawa Y, Guetg N, Kasugai Y, Marker CL, Rigato F, Bettler B, Wickman K, Frotscher M, Shigemoto R. Compartment-dependent colocalization of Kir3.2-containing K^{+} channels and GABAB receptors in hippocampal pyramidal cells. *J. Neurosci*. 2006; 26(16):4289–4297. [PubMed: 16624949]
- Kümmel D, Krishnakumar SS, Radoff DT, Li F, Giraudo CG, Pincet F, Rothman JE, Reinisch KM. Complexin cross-links prefusion SNAREs into a zigzag array. *Nat. Struct. Mol. Biol*. 2011; 18(8):927–933. [PubMed: 21785414]
- Ladera C, del Carmen Godino M, Jose Cabanero M, Torres M, Watanabe M, Lujan R, Sanchez-Prieto J. Pre-synaptic GABA receptors inhibit glutamate release through GIRK channels in rat cerebral cortex. *J. Neurochem*. 2008; 107(6):1506–1517. [PubMed: 19094055]
- Lai AL, Huang H, Herrick DZ, Epp N, Cafiso DS. Synaptotagmin 1 and SNAREs Form a Complex That Is Structurally Heterogeneous. *J. Mol. Biol*. 2011; 405(3):696–706. [PubMed: 21087613]
- Lambright DG, Sondek J, Böhm A, Skiba NP, Hamm HE, Sigler PB. The 2.0 Å crystal structure of a heterotrimeric G protein. *Nature*. 1996; 379(6563):311–319. [PubMed: 8552184]
- Le François B, Czesak M, Steubl D, Albert PR. Transcriptional regulation at a HTR1A polymorphism associated with mental illness. *Neuropharmacology*. 2008; 55(6):977–985. [PubMed: 18639564]
- Lei Q, Jones MB, Talley EM, Schrier AD, McIntire WE, Garrison JC, Bayliss DA. Activation and inhibition of G protein-coupled inwardly rectifying potassium (Kir3) channels by G protein beta gamma subunits. *Proc. Natl. Acad. Sci. USA*. 2000; 97(17):9771–9776. [PubMed: 10944236]
- Lei Q, Talley EM, Bayliss DA. Receptor-mediated inhibition of G protein-coupled inwardly rectifying potassium channels involves Gαq family subunits, phospholipase C, and a readily diffusible messenger. *J. Biol. Chem*. 2001; 276(20):16720–16730. [PubMed: 11279027]
- Lei Q, Jones MB, Talley EM, Garrison JC, Bayliss DA. Molecular mechanisms mediating inhibition of G protein-coupled inwardly-rectifying K^{+} channels. *Mol. Cells*. 2003; 15(1):1–9. [PubMed: 12661754]
- Leveque C, el Far O, Martin-Moutot N, Sato K, Kato R, Takahashi M, Seagar MJ. Purification of the N-type calcium channel associated with syntaxin and synaptotagmin. A complex implicated in synaptic vesicle exocytosis. *J. Biol. Chem*. 1994; 269(9):6306–6312. [PubMed: 8119979]
- Li B, Zhong H, Scheuer T, Catterall WA. Functional Role of a C-Terminal $\text{G}\beta\gamma$ -Binding Domain of Cav2.2 Channels. *Mol. Pharmacol*. 2004; 66(3):761–769. [PubMed: 1532269]
- Li X, Hummer A, Han J, Xie M, Melnik-Martinez K, Moreno RL, Buck M, Mark MD, Herlitze S. G protein beta2 subunit-derived peptides for inhibition and induction of G protein pathways. Examination of voltage-gated Ca^{2+} and G protein inwardly rectifying K^{+} channels. *J. Biol. Chem*. 2005; 280(25):23945–23959. [PubMed: 15824105]
- Lim WK, Myung CS, Garrison JC, Neubig RR. Receptor-G protein gamma specificity: gamma11 shows unique potency for A(1) adenosine and 5-HT(1A) receptors. *Biochemistry*. 2001; 40(35):10532–10541. [PubMed: 11523995]
- Lin HC, Gilman AG. Regulation of Dynamin I GTPase Activity by G Protein $\beta\gamma$ Subunits and Phosphatidylinositol 4,5-Bisphosphate. *J. Biol. Chem*. 1996; 271(45):27979–27982. [PubMed: 8910402]
- Lindorfer MA, Myung CS, Savino Y, Yasuda H, Khazan R, Garrison JC. Differential activity of the G protein beta5 gamma2 subunit at receptors and effectors. *J. Biol. Chem*. 1998; 273(51):34429–34436. [PubMed: 9852110]
- Liu J-P, Yajima Y, Li H, Ackland S, Akita Y, Stewart J, Kawashima S. Molecular interactions between dynamin and G-protein $\beta\gamma$ -subunits in neuroendocrine cells. *Mol. Cell. Endocrinol*. 1997; 132(1–2):61–71. [PubMed: 9324047]

- Liu J, Jordan LM. Stimulation of the Parapyramidal Region of the Neonatal Rat Brain Stem Produces Locomotor-Like Activity Involving Spinal 5-HT₇ and 5-HT_{2A} Receptors. *J. Neurophysiol.* 2005; 94(2):1392–1404. [PubMed: 15872068]
- Logothetis DE, Kurachi Y, Galper J, Neer EJ, Clapham DE. The beta gamma subunits of GTP-binding proteins activate the muscarinic K⁺ channel in heart. *Nature.* 1987; 325(6102):321–326. [PubMed: 2433589]
- Luini A, De Matteis MA. Evidence that the receptor-linked G protein inhibits exocytosis by a post-second messenger mechanism in AtT-20 cells. *J. Neurochem.* 1990; 54:30–38. [PubMed: 1967144]
- Lujan R, Maylie J, Adelman JP. New sites of action for GIRK and SK channels. *Nat. Rev. Neurosci.* 2009; 10(7):475–480. [PubMed: 19543219]
- Luscher C, Jan LY, Stoffel M, Malenka RC, Nicoll RA. G protein-coupled inwardly rectifying K⁺ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron.* 1997; 19(3):687–695. [PubMed: 9331358]
- Luscher C, Slesinger PA. Emerging roles for G protein-gated inwardly rectifying potassium (GIRK) channels in health and disease. *Nat. Rev. Neurosci.* 2010; 11(5):301–315. [PubMed: 20389305]
- Lynch KL, Gerona RR, Larsen EC, Marcia RF, Mitchell JC, Martin TF. Synaptotagmin C2A loop 2 mediates Ca²⁺-dependent SNARE interactions essential for Ca²⁺-triggered vesicle exocytosis. *Mol. Biol. Cell.* 2007; 18(12):4957–4968. [PubMed: 17914059]
- Lynch MJ, Baillie GS, Mohamed A, Li X, Maisonneuve C, Klussmann E, van Heeke G, Houslay MD. RNA silencing identifies PDE4D5 as the functionally relevant cAMP phosphodiesterase interacting with beta arrestin to control the protein kinase A/AKAP79-mediated switching of the beta2-adrenergic receptor to activation of ERK in HEK293B2 cells. *J. Biol. Chem.* 2005; 280(39):33178–33189. [PubMed: 16030021]
- Malsam J, Kreye S, Sollner TH. Membrane fusion: SNAREs and regulation. *Cell. Mol. Life Sci.* 2008; 65(18):2814–2832. [PubMed: 18726177]
- Matteoli M, Pozzi D, Grumelli C, Condiliffe SB, Frassoni C, Harkany T, Verderio C. The synaptic split of SNAP-25: Different roles in glutamatergic and GABAergic neurons? *Neuroscience.* 2009; 158(1):223–230. [PubMed: 18514426]
- Maximov A, Sudhof TC. Autonomous function of synaptotagmin 1 in triggering synchronous release independent of asynchronous release. *Neuron.* 2005; 48(4):547–554. [PubMed: 16301172]
- McMahon HT, Missler M, Li C, Südhof TC. Complexins: Cytosolic proteins that regulate SNAP receptor function. *Cell.* 1995; 83(1):111–119. [PubMed: 7553862]
- Meldolesi J, Huttner WB, Tsien RY, Pozzan T. Free cytoplasmic Ca²⁺ and neurotransmitter release: studies on PC12 cells and synaptosomes exposed to alpha-latrotoxin. *Proc. Natl. Acad. Sci. USA.* 1984; 81(2):620–624. [PubMed: 6141561]
- Michaeli A, Yaka R. Dopamine inhibits GABA(A) currents in ventral tegmental area dopamine neurons via activation of presynaptic G-protein coupled inwardly-rectifying potassium channels. *Neuroscience.* 2010; 165(4):1159–1169. [PubMed: 19944748]
- Millar RP, Newton CL. The year in G protein-coupled receptor research. *Mol. Endocrinol.* 2010; 24(1):261–274. [PubMed: 20019124]
- Miller LC, Swayne LA, Kay JG, Feng Z-P, Jarvis SE, Zamponi GW, Braun JEA. Molecular determinants of cysteine string protein modulation of N-type calcium channels. *Journal of Cell Science.* 2003; 116(14):2967–2974. [PubMed: 12783986]
- Miller RJ. Presynaptic receptors. *Annu. Rev. Pharmacol. Toxicol.* 1998; 38:201–227. [PubMed: 9597154]
- Mirshahi T, Mittal V, Zhang H, Linder ME, Logothetis DE. Distinct sites on G protein beta gamma subunits regulate different effector functions. *J. Biol. Chem.* 2002; 277(39):36345–36350. [PubMed: 12124391]
- Misura KM, Scheller RH, Weis WI. Three-dimensional structure of the neuronal-Sec1-syntaxin 1a complex. *Nature.* 2000; 404(6776):355–362. [PubMed: 10746715]
- Myung CS, Lim WK, DeFilippo JM, Yasuda H, Neubig RR, Garrison JC. Regions in the G protein gamma subunit important for interaction with receptors and effectors. *Mol. Pharmacol.* 2006; 69(3):877–887. [PubMed: 16319284]

- Natochin M, Campbell TN, Barren B, Miller LC, Hameed S, Artemyev NO, Braun JE. Characterization of the G α (s) regulator cysteine string protein. *J. Biol. Chem.* 2005; 280(34): 30236–30241. [PubMed: 15972823]
- Nicola SM, Malenka RC. Dopamine depresses excitatory and inhibitory synaptic transmission by distinct mechanisms in the nucleus accumbens. *J. Neurosci.* 1997; 17(15):5697–5710. [PubMed: 9221769]
- O'Connor VM, Shamotienko O, Grishin E, Betz H. On the structure of the 'synaptosecretosome'. Evidence for a neurexin/synaptotagmin/syntaxin/Ca²⁺ channel complex. *FEBS Lett.* 1993; 326(1–3):255–260. [PubMed: 8100783]
- Oldham WM, Hamm HE. How do receptors activate G proteins? *Adv. Protein Chem.* 2007; 74:67–93. [PubMed: 17854655]
- Oldham WM, Hamm HE. Heterotrimeric G protein activation by G-protein-coupled receptors. *Nat. Rev. Mol. Cell Biol.* 2008; 9(1):60–71. [PubMed: 18043707]
- Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Trong IL, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M. Crystal Structure of Rhodopsin: A G Protein-Coupled Receptor. *Science.* 2000; 289(5480):739–745. [PubMed: 10926528]
- Panchenko MP, Saxena K, Li Y, Charnecki S, Sternweis PM, Smith TF, Gilman AG, Kozasa T, Neer EJ. Sites important for PLC β 2 activation by the G protein betagamma subunit map to the sides of the beta propeller structure. *J. Biol. Chem.* 1998; 273(43):28298–28304. [PubMed: 9774453]
- Park Y, Kim K-T. Short-term plasticity of small synaptic vesicle (SSV) and large dense-core vesicle (LDCV) exocytosis. *Cell. Signal.* 2009; 21(10):1465–1470. [PubMed: 19249357]
- Parpura V, Mohideen U. Molecular form follows function: (un)snaring the SNAREs. *Trends Neurosci.* 2008; 31(9):435–443. [PubMed: 18675467]
- Peng L, Mirshahi T, Zhang H, Hirsch JP, Logothetis DE. Critical determinants of the G protein gamma subunits in the Gbetagamma stimulation of G protein-activated inwardly rectifying potassium (GIRK) channel activity. *J. Biol. Chem.* 2003; 278(50):50203–50211. [PubMed: 12975366]
- Photowala H, Blackmer T, Schwartz E, Hamm HE, Alford S. G protein betagamma-subunits activated by serotonin mediate presynaptic inhibition by regulating vesicle fusion properties. *Proc. Natl. Acad. Sci. U S A.* 2006; 103(11):4281–4286. [PubMed: 16537522]
- Pobhati AV, Razeto A, Böddener M, Becker S, Fasshauer D. Structural Basis for the Inhibitory Role of Tomosyn in Exocytosis. *J. Biol. Chem.* 2004; 279(45):47192–47200. [PubMed: 15316007]
- Polgar J, Chung SH, Reed GL. Vesicle-associated membrane protein 3 (VAMP-3) and VAMP-8 are present in human platelets and are required for granule secretion. *Blood.* 2002; 100(3):1081–1083. [PubMed: 12130530]
- Ponce A, Bueno E, Kentros C, Vega-Saenz de Miera E, Chow A, Hillman D, Chen S, Zhu L, Wu MB, Wu X, Rudy B, Thornhill WB. G-protein-gated inward rectifier K⁺ channel proteins (GIRK1) are present in the soma and dendrites as well as in nerve terminals of specific neurons in the brain. *J. Neurosci.* 1996; 16(6):1990–2001. [PubMed: 8604043]
- Predescu SA, Predescu DN, Shimizu K, Klein IK, Malik AB. Cholesterol-dependent syntaxin-4 and SNAP-23 clustering regulates caveolar fusion with the endothelial plasma membrane. *J. Biol. Chem.* 2005; 280(44):37130–37138. [PubMed: 16118213]
- Pulido IR, Jahn R, Gerke V. VAMP3 is associated with endothelial Weibel-Palade bodies and participates in their Ca(2+)-dependent exocytosis. *Biochim. Biophys. Acta.* 2011; 1813(5):1038–1044. [PubMed: 21094665]
- Qin N, Platano D, Olcese R, Stefani E, Birnbaumer L. Direct interaction of G $\beta\gamma$ with a C-terminal G $\beta\gamma$ -binding domain of the Ca²⁺ channel α 1 subunit is responsible for channel inhibition by G protein-coupled receptors. *Proc. Natl. Acad. Sci. USA.* 1997; 94(16):8866–8871. [PubMed: 9238069]
- Reuveny E, Slesinger PA, Inglese J, Morales JM, Iniguez-Lluhi JA, Lefkowitz RJ, Bourne HR, Jan YN, Jan LY. Activation of the cloned muscarinic potassium channel by G protein beta gamma subunits. *Nature.* 1994; 370(6485):143–146. [PubMed: 8022483]
- Richardson M, Robishaw JD. The α 2A-adrenergic receptor discriminates between Gi heterotrimers of different betagamma subunit composition in Sf9 insect cell membranes. *J. Biol. Chem.* 1999; 274(19):13525–13533. [PubMed: 10224121]

- Richmond JE, Davis WS, Jorgensen EM. UNC-13 is required for synaptic vesicle fusion in *C-elegans*. *Nat. Neurosci.* 1999; 2(11):959–964. [PubMed: 10526333]
- Rizo J, Chen X, Arac D. Unraveling the mechanisms of synaptotagmin and SNARE function in neurotransmitter release. *Trends Cell Biol.* 2006; 16(7):339–350. [PubMed: 16698267]
- Rizo J, Rosenmund C. Synaptic vesicle fusion. *Nat. Struct. Mol. Biol.* 2008; 15(7):665–674. [PubMed: 18618940]
- Rizo, J. SNAREs. In: Larry, RS., editor. *Encyclopedia of Neuroscience*. Oxford: Academic Press; 2009. p. 11-19.
- Robishaw JD, Berlot CH. Translating G protein subunit diversity into functional specificity. *Curr. Opin. Cell Biol.* 2004; 16(2):206–219. [PubMed: 15196565]
- Rosenbaum DM, Rasmussen SG, Kobilka BK. The structure and function of G-protein-coupled receptors. *Nature.* 2009; 459(7245):356–363. [PubMed: 19458711]
- Rosengren AH, Jokubka R, Tojjar D, Granhall C, Hansson O, Li DQ, Nagaraj V, Reinbothe TM, Tuncel J, Eliasson L, Groop L, Rorsman P, Salehi A, Lyssenko V, Luthman H, Renstrom E. Overexpression of $\alpha 2A$ -adrenergic receptors contributes to type 2 diabetes. *Science.* 2010; 327(5962):217–220. [PubMed: 19965390]
- Sadja R, Alagem N, Reuveny E. Gating of GIRK channels: details of an intricate, membrane-delimited signaling complex. *Neuron.* 2003; 39(1):9–12. [PubMed: 12848928]
- Sadja R, Reuveny E. Activation gating kinetics of GIRK channels are mediated by cytoplasmic residues adjacent to transmembrane domains. *Channels (Austin).* 2009; 3(3):205–214. [PubMed: 19574735]
- Sampo B, Tricaud N, Leveque C, Seagar M, Couraud F, Dargent B. Direct interaction between synaptotagmin and the intracellular loop I–II of neuronal voltage-sensitive sodium channels. *Proc. Natl. Acad. Sci. USA.* 2000; 97(7):3666–3671. [PubMed: 10737807]
- Scanziani M, Gahwiler BH, Thompson SM. Presynaptic inhibition of excitatory synaptic transmission by muscarinic and metabotropic glutamate receptor activation in the hippocampus: are Ca^{2+} channels involved? *Neuropharmacology.* 1995; 34:1549–1557. [PubMed: 8606802]
- Schiavo G, Santucci A, Dasgupta BR, Mehta PP, Jontes J, Benfenati F, Wilson MC, Montecucco C. Botulinum neurotoxins serotypes A and E cleave SNAP-25 at distinct COOH-terminal peptide bonds. *FEBS Lett.* 1993; 335(1):99–103. [PubMed: 8243676]
- Schiavo G, Osborne SL, Sgouros JG. Synaptotagmins: More Isoforms Than Functions? *Biochem. Biophys. Res. Commun.* 1998; 248(1):1–8. [PubMed: 9675075]
- Schiöth HB, Fredriksson R. The GRAFS classification system of G-protein coupled receptors in comparative perspective. *Gen. Comp. Endocrinol.* 2005; 142(1–2):94–101. [PubMed: 15862553]
- Schmitz, F.; Fernández-Chacón, R. Cysteine-String Proteins (CSPs). In: Larry, RS., editor. *Encyclopedia of Neuroscience*. Oxford: Academic Press; 2009. p. 285-292.
- Schoch S, Gundelfinger ED. Molecular organization of the presynaptic active zone. *Cell Tissue Res.* 2006; 326(2):379–391. [PubMed: 16865347]
- Scholz KP, Miller RJ. Inhibition of quantal transmitter release in the absence of calcium influx by a G protein linked adenosine receptor at hippocampal synapses. *Neuron.* 1992; 8:1139–1150. [PubMed: 1351733]
- Schwartz EJ, Gerachshenko T, Alford S. 5-HT prolongs ventral root bursting via presynaptic inhibition of synaptic activity during fictive locomotion in lamprey. *J. Neurophysiol.* 2005; 93(2):980–988. [PubMed: 15456802]
- Schwindinger WF, Betz KS, Giger KE, Sabol A, Bronson SK, Robishaw JD. Loss of G protein $\gamma 7$ alters behavior and reduces striatal α (olf) level and cAMP production. *J. Biol. Chem.* 2003; 278(8):6575–6579. [PubMed: 12488442]
- Schwindinger WF, Giger KE, Betz KS, Stauffer AM, Sunderlin EM, Sim-Selley LJ, Selley DE, Bronson SK, Robishaw JD. Mice with Deficiency of G Protein $\gamma 3$ Are Lean and Have Seizures. *Mol. Cell. Biol.* 2004; 24(17):7758–7768. [PubMed: 15314181]
- Seagar M, Leveque C, Charvin N, Marqueze B, Martin-Moutot N, Boudier JA, Boudier JL, Shoji-Kasai Y, Sato K, Takahashi M. Interactions between proteins implicated in exocytosis and voltage-gated calcium channels. *Philos. Transr. Soc. Lond. B Biol. Sci.* 1999; 354(1381):289–297.

- Searl TJ, Silinsky EM. Modulation of Ca(2+)-dependent and Ca(2+)-independent miniature endplate potentials by phorbol ester and adenosine in frog. *British journal of pharmacology*. 2005; 145(7): 954–962. [PubMed: 15880138]
- Sharma M, Burre J, Sudhof TC. CSP[alpha] promotes SNARE-complex assembly by chaperoning SNAP-25 during synaptic activity. *Nat. Cell. Biol.* 2011; 13(1):30–39. [PubMed: 21151134]
- Sheng Z-H, Rettig J, Takahashi M, Catterall WA. Identification of a syntaxin-binding site on N-Type calcium channels. *Neuron*. 1994; 13(6):1303–1313. [PubMed: 7993624]
- Shigemoto R, Kinoshita A, Wada E, Nomura S, Ohishi H, Takada M, Flor PJ, Neki A, Abe T, Nakanishi S, Mizuno N. Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. *J. Neurosci.* 1997; 17(19):7503–7522. [PubMed: 9295396]
- Silinsky EM. On the mechanism by which adenosine receptor activation inhibits the release of acetylcholine from motor nerve endings. *J. Physiol.* 1984; 346(1):243–256. [PubMed: 6321717]
- Silinsky EM. Selective disruption of the mammalian secretory apparatus enhances or eliminates calcium current modulation in nerve endings. *Proc. Natl. Acad. Sci. USA.* 2008; 105(17):6427–6432. [PubMed: 18420824]
- Sillar KT, Wedderburn JF, Simmers AJ. Modulation of swimming rhythmicity by 5-hydroxytryptamine during post-embryonic development in *Xenopus laevis*. *Proc. Biol. Sci.* 1992; 250(1328):107–114. [PubMed: 1361984]
- Simon MI, Strathmann MP, Gautam N. Diversity of G proteins in signal transduction. *Science*. 1991; 252(5007):802–808. [PubMed: 1902986]
- Smrcka AV. G protein betagamma subunits: central mediators of G protein-coupled receptor signaling. *Cell. Mol. Life Sci.* 2008; 65(14):2191–2214. [PubMed: 18488142]
- Sollner T, Bennett MK, Whiteheart SW, Scheller RH, Rothman JE. A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. *Cell*. 1993a; 75(3):409–418. [PubMed: 8221884]
- Sollner T, Whiteheart SW, Brunner M, Erdjument-Bromage H, Geromanos S, Tempst P, Rothman JE. SNAP receptors implicated in vesicle targeting and fusion. *Nature*. 1993b; 362(6418):318–324. [PubMed: 8455717]
- Stanley EF, Mirotnik RR. Cleavage of syntaxin prevents G-protein regulation of presynaptic calcium channels. *Nature*. 1997; 385(6614):340–343. [PubMed: 9002518]
- Stephens GJ, Mochida S. G protein $\beta\gamma$ subunits mediate presynaptic inhibition of transmitter release from rat superior cervical ganglion neurones in culture. *J. Physiol.* 2005; 563(Pt 3):765–776. [PubMed: 15661818]
- Stephens GJ. G-protein-coupled-receptor-mediated presynaptic inhibition in the cerebellum. *Trends Pharmacol. Sci.* 2009; 30(8):421–430. [PubMed: 19632729]
- Stevens DR, Wu ZX, Matti U, Junge HJ, Schirra C, Becherer U, Wojcik SM, Brose N, Rettig J. Identification of the minimal protein domain required for priming activity of Munc13-1. *Curr. Biol.* 2005; 15(24):2243–2248. [PubMed: 16271475]
- Stow JL, Manderson AP, Murray RZ. SNAREing immunity: the role of SNAREs in the immune system. *Nature reviews. Immunology*. 2006; 6(12):919–929. [PubMed: 17124513]
- Sudhof TC. The synaptic vesicle cycle. *Annu. Rev. Neurosci.* 2004; 27:509–547. [PubMed: 15217342]
- Sudhof TC, Rothman JE. Membrane fusion: grappling with SNARE and SM proteins. *Science*. 2009; 323(5913):474–477. [PubMed: 19164740]
- Südhof TC. Synaptotagmins: Why So Many? *J. Biol. Chem.* 2002; 277(10):7629–7632. [PubMed: 11739399]
- Sun J, Pang ZP, Qin D, Fahim AT, Adachi R, Sudhof TC. A dual-Ca²⁺-sensor model for neurotransmitter release in a central synapse. *Nature*. 2007; 450(7170):676–682. [PubMed: 18046404]
- Sutton RB, Fasshauer D, Jahn R, Brunger AT. Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution. *Nature*. 1998; 395(6700):347–353. [PubMed: 9759724]
- Takahashi T, Forsythe ID, Tsujimoto T, Barnes-Davies M, Onodera K. Presynaptic calcium current modulation by a metabotropic glutamate receptor. *Science*. 1996; 274(5287):594–597. [PubMed: 8849448]

- Takao K, Yoshii M, Kanda A, Kokubun S, Nukada T. A region of the muscarinic-gated atrial K⁺ channel critical for activation by G protein beta gamma subunits. *Neuron*. 1994; 13(3):747–755. [PubMed: 7917304]
- Tang J, Maximov A, Shin OH, Dai H, Rizo J, Sudhof TC. A complexin/syntaxin 1 switch controls fast synaptic vesicle exocytosis. *Cell*. 2006; 126(6):1175–1187. [PubMed: 16990140]
- Taylor DP, Eison MS, Riblet LA, Vandermaelen CP. Pharmacological and clinical effects of buspirone. *Pharmacol. Biochem. Behav.* 1985; 23(4):687–694. [PubMed: 2866549]
- Tedford HW, Kisilevsky AE, Peloquin JB, Zamponi GW. Scanning Mutagenesis Reveals a Role for Serine 189 of the Heterotrimeric G-Protein Beta 1 Subunit in the Inhibition of N-Type Calcium Channels. *J. Neurophysiol.* 2006; 96(1):465–470. [PubMed: 16687621]
- Tedford HW, Zamponi GW. Direct G Protein Modulation of Cav2 Calcium Channels. *Pharmacol. Rev.* 2006; 58(4):837–862. [PubMed: 17132857]
- Teng FY, Wang Y, Tang BL. The syntaxins. *Genome Biol.* 2001; 2(11) reviews3012.1–3012.7.
- Toonen RF, Kochubey O, de Wit H, Gulyas-Kovacs A, Konijnenburg B, Sorensen JB, Klingauf J, Verhage M. Dissecting docking and tethering of secretory vesicles at the target membrane. *EMBO J.* 2006; 25(16):3725–3737. [PubMed: 16902411]
- Umemiya M, Berger AJ. Activation of adenosine A1 and A2 receptors differentially modulates calcium channels and glycinergic synaptic transmission in rat brainstem. *Neuron*. 1994; 13(6):1439–1446. [PubMed: 7993635]
- Verhage M, Maia AS, Plomp JJ, Brussaard AB, Heeroma JH, Vermeer H, Toonen RF, Hammer RE, Berg TKvd, Missler M, Geuze HJ, Südhof TC. Synaptic Assembly of the Brain in the Absence of Neurotransmitter Secretion. *Science*. 2000; 287(5454):864–869. [PubMed: 10657302]
- Verhage M, Sorensen JB. Vesicle docking in regulated exocytosis. *Traffic*. 2008; 9(9):1414–1424. [PubMed: 18445120]
- Voets T, Toonen RF, Brian EC, de Wit H, Moser T, Rettig J, Sudhof TC, Neher E, Verhage M. Munc18-1 promotes large dense-core vesicle docking. *Neuron*. 2001; 31(4):581–591. [PubMed: 11545717]
- Weber T, Zemelman BV, McNew JA, Westermann B, Gmachl M, Parlati F, Söllner TH, Rothman JE. SNAREpins: Minimal Machinery for Membrane Fusion. *Cell*. 1998; 92(6):759–772. [PubMed: 9529252]
- Weimer RM, Richmond JE. Synaptic vesicle docking: a putative role for the Munc18/Sec1 protein family. *Curr. Top. Dev. Biol.* 2005; 65:83–113. [PubMed: 15642380]
- Weimer RM, Gracheva EO, Meyrignac O, Miller KG, Richmond JE, Bessereau JL. UNC-13 and UNC-10/rim localize synaptic vesicles to specific membrane domains. *J. Neurosci.* 2006; 26(31):8040–8047. [PubMed: 16885217]
- Wells CA, Betke KM, Lindsley CW, Hamm HE. Label-Free Detection of G Protein–SNARE Interactions and Screening for Small Molecule Modulators. *ACS Chemical Neuroscience*. 2011 [serial on the Internet] Available from: <http://dx.doi.org/10.1021/cn200102d>.
- Weninger K, Bowen ME, Choi UB, Chu S, Brunger AT. Accessory proteins stabilize the acceptor complex for synaptobrevin, the 1:1 syntaxin/SNAP-25 complex. *Structure*. 2008; 16(2):308–320. [PubMed: 18275821]
- Wickman KD, Iniguez-Lluhl JA, Davenport PA, Taussig R, Krapivinsky GB, Linder ME, Gilman AG, Clapham DE. Recombinant G-protein beta gamma-subunits activate the muscarinic-gated atrial potassium channel. *Nature*. 1994; 368(6468):255–257. [PubMed: 8145826]
- Wilding TJ, Womack MD, McCleskey EW. Fast, local signal transduction between the mu opioid receptor and Ca²⁺ channels. *J. Neurosci.* 1995; 15(5 Pt 2):4124–4132. [PubMed: 7538571]
- Wilkie TM, Gilbert DJ, Olsen AS, Chen XN, Amatruda TT, Korenberg JR, Trask BJ, de Jong P, Reed RR, Simon MI, et al. Evolution of the mammalian G protein alpha subunit multigene family. *Nat. Genet.* 1992; 1(2):85–91. [PubMed: 1302014]
- Wojcik SM, Brose N. Regulation of membrane fusion in synaptic excitation-secretion coupling: speed and accuracy matter. *Neuron*. 2007; 55(1):11–24. [PubMed: 17610814]
- Wu LG, Saggau P. GABAB receptor-mediated presynaptic inhibition in guinea-pig hippocampus is caused by reduction of presynaptic Ca²⁺ influx. *J. Physiol.* 1995; 485(Pt 3):649–657. [PubMed: 7562607]

- Xie K, Allen KL, Kourrich S, Colon-Saez J, Thomas MJ, Wickman K, Martemyanov KA. Gbeta5 recruits R7 RGS proteins to GIRK channels to regulate the timing of neuronal inhibitory signaling. *Nat. Neurosci.* 2010; 13(6):661–663. [PubMed: 20453851]
- Yamada M, Ho YK, Lee RH, Kontanill K, Takahashill K, Katadall T, Kurachi Y. Muscarinic K⁺ channels are activated by beta gamma subunits and inhibited by the GDP-bound form of alpha subunit of transducin. *Biochem. Biophys. Res. Commun.* 1994; 200(3):1484–1490. [PubMed: 8185603]
- Yamakuchi M, Ferlito M, Morrell CN, Matsushita K, Fletcher CA, Cao W, Lowenstein CJ. Exocytosis of endothelial cells is regulated by N-ethylmaleimide-sensitive factor. *Methods Mol. Biol.* 2008; 440:203–215. [PubMed: 18369947]
- Yang B, Steegmaier M, Gonzalez LC, Scheller RH. nSec1 binds a closed conformation of syntaxin1A. *J. Cell Biol.* 2000; 148(2):247–252. [PubMed: 10648557]
- Yao J, Gaffaney J, Kwon S, Chapman E. Doc2 is a Ca²⁺ sensor required for asynchronous neurotransmitter release. *Cell.* 2011; 147(3):666–743. [PubMed: 22036572]
- Yokogawa M, Osawa M, Takeuchi K, Mase Y, Shimada I. NMR analyses of the Gbetagamma binding and conformational rearrangements of the cytoplasmic pore of G protein-activated inwardly rectifying potassium channel 1 (GIRK1). *J. Biol. Chem.* 2011; 286(3):2215–2223. [PubMed: 21075842]
- Yokoyama S, Starmer WT. Phylogeny and evolutionary rates of G protein alpha subunit genes. *J. Mol. Evol.* 1992; 35(3):230–238. [PubMed: 1518090]
- Yoon EJ, Gerachshenko T, Spiegelberg BD, Alford S, Hamm HE. Gbetagamma interferes with Ca²⁺-dependent binding of synaptotagmin to the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex. *Mol. Pharmacol.* 2007; 72(5):1210–1219. [PubMed: 17715396]
- Yoon EJ, Hamm HE, Currie KP. G protein betagamma subunits modulate the number and nature of exocytotic fusion events in adrenal chromaffin cells independent of calcium entry. *J Neurophysiol.* 2008; 100(5):2929–2939. [PubMed: 18815342]
- Zamponi GW, Bourinet E, Nelson D, Nargeot J, Snutch TP. Crosstalk between G proteins and protein kinase C mediated by the calcium channel $\alpha 1$ subunit. *Nature.* 1997; 385(6615):442–446. [PubMed: 9009192]
- Zhai RG, Bellen HJ. The architecture of the active zone in the presynaptic nerve terminal. *Physiology.* 2004; 19:262–270. [PubMed: 15381754]
- Zhang, X-l; Upreti, C.; Stanton, PK. G $\beta\gamma$ and the C Terminus of SNAP-25 Are Necessary for Long-Term Depression of Transmitter Release. *PLoS One.* 2011; 6(5):e20500. [PubMed: 21633701]
- Zhang X, Kim-Miller MJ, Fukuda M, Kowalchuk JA, Martin TFJ. Ca²⁺-Dependent Synaptotagmin Binding to SNAP-25 Is Essential for Ca²⁺-Triggered Exocytosis. *Neuron.* 2002; 34(4):599–611. [PubMed: 12062043]
- Zhao Q, Kawano T, Nakata H, Nakajima Y, Nakajima S, Kozasa T. Interaction of G protein beta subunit with inward rectifier K(+) channel Kir3. *Mol. Pharmacol.* 2003; 64(5):1085–1091. [PubMed: 14573757]
- Zhao Y, Fang Q, Straub SG, Lindau M, Sharp GW. Noradrenaline inhibits exocytosis via the G protein betagamma subunit and refilling of the readily releasable granule pool via the alpha(i1/2) subunit. *J Physiol.* 2010; 588(Pt 18):3485–3498. [PubMed: 20643776]
- Zhu Y, Ikeda SR. VIP inhibits N-type Ca²⁺ channels of sympathetic neurons via a pertussis toxininsensitive but cholera toxin-sensitive pathway. *Neuron.* 1994; 13(3):657–669. [PubMed: 7917296]

Abbreviations

5-HT	serotonin
ADRA2A	α_{2a} adrenergic receptor gene
ATP	adenosine triphosphate

CSP	cysteine string protein
GABA	gamma-aminobutyric acid
GIRK	G protein-coupled inward rectifier K ⁺ channels
GPCR	G protein coupled receptors
GRK2	G protein-coupled receptor kinase 2
K_{ir}	inwardly rectifying potassium channel
LTD	long-term depression
LTP	long-term potentiation
mGluR	metabotropic glutamate receptor
MHD	Munc homology domain
RGS	regulator of G protein signaling
SM	Sec-1/Munc-18
SNAP	synaptosome-associated protein
SNARE	soluble N-ethylmaleimide-sensitive factor attachment protein receptor
VAMP	vesicle-associated membrane protein
VDCC	voltage-dependent calcium channels

Figure 1. Overview of neurotransmission across a synapse

In the presynaptic neuron, synaptic vesicles expressing VAMP2, among other proteins embedded in their membrane, are loaded with neurotransmitters. Simplistically, docking of the vesicle occurs via interaction of VAMP2 with t-SNARE (SNAP-25/syntaxin 1A), a process that is guided and regulated by numerous proteins, while priming has been described as further zippering of the SNARE complex with interaction of other major components such as synaptotagmin, MUNC13, and MUNC18. Upon the arrival of an action potential, VDCC facilitate a large increase in intracellular calcium concentration. This rise is sensed by proteins within the synaptic complex of the vesicle at the membrane such that fusion of the vesicle membrane with the presynaptic membrane occurs, resulting in release of neurotransmitter into the synaptic cleft to activate its respective receptor on the postsynaptic neuron ensuring propagation of action potentials from one neuron to another.

Figure 2. Structure of G protein Coupled Receptors and Heterotrimeric G proteins

Shown in ribbon diagrams are representative examples of GPCRs and heterotrimeric G proteins. A) The structure of rhodopsin (PDB ID: 1F88) (Palczewski et al., 2000) is shown in orange, and it depicts the seven transmembrane α -helices and the respective extracellular and intracellular surfaces that interact with agonists and heterotrimeric G proteins, respectively. B) The structure of the heterotrimer transducin (PDB ID: 1GOT) (Lambright et al., 1996) is shown. The $G\alpha$ subunit (green) has two domains, a GTPase domain and an α -helical domain. Within the GTPase domain, there are three regions termed Switch I, II, and III, (C), that have different orientations depending on which guanine nucleotide is present. In the GDP-bound form, Switches I–III form the major interface that interacts with the $G\beta\gamma$

dimer. The structure of the $G\beta$ subunit (red) is an N-terminal α -helix followed by a series of β sheets that make a seven-bladed propeller or toroid. A single blade (light blue) is based on the amino acid motif termed WD-40 repeat. The structure of the $G\gamma$ subunit (dark blue) is two tandem α -helices that form interactions with the N-terminal α -helix of $G\beta$ and a surface of the $G\beta$ toroid on the opposite face from where $G\alpha$ interacts with $G\beta$.

Figure 3. The interactions with Gβγ during exocytosis

Gβγ subunits are known to have a variety of interactions during exocytosis - GPCRs and Gα subunits during its activation, as well as a number of effectors. Shown are the structures, if known, of those proteins that interact with Gβγ and play a role in exocytosis. THE GPCRs and Gα are discussed in Figure 2. The SNARE proteins (SNAP-25/Syntaxin 1A/VAMP2) form a trimeric complex through interaction of their α-helical SNARE motifs, SNAP-25 having 2 two of them (PDB ID: 1SFC)(Sutton et al., 1998). VAMP2 (purple) and syntaxin 1A (cyan) have transmembrane domains through which they interact with membranes. SNAP-25 (green) has a palmitoylation site near the C-terminal portion of its first SNARE motif by which it is tethered to the membrane. Not shown is the N-terminal helical domain

of syntaxin 1A, H_{abc} . The crystal structure of syntaxin 1A without the transmembrane domain confirms the triple helix H_{abc} domain at its N-terminus. The GIRK channel is represented by the crystal structure for the structurally similar ATP-sensitive K_{ir} channel 10 which is a tetramer with both transmembrane and intracellular domains (PDB ID: 2X6A) (Clarke et al., 2010). There is no structure determined yet for VDCC; it is shown as a simple transmembrane cartoon.

Figure 4. Hypothesis of Gβγ regulation of presynaptic vesicle release

Synaptic vesicles are primed by a tethering interaction between VAMP2 on the vesicle and the SNAP-25/syntaxin 1A dimer at the plasma membrane. At low intracellular concentrations of calcium, activation of G_{i/o}-coupled receptors results in release of Gβγ that will bind to the SNARE proteins and prevent binding of synaptotagmin. However, high enough intracellular calcium concentrations, such as with repetitive neuronal stimulation, synaptotagmin is able to compete with Gβγ for binding to SNARE, and thereby promote fusion of the vesicles with the plasma membrane. Figure adapted from Wells et al. (Wells et al., 2011).

Table 1
Inhibitory GPCR function at synapses and cell bodies in the central nervous system

Listed are examples of $G_{i/o}$ -coupled GPCRs known to inhibit synaptic transmission via actions on voltage-dependent calcium channels or exocytotic machinery downstream of calcium entry. Receptors are separated depending on localization to the synapse or soma of neurons in the central nervous system.

	Synapse		Soma
	VDCC	Exocytotic fusion apparatus	VDCC
M2/M4 Ach receptor	(Higley et al., 2009)	(Bellingham and Berger, 1996; Scanziani et al., 1995)	(Allen and Brown, 1993; Delmas et al., 1998)
Group II mGlu receptor	(Capogna, 2004; Glitsch, 2006; Scanziani et al., 1995)		
Group III mGlu receptor	(Giustizieri et al., 2005)	(Gereau and Conn, 1995)	
GABA _B receptor	(Dittman and Regehr, 1996; Isaacson, 1998; Wu and Saggau, 1995)	(Dittman and Regehr, 1996; Harvey and Stephens, 2004; Wu and Saggau, 1995)	
μ opioid receptor	(Heinke et al., 2011)	(Heinke et al., 2011)	(Wilding et al., 1995)
CB1 cannabinoid receptor	(Brown et al., 2004)		
A1 adenosine receptor	(Dittman and Regehr, 1996; Scholz and Miller, 1992; Umekiya and Berger, 1994)	(Scholz and Miller, 1992)	(Umekiya and Berger, 1994)
D2 dopamine receptor	(Hamilton and Smith, 1991; Higley and Sabatini, 2010)	(Nicola and Malenka, 1997)	(Cabrera-Vera et al., 2004)
α_2 adrenergic receptor	(Boehm, 1999)	(Delaney et al., 2007)	(Boehm, 1999)