Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

G-protein regulation of neuronal calcium channels: back to the future

Juliane Proft and Norbert Weiss

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

Running title: Regulation of calcium channels by G-proteins

Correspondence to: Norbert Weiss
Institute of Organic Chemistry and Biochemistry
Academy of Sciences of the Czech Republic, v.v.i.
Flemingovo nám. 2.
166 10 Prague 6 - Dejvice
Czech Republic
E-mail: weiss@uochb.cas.cz

Document statistics:
Text pages: 63
Tables: 1
Figures: 5
References: 191
Abstract: 118
Introduction: 527
Discussion: 434 (see Concluding remarks and perspectives)

Non-standard abbreviations

AA, arachidonic acid; AID, alpha interaction domain; AMPc, cyclic adenosine monophosphate; CSP, cysteine string protein; DAG, diacyglycerol; DRG, dorsal root ganglion; FHM-1, familial hemiplegic migraine type-1; FRET, fluorescence resonance energy transfer; GDP, guanosine-5'-diphosphate; GK, guanylate kinase; GPCR, G-protein coupled receptor; GTP, guanosine-5'-trisphosphate; HEK293, human embryonic kidney 293; IP3, inositol trisphosphate; M2, muscarinic receptor 2; MAGUK, membrane-associated guanylate kinase; NOP, nociception receptor; ORL-1, opioid receptor like-1; PIP2, Phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; RGS, regulator of G-protein signaling; RIM, rab3-interacting molecule; SNAP-25, synaptosomal-associated protein 25; SNARE, soluble NSF (N-ethylmaleimide-sensitive fusion protein) attachment protein receptor; *Synprint*, synaptic protein interaction; VGCC, voltage-gated calcium channel.

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Neuronal voltage-gated calcium channels have evolved as one of the most important players for calcium entry into presynaptic endings responsible for the release of neurotransmitter. In turn, and in order to fine tune synaptic activity and neuronal communication, numerous neurotransmitters exert a potent negative feedback over the calcium signal provided by G-protein-coupled receptors. This regulation pathway of physiological importance is also extensively exploited for therapeutic purposes, for instance in the treatment of neuropathic pain by morphine and other mu-opioid receptor agonists. However, despite more than three decades of intensive research, important questions remain unsolved regarding the molecular and cellular mechanisms of direct G-protein inhibition of voltage-gated calcium channels. Here, we revisit this particular regulation and explore new considerations.

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Within neurons, calcium ion (Ca^{2+}) represents an essential important signaling molecule, responsible for regulation of a large number of diverse cellular functions (Berridge, 1998). Voltage-gated Ca^{2+} channels (VGCCs) have evolved as one of the most important players in the initiation of the Ca^{2+} signal by converting electrical impulse into intracellular Ca^{2+} elevation (Catterall, 2011). VGCCs are pore-forming multi-subunits plasma membrane complexes that are activated upon membrane depolarization (i.e. action potentials) to permit entry of Ca^{2+} along its electrochemical gradient. To date, ten genes encoding the pore-forming subunits of mammalian VGCCs have been identified (Figure 1). Seven genes encode the high-voltage activated (HVA) channel subfamily consisting of L-type ($Ca_v1.1$ to $Ca_v1.4$), P/Q-type ($Ca_v2.1$), N-type ($Ca_v2.2$), R-type ($Ca_v2.3$), and three genes encode the low-voltage-activated (LVA) channel subfamily composed exclusively of T-type channels ($Ca_v3.1$ to $Ca_v3.3$) (Ertel et al., 2000). The Ca_v pore-forming subunits of VGCCs share a similar transmembrane topology built of four homologous domains each of them containing six putative transmembrane helices (S1-S6), plus a re-entrant loop (P-loop) that forms the pore of the channel. The four domains are connected via large cytoplasmic linkers (loops I-II, II-III, III-IV), and cytoplasmic amino- and carboxy-terminal domains, which form interaction sites for various regulatory proteins. In addition to the Ca_v pore-forming subunit, HVA channels contain ancillary subunits (Arikkath and Campbell, 2003): β (β_1 to β_4, a 55 kDa cytosolic protein of the membrane-associated guanylate kinase (MAGUK) family), $\alpha_2\delta$ ($\alpha_2\delta_1$ to $\alpha_2\delta_4$, a 170 kDa highly glycosylated extracellular protein with a single transmembrane domain), and in some cases γ (γ_1 to γ_8, a 33 kDa transmembrane protein), that control channel trafficking, gating and function at the plasma membrane.

In order to make use and regulate the amplitude, duration and subcellular localization of the Ca^{2+} signal, VGCCs are under tight regulatory control. One of the most important regulatory mechanisms involves G-protein coupled receptors (GPCRs), also known as seven-transmembrane domain receptors. GPCRs are a large protein family of integral membrane receptors (Vassilatis et al., 2003) that sense extracellular molecules such as neurotransmitters and in turn activate intracellular signaling pathways by regulating the activity of heterotrimeric G-proteins. Heterotrimeric G-proteins consist of a Gα-subunit that binds and hydrolyses GTP into GDP, and Gβ- and Gγ-subunits that remain constitutively associated and form the Gβγ-dimer (Wettschureck and Offermanns, 2005). In the absence of stimulus, GDP-bound Gα-subunit and Gβγ-dimer are associated with the receptor. Binding of an extracellular ligand onto the GPCR induces a conformational change that promotes the exchange of GDP for GTP from the Gα-subunit, resulting in the dissociation of the GTP-bound Gα and Gβγ-dimer from the receptor. Intrinsic hydrolysis of GTP by Gα-subunit can be speeded up by GTPase activating proteins such as regulators of G-protein signaling (RGS) that allows reassociation of GDP-bound Gα-subunit with the Gβγ-dimer, which terminates G-protein signaling (Figure 2). Although both GTP-bound Gα-subunit and the Gβγ-dimer mediate intracellular signaling by modulating the activities of neuronal VGGCs, this review is focused on the so-called direct "voltage-dependent" regulation mediated by the Gβγ-dimer. Interested readers may also refer to the recent review of Zamponi and Currie for an interesting discussion on the regulation of VGCCs by Gα- and protein kinase-dependent signaling (Zamponi and Currie, 2013).

Description of the phenomenon

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

Interestingly enough, while Ca^{2+} entry at synaptic endings can trigger transmitter release, numerous neurotransmitters released from synaptic contacts and hormones secreted at proximity of the synaptic cleft are in turn able to modulate presynaptic VGCCs via activation of G-protein-coupled receptors (GPCRs) to terminate the Ca^{2+} signal and neurotransmitter discharge. This regulation is not only of physiological importance but it is also extensively exploited as a therapeutic avenue. For example, one of the most remarkable usages of G-protein-mediated inhibition of VGCCs is the management of pain symptoms by specific opioid receptor agonists (e.g. natural opiates like morphine and its synthetic opioid derivatives).

The first observation that synaptic activity is modulated by neurotransmitters goes back to the late 1970s with the pioneer work of Dunlap and Fischbach on sensory neurons (Dunlap and Fischbach, 1978), and later attributed the phenomena to the inhibition of VGCCs (Dunlap and Fischbach, 1981). To date, up to 20 neurotransmitters and corresponding receptors have been described to modulate VGCCs (Table 1), including noradrenaline (Bean, 1989) (Docherty and McFadzean, 1989) (McFadzean and Docherty, 1989) (Lipscombe et al., 1989), somatostatin (Bean, 1989) (Ikeda and Schofield, 1989a, Ikeda and Schofield, 1989b), gamma-aminobutyric acid (GABA) (Deisz and Lux, 1985) (Dolphin and Scott, 1987) (Grassi and Lux, 1989) and acetylcholine (Bernheim et al., 1991) (Shapiro et al., 1999).

Based on the observation that application of pertussis toxin on rat dorsal root ganglion neurons (DRG), or intracellular injection of non-hydrolysable GDPβS, prevents inhibition of voltage-activated Ca^{2+} currents by noradrenaline or GABA, it was proposed that heterotrimeric G-proteins certainly mediate GPCR-dependent modulation of VGCCs (Holz et al., 1986) (Scott and Dolphin, 1986). Using an original approach, Forscher and Collaborators

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

further demonstrated that this regulation is spatially delimited and does not involve diffusible second messengers, suggesting proximity between the Ca^{2+} channel and the GPCR (Forscher et al., 1986). The functional importance of channel / GPCR coupling in G-protein mediated inhibition of Ca^{2+} currents will be further discussed later in this review. A period of intensive work and controversy followed (1989-1996) to determine which of the G-protein subunits mediate inhibition of the Ca^{2+} channel. Using specific antibodies and antisense oligonucleotides to block or knockdown Gα-subunits of heterotrimeric G-proteins, it was initially proposed that Gαo is the mediator of the inhibition (McFadzean et al., 1989) (Baertschi et al., 1992) (Campbell et al., 1993) (Menon-Johansson et al., 1993). However, during the same period of time, various studies suggested as well an implication of Gαi (Ewald et al., 1989) or Gαs and Gαq (Shapiro and Hille, 1993) (Golard et al., 1994) (Zhu and Ikeda, 1994). The divergent results led to the hypothesis that GPCR-mediated inhibition of VGCCs is not mediated by Gα-subunits but rather by a common G-protein determinant, and Herlitze and Colleagues (Herlitze et al., 1996) and Ikeda (Ikeda, 1996) eventually established that inhibition of Ca_v2 channels is mediated by the Gβγ-dimer concomitantly produced with Gα-GTP following GPCR activation. Indeed, the overexpression of Gβ1γ2- or Gβ2γ3-dimers in sympathetic neurons is sufficient to mimic noradrenaline-mediated inhibition of N-type currents, and prevents subsequent inhibition by α_2-adrenergic agonists. The question is how can we incorporate those results in the observation of other groups suggesting a functional implication of Gα-subunits? Interestingly enough, it appears that even though Gβγ-dimer is the mediator of the inhibition, Gα-subunits might have an important role in the ability and specificity of GPCRs to functionally couple with VGCCs. Hence, inhibition of neuronal VGCCs is usually mediated by GPCRs coupled to Gαi or Gαo (Holz et al., 1986, Scott and Dolphin, 1986) such as α_2-

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

adrenergic receptors, clarifying why depletion of the Gαo-subunit in NG108-15 cells prevents noradrenaline-mediated inhibition of Ca^{2+} currents (McFadzean et al., 1989).

Landmarks of GPCR-mediated inhibition of VGCCs

Inhibition of VGCCs by GPCRs involves the direct binding of G-protein βγ-dimer onto various structural molecular determinants of the Ca_v2-subunit (see next section). At the whole-cell level, this regulation is characterized by various phenotypical modifications of the Ca^{2+} current properties (Figure 3). The most obvious is a decrease of the inward current amplitude (Boland and Bean, 1993) (Wu and Saggau, 1997) that usually varies from 15 to 80% depending of the Ca_v channel / GPCR involved. Based on the observation that Gβγ-mediated inhibition of VGCCs is less pronounced at depolarized membrane potential, this regulation was named "voltage-dependent". In some cases, this inhibition is also accompanied by a depolarizing shift of the voltage-dependence curve of current activation (Bean, 1989), and a slowing of activation (Marchetti et al., 1986) and inactivation kinetics (Zamponi, 2001). In addition, short highly depolarizing voltage step, usually applied around +100mV before the current eliciting pulse (double-pulse protocol), is sufficient to reverse, at least partially, most of the landmarks of G-protein inhibition and producing a so-called "prepulse facilitation" (Scott and Dolphin, 1990) (Ikeda, 1991) (Doupnik and Pun, 1994). Current inhibition has been attributed to the direct binding of Gβγ-dimer to the Ca_v2-subunit (referred as "*ON*" landmark), whereas all the other landmarks including the slowing of current kinetics and prepulse facilitation can be described as variable time-dependent dissociation of Gβγ-dimer from the channel (referred as "*OFF*" landmarks) and consequent current recovery from inhibition (Elmslie and Jones, 1994, Stephens et al., 1998, Weiss et al., 2006). It is worth noting that "*ON*" and "*OFF*" landmarks do not represent two

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

independent regulations but rather the transition from Gβγ-bound channels to Gβγ-unbound channels, and *vice versa*. Furthermore, although the dissociation of Gβγ-dimer was previously defined as "voltage-dependent", it was then proposed that channel opening after membrane depolarization and associated conformational changes of the Ca_v2-subunit was likely the trigger for Gβγ dissociation from the channel (Patil et al., 1996). More recently, this concept was further analyzed and it was shown that the voltage-dependence of the kinetic of Gβγ dissociation correlates to the voltage-dependence of the channel activation (Weiss et al., 2006). Hence, it is likely that the trigger of Gβγ dissociation is not the electrical membrane potential *per se*, but rather the conformational change that occurs within the Ca_v2-subunit during the opening of the channel, making the regulation intrinsically "channel-opening-dependent" rather than "voltage-dependent".

What are the Ca_v channel molecular determinants of G-protein inhibition?

Various structural determinants of the Ca_v2-subunit have been characterized either as direct biochemical binding loci for Gβγ-dimer or of functional importance for GPCR-mediated regulation of Ca^{2+} currents. These determinants are located within the I-II loop of the Ca_v2-subunit, as well as in the amino- and carboxyl-terminal regions of the channel.

Role of the I-II loop of Ca_v2 channels

The observation that Gβγ-dimer is able to functionally interact with the Ca_vβ-subunit (Campbell et al., 1995) led some groups to question the role of the I-II loop of Ca_v2 channels in G-protein regulation. It is well established that Gβγ-dimer is able to interact with the type II adenylate cyclase and the phospholipase $C\beta_2$ via a consensus motif QXXER (Chen et al., 1995). Interestingly, this consensus site is also present within the I-II loop of Ca_v2 channels,

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

and is located in a proximal region of the so-called AID (Alpha Interaction Domain), a 18 amino-acids sequence (**QQIER**ELNGY--WI--AE)) initially described as the main interaction locus of the $Ca_v\beta$-subunit (Pragnell et al., 1994), suggesting a possible interaction between the I-II loop and the Gβγ-dimer. Consistent with this idea, injection in HEK293 cells of the synthetic peptide of the $Ca_v2.2$-subunit FLKRRQ**QQIER**ELNGYL (peptide I-II_{S2}, Figure 4) prevents Gβγ-mediated inhibition of $Ca_v2.2/\alpha_2/\beta_{1b}$ channel, suggesting that Gβγ-dimer is able to interact with the peptide (Zamponi et al., 1997). This interaction was then demonstrated by in vitro binding of Gβ1γ2-dimer onto the GST-AID_A fusion protein with an affinity of 63 nM (De Waard et al., 1997). For comparison, $Ca_v\beta_{1b}$ binds to the GST-AID_A fusion protein with an apparent Kd of 5 nM that is a dozen times more efficient than the binding of Gβγ-dimer (De Waard et al., 1995). In addition, the role of the QXXER domain in the binding of Gβγ-dimer was further analyzed by site-directed mutagenesis. Hence, substitution of the arginine (R) by a glutamic acid (E) is sufficient to prevent the binding of Gβ1γ2-dimer to the GST-AID_A fusion protein, but also prevents GTPγS-induced inhibition of $Ca_v2.1/\beta_4$ channels expressed in *Xenopus* oocyte (De Waard et al., 1997). This functional result is however in contradiction with another observation that the same amino acid substitution in the $Ca_v2.1$ channel expressed in tsA-201 cells together with the $Ca_v\beta_{1b}$ subunit promotes channel inhibition by GTPγS (Herlitze et al., 1997). At this point, it is worth noting that substitution of the arginine located in the QXXER motif to a glutamic acid not only prevents the binding of the Gβγ-dimer, but also slows down the inactivation kinetics of the channel (Herlitze et al., 1997). Hence, the QXXER domain appears to be not only a binding locus of the Gβγ-dimer within the I-II loop of the Ca_v2-subunit, but also an important molecular determinant of the fast inactivation of the channel. Considering that channel inactivation is by itself an important component for back modulation of G-protein

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

inhibition, as we will see later, it is delicate to conclude on the exact functional role of the QXXER domain. It was also shown that substitution of the isoleucine (I) to a leucine (L) within the QQIER domain of the $Ca_v2.1$-subunit decreases GTPγS-induced inhibition of $Ca_v2.1/\beta_{1b}$ channel (Herlitze et al., 1997). Altogether, these results provide a structural explanation for the weak sensibility of Ca_v1 channels, which instead contain a QQLEE motif, to GPCR inhibition (Bell et al., 2001). However, insertion of the consensus QXXER domain into the $Ca_v1.2$-subunit is not sufficient to make the channel sensitive to G-protein inhibition (Herlitze et al., 1997), suggesting that despite an important role in the biochemical coupling of Gβγ-dimer with the channel, the QXXER domain is not the sole molecular determinant for potent channel inhibition by G-proteins. Hence, additional molecular loci of the I-II loop have been identified. For example, injection in HEK293 cells of the GVLGEFAKERERVENRRA peptide of the $Ca_v2.2$-subunit (peptide $I\text{-}II_{S1}$, Figure 4), localized upstream the AID domain and straddling the IS6 transmembrane segment and the I-II loop, is able to prevent Gβγ-dependent inhibition of $Ca_v2.2/\alpha_2/\beta_{1b}$ channels, suggesting a second Gβγ-dimer binding locus (Zamponi et al., 1997). Finally, the GID domain (G-protein Interaction Domain) localized 13 amino acid residues upstream the AID domain has also been proposed as a molecular locus for Gβγ binding with an affinity of 20 nM (De Waard et al., 1997), and the corresponding synthetic peptide is likewise able to prevent Gβγ modulation of Ca^{2+} currents (Zamponi et al., 1997). Interestingly enough, PKC-dependent phosphorylation of the GID domain on a threonine residue prevents binding of Gβγ, suggesting a functional crosstalk between direct (i.e. mediated by Gβγ-dimer) and indirect (i.e. involving second messengers) GPCR modulation of VGCCs (Zamponi et al., 1997).

Altogether, it is unambiguous that the cytoplasmic I-II loop of Ca_v2 channels contains various structural determinants for Gβγ-dimer binding. However, the functional importance

of those determinants in the modulation of the Ca^{2+} currents remains largely discussed and other groups rather suggested that the I-II loop was not critical for Gβγ-mediated inhibition of Ca^{2+} channels. Hence, it has been shown that the substitution of the I-II loop of the $Ca_v2.2$-subunit by the I-II loop of the $Ca_v2.1$ channel (less sensitive to Gβγ inhibition) or by the I-II loop of the $Ca_v1.2$ channel (insensitive to direct G-protein modulation) does not alter somatostatine-mediated inhibition of the $Ca_v2.2/\beta_{1b}$ channel (Zhang et al., 1996). Conversely, the replacement of the I-II loop of the $Ca_v1.2$-subunit by the I-II loop of the $Ca_v2.2$-subunit is not sufficient to restore a direct G-protein modulation of the $Ca_v1.2/\beta_{2a}$ channel by D2 dopaminergic receptors (Canti et al., 1999). Finally, the substitution of the I-II loop of the $Ca_v2.3$ channel (rat rbE-II isoform (Soong et al., 1993)) - that is less sensitive to direct G-protein modulation - by the I-II loop of the $Ca_v2.2$-subunit, induces a slowing of activation kinetics of $Ca_v2.3/\beta_{2a}$ channel upon activation of G-proteins by injection of GTPγS, but no net inhibition of the maximal amplitude of the Ca^{2+} current (Page et al., 1997). Hence, it was proposed that the I-II loop could be the channel molecular determinant mediating the slowing of current kinetics under direct G-protein regulation, and that current inhibition *per se* could require other molecular determinants (Page et al., 1997). Considering that the apparent slowing of current activation kinetics is attributed to the dissociation of Gβγ-dimer from the channel ("*OFF*" landmarks) (Elmslie and Jones, 1994) (Stephens et al., 1998) (Weiss et al., 2006), the I-II loop might be the channel molecular determinant involved in the dissociation of Gβγ-dimer from the Ca_v2-subunit.

Role of the amino-terminal domain of Ca_v2 channels

Interestingly enough, while Ca^{2+} currents generated by the BII-2 brain rabbit isoform of the $Ca_v2.3$-subunit are subject to direct G-protein modulation (either upon somatostatin

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

application (Yassin et al., 1996) or by direct injection of GTPγS (Meza and Adams, 1998)), currents generated by the rat rbE-II isofom that presents a truncated amino-terminal domain remain insensitive (Page et al., 1997), suggesting an implication of the amino-terminal domain of the Ca_v2 channel in direct G-protein modulation. Consistent with this idea, extension by PCR of the amino-terminal region of the rbE-II isoform, leading to a $Ca_v2.3$-subunit homologue to the rabbit one, is sufficient to restore quinpirole (D2 dopaminergic agonist)-mediated inhibition of Ca^{2+} currents (Page et al., 1998). Moreover, the substitution of the amino-terminal region of the $Ca_v1.2$-subunit by the amino-terminal domain of the $Ca_v2.2$-subunit makes the $Ca_v1.2$ channel sensitive to quinpirole-mediated inhibition (Canti et al., 1999). Further work identified a highly conserved 11 amino-acids sequence (**YKQ**SIAQ**RAR**T) in the amino-terminal region of Ca_v2 channels critical for direct G-protein modulation (Canti et al., 1999) (Figure 4A), and alanine scan of the YKQ and RAR motifs abolished quinpirole-induced inhibition of $Ca_v2.3/\beta_{2a}/\alpha_2\delta$ channels (Canti et al., 1999). In addition, it was proposed that the amino-terminal region of the $Ca_v2.2$-subunit might constitute a G-protein-gated inhibitory module acting via interaction with the cytoplasmic I-II linker of the channel (Agler et al., 2005). More recently, $Ca_v2.2$ amino-terminal-derived peptides have been shown to prevent noradrenaline-induced G-protein inhibition of Ca^{2+} currents in superior cervical ganglion neurons (SCGNs) strengthening the implication of the Ca_v2 amino-terminal region in G-protein modulation in native environment (Bucci et al., 2011).

Role of the carboxy-terminal domain of Ca_v2 channels

The carboxy-terminal region of the Ca_v2-subunits has also been proposed as an important determinant for direct-G-protein regulation and a binding domain of Gβγ-dimer was

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

identified in the distal carboxy-terminal region of the $Ca_v2.3$-subunit (Qin et al., 1997) that presents some homologies with the corresponding region of the $Ca_v2.1$ and $Ca_v2.2$-subunits (Simen et al., 2001) (Figure 4A). Hence, the substitution of the carboxy-terminal region of the $Ca_v2.3$-subunit by the corresponding one of the $Ca_v1.2$-subunit is sufficient to abolish M2 muscarinic receptor-mediated inhibition of the $Ca_v2.3/\beta_{2a}$ channel (Qin et al., 1997). Association of G-protein β_{2}-subunit with the carboxy-terminal region of the $Ca_v2.1$ channel was also observed using a fluorescence resonance energy transfer approach (FRET) (Hummer et al., 2003). In addition, based on the observation that FRET signal between $Ca_v2.1/Ca_v\beta_{1b}$ FRET pairs is increased in the presence of $G\beta_2$-subunit and requires the presence of the carboxy-terminal region of the channel, it was proposed that binding of G-proteins to the carboxy-terminal region may produce a conformational change of the channel that might contribute to channel inhibition (Hummer et al., 2003). However, deletion of the carboxy-terminal region of the $Ca_v2.2$-subunit containing the binding determinant of $G\beta\gamma$-dimer does not alter inhibition of Ca^{2+} currents mediated by injection of GTPγS (i.e. agonist/receptor-independent) (Meza and Adams, 1998), but only alters somatostatin-mediated inhibition (i.e. agonist/receptor-dependent) of $Ca_v2.2$ channels (Hamid et al., 1999) suggesting that the carboxy-terminus might rather be involved in the functional coupling of the channel with agonist-dependent activation of G-proteins. Interestingly, the binding determinant of $G\beta\gamma$-dimer in the carboxy-terminal region of Ca_v2-channels is localized close to the binding domain of $G\alpha o$ on $Ca_v2.1$- and $Ca_v2.2$-subunits (Furukawa et al., 1998b) and $G\alpha q$ on the $Ca_v2.2$-subunit (Simen et al., 2001) (Figure 4A), suggesting that the carboxy-terminal region could be involved in the functional and biochemical coupling of Ca_v2 channels with the GPCR via the $G\alpha/\beta\gamma$ trimmer (Kitano et al., 2003)(Beedle et al., 2004, Kisilevsky et al., 2008) (Weiss, 2009). Paradoxically, the

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

importance of the carboxy-terminal region of Ca_v2 channels in the direct regulation by G-proteins was poorly investigated compared to the intensive work that was done for the I-II loop and the amino-terminal region. One possible reason is that injection of GTPγS, or overexpression of Gβγ-dimer to trigger direct G-protein modulation of Ca_v2 channels was not suitable to highlight the functional importance of the carboxy-terminal region.

Toward the notion of Gβγ-Protein-Binding Pocket

As previously seen, the Ca_v2-subunit contains various molecular determinants for Gβγ-dimer binding, and it was initially proposed that several Gβγ-dimers could interact simultaneously with the Ca_v2-subunit in a cooperative manner (Boland and Bean, 1993). However, further analysis revealed that the kinetics of Gβγ-dimer interaction with the $Ca_v2.2$ channel (evidenced by the functional modulation of the Ca^{2+} current) can be described by a mono-exponential function with a time constant directly correlated to the free Gβγ-dimer concentration, suggesting that only a single Gβγ-dimer interacts with the Ca_v2-subunit (Zamponi and Snutch, 1998). Based on this observation, it was proposed that the various Gβγ-binding sites located within the Ca_v2-subunit could be spatially structured to form a unique interaction domain called the Gβγ-Protein-Binding Pocket (GPBP) (De Waard et al., 2005) (Figure 4B), where various binding loci of the GPBP could be responsible of a particular feature of the G-protein regulation. Hence, the carboxy-terminal region of the Ca_v2-subunit would play a critical role in the inhibition of Ca^{2+} currents by GPCRs (Qin et al., 1997) (Furukawa et al., 1998b) (Furukawa et al., 1998a) through favoring the biochemical and functional coupling of the receptor with the channel via the Gα/βγ-trimmer (Kitano et al., 2003) (Beedle et al., 2004). In that context, the carboxy-terminal domain of the Ca_v2-subunit would not be directly involved in the inhibition of the Ca^{2+}

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

currents but would rather allow the rapprochement of the GPCR with the channel required for the direct G-protein modulation (Forscher et al., 1986). The I-II loop of the Ca_v2-subunit represents an important domain of interaction with the Gβγ-dimer via three motifs clearly identified. However, the binding of Gβγ-dimer onto the I-II loop does not appear critical for the inhibition of the Ca^{2+} current ("*ON*" landmark) (Zhang et al., 1996) (Qin et al., 1997), but rather seems to be involved in the relaxation of the inhibition ("*OFF*" landmarks, i.e. unbinding of Gβγ-dimer from the Ca_v2-subunit) in response for example to a depolarizing prepulse (Herlitze et al., 1997) (Simen and Miller, 2000). Finally, the amino-terminal region of the Ca_v2-subunit appears as the main determinant of direct G-protein inhibition of Ca^{2+} currents by Gβγ-dimer (Page et al., 1998) (Canti et al., 1999). In that context, the GPBP emerges as a dynamic structure, composed of various loci that on one hand bring the Ca^{2+} channel and the GPCR together, and on the other hand differentially mediates the "*ON*" and "*OFF*" G-protein landmarks.

How Gβγ-dimer inhibits Ca_v2 channels?

Mutagenesis studies have identified a number of amino acid residues on the surface of the Gβ–subunit important for inhibition of the Ca^{2+} channel (Ford et al., 1998) (Tedford et al., 2006) (Mirshahi et al., 2002). Interestingly, most of these residues are located on the G_b surface that interacts with Gα and are essentially masked when Gα is present. However, the molecular mechanism by which binding of Gβγ-dimer to the channel inhibits the Ca^{2+} current remains largely unknown. It has to be mentioned that beside the extensive work that was done to understand the molecular mechanisms of G-protein regulation of VGCCs, it still remains unclear how the binding of Gβγ-dimer to the Ca_v2-subunit inhibits the Ca^{2+} current. Based on the observation that the voltage-dependence curve of the Ca^{2+} current

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

under G-protein modulation is significantly shifted to more depolarized potentials, it was initially proposed that the Ca^{2+} channel undergoes a switch from a "*willing*" mode (i.e. easily activated) to a "*reluctant*" mode (i.e. hardly activated) upon GPCR activation (Bean, 1989). The channel returns to the "*willing*" state after dissociation of the Gβγ-dimer from the Ca_v2-subunit (Bean, 1989) (Elmslie et al., 1990). Consistent with this idea, single channel recordings revealed latency in the first opening of the $Ca_v2.2$ channel upon M2 muscarinic receptor activation, after what the channel presents a similar behavior as the non regulated control channel (Patil et al., 1996). Hence, it was proposed that the first opening latency could correspond to the time for Gβγ-dimer to dissociate from the Ca_v2-subunit in response to the membrane depolarization prior to channel opening (Patil et al., 1996). It is worth noting that low-probability openings of $Ca_v2.2$ channels under G-protein modulation have been observed, corresponding most likely to transient dissociations of the Gβγ-dimer from the channel upon moderate membrane depolarizations (30 mV) (Lee and Elmslie, 2000). This simple model of regulation appears to be sufficient to support both the inhibition of the Ca^{2+} current and the depolarizing shift of the current/voltage activation curve. However, the observation that the amplitude of gating currents of $Ca_v2.2/\alpha_2/\beta_{1b}$ channels is reduced upon injection of GTPγS led the authors to propose that G-proteins mediate inhibition of Ca^{2+} current by altering intrinsic gating properties of the channel (Jones et al., 1997). Gating currents are produced by the movement of the charged voltage-sensor domains, including positively charged S4 segments, that move into the lipid bilayer in response to electrical membrane depolarizations and leading to the opening of the channel. It is conceivable that Gβγ-dimer could bind either to the intracellular end of the S4 segment of the Ca_v2-subunit, or to another structural determinant of the voltage-sensor exposed on the intracellular surface of the channel, preventing the proper movement of the voltage-

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

sensor and thus inhibiting the channel. Consistent with this idea, it was shown that the point mutation G177E localized in the third transmembrane segment of the first domain (IS3) of the $Ca_v2.2a$-subunit (rat rbB-I isoform) and induces a tonic inhibition of the channel that can be reversed by a depolarizing prepulse as the classical G-protein inhibition (Zhong et al., 2001). Interestingly, while this inhibition shares some of the features of the G-protein modulation, activation of G-proteins does not produce additional inhibition. Similarly, introduction of the G177E mutation into the $Ca_v2.2b$-subunit (rat rbB-II isoform) produces a tonic inhibition of the channel and prevents G-protein-mediated modulation of the Ca^{2+} current (Zhong et al., 2001). In contrast, the E177G mutation introduced into the $Ca_v2.2a$-subunit reverses tonic inhibition of the channel and restores a normal G-protein regulation. Hence, it was proposed that the negative charge introduced by the mutation G177E could interact with a positively charged residue of the S4 segment of the first domain of the Ca_v2-subunit, pushing the channel to a "*reluctant*" mode via a mechanism of "*voltage-sensor-trapping*", suggesting that Gβγ-dimer could possibly regulate the channel in a similar way (Zhong et al., 2001) (Flynn and Zamponi, 2010). Intriguingly, Gem2 that belongs to the RGK (Rad, Gem and Kir) family of small G-proteins is also able to inhibit $Ca_v2.2$ channels (Chen et al., 2005). However, in contrast to the direct G-protein inhibition by Gβγ-dimer, depolarizing prepulses do not reverse Gem2-mediated inhibition (Chen et al., 2005). Remarkably, structure analyses of Rad, an homologue of Gem, indicates the presence of a molecular motif similar to the seven repeated motifs of the Gβ-subunit, suggesting a common mechanism by which Gβγ-dimer and RGK proteins modulate VGCCs. Another possible model of channel regulation by G-proteins that does not require any alteration of gating parameters was also proposed in which "willing" and "reluctant" modes are intrinsic to the channel, whereas G-proteins and other effectors simply shift the fraction of channels

in these two states without modifying their intrinsic gating properties (Herlitze et al., 2001). This "willing" to "reluctant" model could also potentially explain the decrease in gating currents initially observed during G-protein regulation (Jones et al., 1997). Finally, based on the observation that sodium currents recorded through $Ca_v2.2$ channels are less affected than calcium currents by G-protein inhibition, it was proposed that binding of Gβγ-dimer onto the Ca_v2-subunit alters ion permeation of the channel (Kuo and Bean, 1993). Hence, consistent with the observation that a 35 amino-acids peptide (residues 271-305) of the $G\beta_2$-subunit is sufficient to inhibit Ca^{2+} currents (Li et al., 2005), it is also conceivable that Gβγ-dimer simply inhibits the channel via a "pore-modifier" mechanism. However, such a mechanism could only contribute partially to the overall current inhibition given the effect of Gβγ-dimer on single channel gating properties (essentially latency of the first opening). Either way, the exact molecular mechanisms by which Gβγ-dimer inhibits VGCCs remains essentially unknown and certainly deserves further investigation. Recent structural information obtained from voltage-gated sodium channels (Payandeh et al., 2011, Zhang et al., 2012) will certainly provide key information to further analyze the biding of G-proteins to the channel subunit and find out the molecular determinant of the inhibition.

What is the functional role of the Ca_vβ-subunit in direct G-protein inhibition?

The involvement of the Ca_vβ-subunit in the direct regulation of VGCCs by GPCRs was extensively examined. Hence, the Ca_vβ-subunit and Gβγ-dimer share a structural binding site (QXXER located in the AID domain of the I-II loop) on the Ca_v2-subunit suggesting a possible cross talk. The initial observations that expression of the $Ca_v\beta_3$-subunit in *Xenopus* oocytes decreases G-protein inhibition of $Ca_v2.1$ and $Ca_v2.2$ channels (Roche et al., 1995),

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

and conversely that inhibition of $Ca_v2.1$ channels by μ-opioid and M2 muscarinic receptors is increased in the absence of Ca_vβ-subunit (Bourinet et al., 1996, Roche and Treistman, 1998a) led the authors to propose that the Ca_vβ-subunit is antagonistic to the direct G-protein modulation of Ca_v2 channels. In addition, it was shown that binding of Gβγ-dimer to the carboxy-terminal region of the Ca_v2-subunit is abolished by the GK domain of the $Ca_v\beta_{2a}$-subunit, which also prevents M2 muscarinic receptor-dependent inhibition of the Ca^{2+} current, providing a possible explanation for the functional antagonism existing between the Ca_vβ-subunit and direct G-protein inhibition of VGCCs (Qin et al., 1997). However, considering that the carboxy-terminal domain of the Ca_v2-subunit plays an important role in the functional coupling with the GPCR, it is likely that the Ca_vβ-dependent antagonism observed relies on an altered coupling of the channel with the GPCR rather than on the direct alteration of Gβγ-dimer binding to the channel. In contrast to the antagonistic hypothesis, it was also proposed that the Ca_vβ-subunit could rather be essential for potent G-protein modulation of the Ca_v2 channels (Meir et al, 2000). Consistent with this idea, it was proposed using a FRET approach that Gβγ-dimer induces $Ca_v2.1$ channel reluctance by displacement of the Ca_vβ-subunit form the channel (Sandoz et al., 2004). However, besides the fact that direct G-protein modulation of Ca_v2 channels has been observed numerous times in the absence of the Ca_vβ-subunit (Bourinet et al., 1996, Roche et al., 1995)(Canti et al., 2000), this observation is balanced by a study using a similar FRET approach showing that binding of $Ca_v\beta_{1b}$ and Gβ to the $Ca_v2.1$-subunit is not exclusive but rather synergetic (Hummer et al., 2003). In addition, it was shown that recovery of the $Ca_v2.2$ channel from G-protein inhibition is not only influenced by the presence of a Ca_vβ-subunit (Roche and Treistman, 1998b) but also depends on the Ca_vβ isoform ($\beta_3 > \beta_4 > \beta_{1b} > \beta_{2a}$) (Canti et al.,

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

2000). Considering that channel activity is an important factor of the "*OFF*" G-protein landmark, these results suggest that the $Ca_v\beta$-subunit could modulate G-protein inhibition indirectly by biophysical changes induced on the channel. Consistent with this idea, the respective kinetics of channel inactivation induced by various $Ca_v\beta$-subunits nicely correlates with the kinetic of recovery from G-protein inhibition (Weiss et al., 2007a). It was thus proposed that the $Ca_v\beta$-subunit, by controlling channel inactivation, indirectly influence the speed of G-protein dissociation from the channel. Hence, fast inactivating $Ca_v\beta$-subunits (example $Ca_v\beta_3$) act as a cofactor to speed the recovery from the G-protein inhibition. However, the situation becomes more complicated considering the fact that fast inactivating $Ca_v\beta$-subunits, by accelerating the inactivation kinetics of the channel, also reduce the temporal window for G-protein dissociation (Figure 5). Hence, fast inactivating $Ca_v\beta$-subunits, while speeding up the rate of G-protein dissociation, also reduce the maximal extent of current recovery from inhibition that leads to an apparent decrease of the prepulse facilitation. In contrast, a slow inactivating $Ca_v\beta$-subunit (for instance $Ca_v\beta_{2a}$) that slows down channel inactivation, has minor effect on the kinetic of G-protein dissociation, but prolong the temporal window of opportunity for G-protein dissociation and leads to a far more complete current recovery from inhibition, evidenced by an apparent increased prepulse facilitation (Weiss et al., 2007a). It is worth noting that the amino-terminal domain of the Ca_v2 channels that has ben implicated in the direct G-protein inhibition of VGCCs mediates $Ca_v\beta$-dependent fast inactivation of $Ca_v2.2$ channel (Stephens et al., 2000). In addition, the R387E mutation in the QXXER motif of the I-II loop that alters G-protein regulation (De Waard et al., 1997) (Herlitze et al., 1997), also affects channel inactivation. Hence, it is possible that these molecular channel determinants contribute to

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

G-protein regulation not only by providing an anchor for the Gβγ-dimer but also via their intrinsic effect on channel gating.

How synaptic proteins modulate direct G-protein inhibition?

The two types of Ca^{2+} channels ($Ca_v2.1$ and $Ca_v2.2$) that are most responsible for voltage-dependent release of neurotransmitter at nerve terminal endings biochemically associate with presynaptic vesicles of transmitters (Bennett et al., 1992) (Yoshida et al., 1992) (Leveque et al., 1994) by interacting with some of the proteins of the vesicular machinery release complex (SNAREs) including syntaxin-1A and SNAP-25. Molecular characterization of channel / SNAREs interaction has identified a *synprint* (*synaptic protein interaction*) locus in $Ca_v2.1$ and $Ca_v2.2$ located within the intracellular loop between domains II and III of the channels (Sheng et al., 1994) (Rettig et al., 1996). It is believed that the functional relevance of this interaction is to bring presynaptic vesicles close to the Ca^{2+} source for efficient and fast neurotransmitter release and disruption of Ca_v2 / SNARE complex alters Ca^{2+}-dependent release of neurotransmitter (Mochida et al., 1996) (Rettig et al., 1997) (Harkins et al., 2004) (Keith et al., 2007). Furthermore, direct binding of syntaxin-1A and SNAP-25 to the $Ca_v2.1$ and $Ca_v2.2$ subunits also potently modulates channel gating by shifting the voltage-dependence of channel inactivation toward more negative membrane potentials (Bezprozvanny et al., 1995) (Wiser et al., 1996) (Zhong et al., 1999) (Degtiar et al., 2000) (Weiss and Zamponi, 2012) (Zamponi and Currie, 2013), although the physiological relevance of this regulation is not fully understood. Interestingly, while the *synprint* site of $Ca_v2.1$ and $Ca_v2.2$ subunits, and in general the II-III intracellular linker, are not essential molecular determinants of Gβγ-mediated modulation of the channel activity, proteolytic cleavage of synatxin-1A with botulinium neurotoxin C1 in primary neurons was shown to

completely prevent GPCR-dependent inhibition of presynaptic Ca^{2+} channels (Stanley and Mirotznik, 1997). However, because G-protein modulation of transiently expressed $Ca_v2.2$ channels in *Xenopus* oocytes (Bourinet et al., 1996) or HEK cells (Zamponi et al., 1997) does not require coexpression with syntaxin-1A, it is unclear why the cleavage of syntaxin-1A in native neuronal environment would lead to a total loss of G-protein modulation. In contrast, Jarvis et al., reported that syntaxin-1A is not essential but facilitates G-protein modulation of the $Ca_v2.2$ channel. Indeed, while $Ca_v2.2$ transiently expressed in tsA201 cells in combination with Gβ1γ2 dimer is susceptible to a tonic inhibition that can be assessed (relieved) by a preceding strong membrane depolarization, coexpresion of syntaxin-1A produced an even larger tonic G-protein inhibition, suggesting that syntaxin-1A facilitates Gβγ-dependent inhibition of the channel (Jarvis et al., 2000). However, it remains unclear whether syntaxin-1A facilitates G-protein inhibition or promotes Gβγ-dimer dissociation from the channel upon prepulse depolarization. Indeed, as previously discuss in the case of the Ca_vβ-subunit, it is possible that syntaxin-1A, by affecting channel gating, indirectly influence the kinetic of G-protein dissociation from the channel, producing a larger prepulse facilitation that could be misinterpreted as the result of an increased initial G-protein inhibition. In support of this idea, coexpression of SNAP-25 that reverses syntaxin-1A-dependent channel gating also reduces the magnitude of the prepulse-induce current recovery upon tonic G-protein inhibition (Jarvis and Zamponi, 2001). However, this concept should also be toned-down as coexpression of a mutant syntaxin-1A that is locked permanently in an "open" conformation (the conformation adopted by syntaxin-1A when in interaction with SNAP-25 or synaptobrevin-2) (Dulubova et al., 1999) (Brunger, 2001) has no effect on $Ca_v2.2$ gating but still modulates Gβγ-dependent inhibition of the channel (Jarvis et al., 2002). Conversely, syntaxin-1B affects $Ca_v2.2$ channel gating but does not

support G-protein modulation (Jarvis and Zamponi, 2001). Altogether, these results suggest that syntaxin-1A may present specific features responsible for G-protein modulation independently of its effect on the channel gating, possibly by facilitating the co-localization of G-proteins with the Ca^{2+} channel. Consistent with this idea, a syntaxin 1 / Gαo / $Ca_v2.2$ complex has been described at presynaptic nerve terminals of chick ciliary ganglion cells (Li et al., 2004).

Several other types of presynaptic proteins have been documented as important in the modulation of G-protein inhibition of VGCCs. For example, co-expression of Rim1 significantly decrease direct DAMGO-induced direct G-protein inhibition of $Ca_v2.2$ channels expressed in HEK293 cells (Weiss et al., 2011). Interestingly, careful kinetic analyses revealed that Rim1 dos not prevent inhibition of the channel ("*ON*" landmark, i.e. binding of Gβγ-dimer to the channel) but rather favor channel recovery from inhibition ("*OFF*" landmarks, i.e. unbinding of Gβγ-dimer from the channel), likely by slowing-down channel inactivation (Kiyonaka et al., 2007) increasing the time window for functional recovery from G-protein inhibition similarly to Ca_vβ-subunits (Weiss et al., 2011)(Weiss et al., 2007a). Modulation of G-protein inhibition of VGCCs has been also documented with cysteine string protein (CSP). Although CSP has been shown to stimulate Gα-subunit activity by promoting the exchange of GTP for GDP (Natochin et al., 2005) that in turn is expected to reduce free Gβγ-dimers, coexpression of CSP increases apparent Gβγ-dependent inhibition of $Ca_v2.2$ channel similarly to what was observed with synatxin-1A (Magga et al., 2000). Although the molecular mechanism underlying CSP-dependent modulation of G-protein inhibition remains to be further explored, it is possible that CSP brings G-proteins together with the Ca^{2+} channel since binding of CSP has been documented with $Ca_v2.1$ and

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

$Ca_v2.2$ subunits (Leveque et al., 1998)(Magga et al., 2000), as well as with G-proteins Gα-subunit and Gβγ-dimer (Magga et al., 2000).

How the termination of the signal occurs?

In order to be efficient, cellular events have to be localized and timely regulated. Hence, we have seen that direct G-protein modulation of VGCCs requires 1) the activation of the GPCR by specific extracellular ligands, 2) the release and binding of Gβγ-dimer to the channel leading to a complex regulatory phenotype, and finally 3) that this regulation can be temporally relieved, beside the continuous presence of the extracellular agonist, in response for example to a train of action potentials. However, how does this regulation definitively end? While the recapture and/or degradation of the agonist terminate the activation of the GPCR, how does the channel bound Gβγ-dimer reassociates with the Gα-subunit? Because channel recovery from G-protein inhibition can occur at membrane potentials above the reversal potential of Ca^{2+}, ion influx is not the driving force for Gβγ-dimer dissociation from the channel. From a biochemical and thermodynamic point of view, it is conceivable that intrinsic hydrolysis of GTP by the Gα-subunit and formation of GDP-bound Gα-subunit - which presents a high affinity for Gβγ-dimer - could chelate free Gβγ when dissociating from the channel according to a binding/unbinding thermodynamics equilibrium. It is also possible that the GDP-bound Gα-subunit is able to bind Gβγ-dimer while still on the Ca_v-subunit. This concept implies the molecular determinants of Gα and Gβγ binding being accessible when the Gβγ-dimer is bound to the channel. Binding of GDP-Gα to Gβγ could produce a conformational change of the channel Ca_v-subunit and/or Gβγ-dimer, unfavorable to the formation of Ca_v / Gβγ complex, possibly by altering an essential channel molecular determinant important for Gβγ binding. Although this molecular

determinant has not been identified yet, it is likely to contribute to Gβγ dissociation upon channel activation, and possibly involves channel structures sensitive to electrical membrane potential, like S4 segments for example. However, this concept remains to be investigated to be able to better understand the molecular dynamic of GPCR-induced inhibition of VGCCs.

Are Ca_v2 channels the only calcium channels susceptible to direct G-protein inhibition?

It is thought that direct G-protein modulation of VGCCs is mostly confined to neuronal Ca_v2 channels. Indeed, structure/function studies indicate that Ca_v1 channels that lack most of the believed important structural determinants involved in the binding of the Gβγ-dimer to the channel, such as the QXXER motif of the I-II loop (Herlitze et al., 1997), are not subject to direct G-protein regulation (Roche et al., 1995) (Bourinet et al., 1996) (Zhang et al., 1996) (Meza and Adams, 1998). However, additional studies on native channels clearly demonstrated an inhibition of L-type Ca^{2+} currents mediated by heterotrimeric G-proteins. For example, injection of GTPγS in cerebellar granule cells induces inhibition of L-type Ca^{2+} currents that is partly reversed upon channel activation by the dihydropyridine agonist +(S)-202-791 (Haws et al., 1993). Moreover, inhibition of L-type Ca^{2+} currents has been documented upon agonist stimulation of GPCRs. Indeed, baclofen activation of GABA-B receptors in retinal bipolar neurons induces inhibition of L-type Ca^{2+} currents, an inhibition that is potentiated by GTPγS injection (Maguire et al., 1989). In addition, activation of mGluR2/3 receptors by application of (2S,1'S,2'S)-2-(carboxycyclopropyl) glycine (L-CCG-I) on cerebellar granule cells also inhibits L-type Ca^{2+} currents (Chavis et al., 1994). Although these results do not provide evidence for a direct G-protein inhibition, it is worth noting

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

that GPCR-mediated inhibition of L-type Ca^{2+} currents in nerve cells is fully abolished by pertussis toxin treatment indicating the implication of Gαi/o proteins. However, evidence for a direct G-protein modulation of Ca_v1 channels comes from the observation that inhibition of L-type Ca^{2+} currents in pancreatic β-cells can be reversed by application of a depolarizing prepulse (Ammala et al., 1992). Though work performed on nerve and pancreatic cells mostly involved $Ca_v1.2$ and $Ca_v1.3$ channels, G-protein modulation of native $Ca_v1.1$ channel isoform has also been described in skeletal muscle cells where L-type Ca^{2+} current inhibition was observed upon β-adrenergic stimulation by isoproterenol or GTPγS injection (Somasundaram and Tregear, 1993). In addition, it was shown that expression of $G\beta_1\gamma_2$-dimer *in vivo* in adult skeletal muscle fibers specifically inhibits L-type Ca^{2+} currents and voltage-induced Ca^{2+} release (Weiss et al., 2010). Although this inhibition is not reversed by a classical depolarizing prepulse, the observation that expression of some other Gβγ-dimer combinations (for instance $G\beta_2\gamma_2$-, $G\beta_3\gamma_2$- or $G\beta_4\gamma_3$) has no effect on the Ca^{2+} current strongly suggest that $G\beta_1\gamma_2$-dimer specifically mediates modulation of $Ca_v1.1$ channels. The situation is similar regarding the low-voltage-activated Ca_v3 channels. Hence, application of baclofen on dorsal root ganglion neurons (DRG) inhibits T-type Ca^{2+} currents, this inhibition being often counterbalance by the concomitant presence of a current potentiation (likely mediated by diffusible second messengers) (Scott and Dolphin, 1990). Similarly, it was shown that inhibition of T-type Ca^{2+} current by dopamine D1 receptor in rat adrenal glomerulosa cells requires the combined action of Gβγ-dimer and cAMP (Drolet et al., 1997). More recently, the molecular mechanism of T-type Ca^{2+} current inhibition by G-proteins was specified. It specifically affects $Ca_v3.2$ channel over the $Ca_v3.1$ isoform, and in contrast to $Ca_v1.1$ channels specifically requires the $G\beta_2\gamma_2$ combination ($G\beta_1\gamma_2$-dimer having no significant effect on the Ca^{2+} current) (Wolfe et al., 2003). The inhibition relies on

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

the direct binding of $G\beta_2\gamma_2$-dimer to the II-III loop of the $Ca_v3.2$-subunit, replacement of this loop by the corresponding $Ca_v3.1$ region preventing G-protein modulation. This inhibition has been attributed to a diminution of the opening probability of $Ca_v3.2$ channels, without other alteration of channel gating or expression (DePuy et al., 2006). It is worth noting that similarly to what was observed for Ca_v1 channels, G-protein-mediated inhibition of the $Ca_v3.2$ channel is not reversed by depolarizing prepulse, although mediated by the direct binding of Gβγ-dimer to the channel.

Taken together, these results suggest that likely most of the VGCCs are affected by G-protein inhibition. However, in contrast to Ca_v2 channels, it remains unclear if inhibition of Ca_v1 and Ca_v3 channels is mediated by a direct binding of Gβγ-dimer to the channel or required activation of secondary signaling pathways. Inhibition of Ca_v1 and Ca_v3 channels that is usually not reversed by a depolarizing prepulse, is also not characterized by a depolarizing shift of the current / voltage activation curve (DePuy et al., 2006), typical feature of the direct Gβγ-dependent inhibition. However, considering the presence of numerous molecular channel determinants that contribute to the phenotype of the direct Gβγ-regulation of Ca_v2 channels, it is possible the Ca_v1 and Ca_v3 channels lack important molecular determinants required to reveal "*OFF*" landmarks of the regulation. Either way, future studies will certainly uncover the molecular mechanisms by which G-proteins modulate Ca_v1 and Ca_v3 channels.

Voltage-independent inhibition of Ca_v2 channels

While this review is essentially focused on the so-called fast voltage-dependent regulation mediated by direct binding of G-proteins onto the channel, various studies on native neurons and heterologous expression systems have identified other types of inhibition that

usually take tens of second to develop and involve diffusible second messengers or surface remodeling of GPCR / channel complexes.

Inhibition of VGCCs by phosphoinositides

Direct Gβγ-dependent inhibition of VGCCs is usually fast and requires the activation of Gi/o-coupled receptors. In contrast, initial recordings performed on sympathetic neurons have identified a relatively slow and voltage-independent form of inhibition that is mediated by the activation of Gq-coupled receptors (Gamper et al., 2004). While various studies have ruled out the implication of typical Gq-dependent signaling pathways downstream of phospholipase Cβ, inositol trisphosphate (IP3), diacyglycerol (DAG) and protein kinase C (PKC), it was proposed that depletion of membrane phosphatidylinositol 4,5-bisphosphate (PIP2) is the mediator of Gq-dependent inhibition of VGCCs (Delmas et al., 2005) (Roberts-Crowley et al., 2009) (Michailidis et al., 2007) (Rodriguez-Menchaca et al., 2012). Consistent with this idea, loss of $Ca_v2.2$ channel activity typically observed in excised membrane patches can be either reduced by application of PIP2 or in contrast enhanced by depletion of PIP2 (Gamper et al., 2004). In addition, the time course of the slow inhibition of Ca^{2+} currents produced by activation of muscarinic M1 receptors (M1R) in sympathetic neurons nicely correlate with the kinetics of PIP2 hydrolysis, while infusing of PIP2 into the cell via the patch pipette is sufficient to prevent M1R-dependent inhibition of $Ca_v2.2$ channels (Gamper et al., 2004). More recently, using an exogenous voltage-sensitive 5-phosphatase (VSP) that allows rapid hydrolysis of PIP2 into Pi(4)P, it was shown that membrane depletion of PIP2 suppresses $Ca_v1.2$, $Ca_v1.3$, $Ca_v2.1$ and $Ca_v2.2$ currents, supporting the idea that depletion of PIP2 is sufficient to mimic the slow inhibition of calcium currents observed under Gq-coupled receptor activation (Suh et al., 2010). However, it is worth

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

noting that beside VSP-induced PIP2 depletion is similar to the depletion produced by activation of muscarinic receptors, the amplitude of the Ca^{2+} current inhibition is significantly less, suggesting that another signaling pathway might also contribute to the slow Gq-dependent inhibition. Hence, it was proposed that production of arachidonic acid (AA) by the action of phospholipase A2 on PIP2 and other membrane phospholipids elicits modulation of $Ca_v2.2$ channels (Liu et al., 2001) (Liu and Rittenhouse, 2003). Interestingly, it was also proposed that depletion of membrane PIP2 might reduce Gβγ-dependent inhibition of $Ca_v2.1$ channels expressed in *Xenopus* oocytes, suggesting a crosstalk between voltage-dependent and voltage-independent inhibitions (Rousset et al., 2004). Although the molecular mechanism by which PIP2 interfere with Gβγ regulation is not fully understood, it was proposed that binding of the carboxy-terminal tail of the $Ca_v2.1$ subunit to membrane phosphoinositides might stabilize a Gβγ-sensitive state of the channel (Rousset et al., 2004). However, considering that depletion of membrane PIP2 upon Gαq-dependent activation takes several minutes, it is unlikely that this mechanism account for the fast initial inhibition phase produced by Gβγ-dimer.

Inhibition of Ca_v2 channels by channel/GPCR complex internalization

Initially proposed by Forscher et al., G-protein-dependent inhibition of VGCCs may require a tight GPCR-channel coupling (Forscher et al., 1986), and evidence exist for direct biochemical interaction between the Ca^{2+} channel and the GPCR. Hence, physical association of $Ca_v2.1$ channels with metabotropic glutamate receptors mGluR1 has been documented in cerebellar Purkinje neurons as well as in cellular expression systems and involves the direct binding the of carboxy-terminal domain of the $Ca_v2.1$-subunit with the carboxy-terminal region of the receptor (Kitano et al., 2003). Similarly, a $Ca_v2.2$-ORL-1

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

(opioid receptor like-1 also known as nociceptin receptor or NOP) signaling complex has been documented in small dorsal root ganglion (DRG) neurons, and support a tonic agonist-independent G-protein inhibition of the Ca^{2+} channel evidenced by prepulse facilitation (Beedle et al., 2004). Similar observations have been reported for μ- and δ-opioid receptors transiently expressed with $Ca_v2.2$ channel in tsA201 cells although the existence of these protein complexes remains to be explored in native conditions (Chee et al., 2008) (Evans et al., 2010). Also, a physical interaction exist between $Ca_v2.2$ channels and dopamine D1 and D2 receptors and requires other channel structural determinants including the II-III intracellular linker (Kisilevsky et al., 2008) (Kisilevsky and Zamponi, 2008) (Weiss, 2009). Although the existence of Ca_v2-GPCR signaling complexes is unambiguous, the physiological relevance of these interactions is not fully understood. It was proposed that association of the Ca^{2+} channels with GPCRs might control channel density at the plasma membrane, providing an additional level of control of the Ca^{2+} influx. Indeed, activation of ORL-1 receptors triggers an agonist-dependent co-internalization of $Ca_v2.2$-ORL-1 complexes into vesicular compartments both in tsA201 cells and DRG neurons (Altier et al., 2006, Evans et al., 2010). However, internalization of $Ca_v2.2$ channels is not accompanied by a diminution of the membrane Ca^{2+} current, questioning the physiological relevance of this regulation (Murali et al., 2012). In addition, although μ-opioid receptors also physically interact with $Ca_v2.2$ channels they do not co-internalized indicating that biochemical coupling of the channel with the GPCR is not sufficient to mediate agonist-mediated internalization of the Ca^{2+} channel (Evans et al., 2010). It is also possible that the assembly of Ca_v2 channels and GPCRs provides a mechanism that ensures spatiotemporal regulation of the Ca^{2+} entering synaptic nerve terminals. In addition, tonic channel inhibition mediated by channel-GPCR complexes could also represent a mean to dynamically adjust the Ca^{2+} influx to the electrical

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

input signal coming to the synaptic ending. Indeed, it was shown that the extent of current facilitation (i.e. current recovery from G-protein inhibition) is dependent on both the duration (Brody et al., 1997) and the frequency of action potentials (AP) (Penington et al., 1991) (Williams et al., 1997). While low frequency AP produces no or little recovery, increasing AP frequency significantly enhances recovery from G-protein inhibition and Ca^{2+} influx and could contribute to short-term synaptic facilitation or depression (Bertram et al., 2003).

Contribution of G-protein modulation to channelopathies

As previously discussed, gating properties of the Ca^{2+} channel significantly affects direct G-protein inhibition, essentially the "*OFF*" landmarks (i.e. the dissociation of Gβγ-dimer from the Ca_v2-subunit). Hence, alteration of channel gating is likely to affect G-protein regulation and synaptic activity. A number of congenital mutations in the gene CACNA1A encoding the $Ca_v2.1$ channel cause familial hemiplegic migraine type-1 (FHM-1), a rare and severe monogenic subtype of migraine with aura, characterize by at least some degree of hemiparesis during aura (Ophoff et al., 1996) (Weiss et al., 2007b)(Ducros, 2013) (Pietrobon, 2013). FHM-1 mutations generally affect structural determinants that are essential for channel gating including the S4 transmembrane segments thought to carry the voltage-sensor controlling channel activation, the S6 transmembrane segment involved in the control of channel inactivation, and the poor-forming loops. Biophysical analyses of channel gating revealed a hyperpolarizing shift of the voltage-dependence of activation, as well as additional effects on channel inactivation kinetics, open probability, and unitary conductance (Hans et al., 1999) (Kraus et al., 1998) (Kraus et al., 2000) (Mullner et al., 2004) (Tonelli et al., 2006) (Tottene et al., 2005). In addition, a knock-in mouse model

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

expressing the human pathogenic FHM-1 mutation R192Q located in the first S4 segment of the $Ca_v2.1$-subunit revealed a decreased neuronal excitability threshold, increased Ca^{2+} influx and cortical spreading depression (i.e. the mechanism underlying migraine with aura (Pietrobon and Moskowitz, 2013)) (van den Maagdenberg et al., 2004), and enhanced excitatory transmission at cortical synapses (Tottene et al., 2009). Similar alterations have been also documented for the S218L mutation (van den Maagdenberg et al., 2010). While intrinsic alteration of $Ca_v2.1$ channel gating likely contributes to neuronal hyperexcitability, alteration of the G-protein-dependent inhibitory pathway of presynaptic Ca^{2+} channels may also contribute to synaptic hyperexcitability. Consistent with this idea, a decreased G-protein inhibition of R192Q $Ca_v2.1$ channels was reported (Melliti et al., 2003). Careful analysis revealed that the R192Q mutation does not affect the "*ON*" landmark but rather favor the dissociation of Gβγ-dimer following channel activation ("*OFF*" landmarks), thereby decreasing the inhibitory G-protein pathway (Weiss et al., 2008). Similar results were observed with various other FHM-1 mutations (Weiss et al., 2008) (Garza-Lopez et al., 2012)(Garza-Lopez et al., 2013) indicating that alteration of G-protein regulation of the $Ca_v2.1$ channel caused by FHM-1 mutations is a common underlying mechanism that certainly contribute to synaptic defects observed during the disease.

Alteration of channel gating can also be caused by change in regulatory subunits. Hence, an epileptic lethargic phenotype in mouse resulting from the loss of expression of the $Ca_v\beta_4$-subunit is accompanied by a $Ca_v\beta$-subunit reshuffling (Burgess et al., 1997). Considering that $Ca_v\beta$-subunits significantly affect G-protein inhibition of Ca_v2 channels in a $Ca_v\beta$ isoform-dependent manner (Weiss et al., 2007a), it is likely to contribute to the altered excitatory synaptic transmission observed in those animals (Hosford et al., 1999) (Caddick et al., 1999).

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

Concluding remarks and perspectives

Since the first description of the phenomena by Dunlap and Fischbach in the late seventies (Dunlap and Fischbach, 1978), great advances have been made in our understanding of the underlying molecular regulation of neuronal VGCCs by GPCRs and its importance in physiology. In this review, we provided an appreciation of its tremendous complexity, arising not only from the numerous molecular channel and G-protein determinants involved in the regulation, but also from the channel subunit composition, GPCR subtype, interactions with synaptic proteins and other intracellular signaling pathways, and likely many more factors that have not been yet characterized. Although numerous channel / G-protein binding determinants have been described, the molecular mechanism by which Gβγ-dimer mediates inhibition of the Ca^{2+} current remains incompletely ununderstood. It is likely that more discrete interactions that have not been yet characterized support this inhibition, and the recent structural information obtained from structurally similar channels will certainly help to find out the molecular basis of G-protein inhibition. In addition, the use of small molecules and peptides to selectively disrupt interaction of G-protein βγ-dimer with some effectors has been demonstrated *in vitro* and *in vivo* on various models of heart failure and morphine tolerance (Bonacci et al., 2006) (Mathews et al., 2008) (Casey et al., 2010). A deeper biochemical and functional characterization of Gβγ-channel interaction will certainly provide important information to identified molecules targeting G-protein inhibition of VGCCs with potential therapeutic benefits. From a more physiological point of view, although the most evident outcome of G-protein regulation of presynaptic Ca^{2+} channel is a reduction of the Ca^{2+} influx entering nerve terminals ("*ON*" landmark), the observation that "*OFF*" landmarks might play an important role in fine tuning synaptic

strength, possibly contributing to short-term synaptic facilitation / depression, represents an interesting concept in molecular neuroscience which certainly merits to be further investigated. In addition, the notion that G-protein regulation is altered by pathological mutations in the Ca^{2+} channel complex not only contributes to our understanding of the pathogenesis of neuronal Ca_v channelopathies, but also emerges as an important signaling pathway for potential new therapeutic strategies.

Finally, merely twenty GPCRs from over nearly 1000 estimated from the sequencing of the human genome (including many orphan receptors) (Fredriksson et al., 2003) (Vassilatis et al., 2003) (Zhang et al., 2006) have been described for modulating VGCCs. While investigations into this extraordinary field continue, it is likely that many new GPCRs can underlie modulation of not only neuronal VGCCs but also channels expressed in other tissues like heart and skeletal muscle, and certainly represent a considerable source of potential therapeutic targets for the treatment of channelopathies in general.

Authorship contributions

Wrote or contributed to the writing of the manuscript: JP and NW

References

Agler, H. L., J. Evans, L. H. Tay, M. J. Anderson, H. M. Colecraft, and D. T. Yue (2005) G protein-gated inhibitory module of N-type (ca(v)2.2) ca2+ channels. Neuron **46:**891–904.

Altier, C., H. Khosravani, R. M. Evans, S. Hameed, J. B. Peloquin, B. A. Vartian, L. Chen, A. M. Beedle, S. S. Ferguson, A. Mezghrani, S. J. Dubel, E. Bourinet, J. E. McRory, and G. W. Zamponi (2006) ORL1 receptor-mediated internalization of N-type calcium channels. Nat Neurosci **9:**31–40.

Ammala, C., P. O. Berggren, K. Bokvist, and P. Rorsman (1992) Inhibition of L-type calcium channels by internal GTP [gamma S] in mouse pancreatic beta cells. Pflugers Arch **420:**72–77.

Arikkath, J., and K. P. Campbell (2003) Auxiliary subunits: essential components of the voltage-gated calcium channel complex. Curr Opin Neurobiol **13:**298–307.

Baertschi, A. J., Y. Audigier, P. M. Lledo, J. M. Israel, J. Bockaert, and J. D. Vincent (1992) Dialysis of lactotropes with antisense oligonucleotides assigns guanine nucleotide binding protein subtypes to their channel effectors. Mol Endocrinol **6:**2257–2265.

Bean, B. P. (1989) Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. Nature **340:**153–156.

Beedle, A. M., J. E. McRory, O. Poirot, C. J. Doering, C. Altier, C. Barrere, J. Hamid, J. Nargeot, E. Bourinet, and G. W. Zamponi (2004) Agonist-independent modulation of N-type calcium channels by ORL1 receptors. Nat Neurosci **7:**118–125.

Bell, D. C., A. J. Butcher, N. S. Berrow, K. M. Page, P. F. Brust, A. Nesterova, K. A. Stauderman, G. R. Seabrook, B. Nurnberg, and A. C. Dolphin (2001) Biophysical properties, pharmacology, and modulation of human, neuronal L-type (alpha(1D), Ca(V)1.3) voltage-dependent calcium currents. J Neurophysiol **85:**816–827.

Bennett, M. K., N. Calakos, and R. H. Scheller (1992) Syntaxin: a synaptic protein implicated in

docking of synaptic vesicles at presynaptic active zones. Science **257:**255–259.

Bernheim, L., D. J. Beech, and B. Hille (1991) A diffusible second messenger mediates one of the pathways coupling receptors to calcium channels in rat sympathetic neurons. Neuron **6:**859–867.

Berridge, M. J. (1998) Neuronal calcium signaling. Neuron **21:**13–26.

Bertram, R., J. Swanson, M. Yousef, Z. P. Feng, and G. W. Zamponi (2003) A minimal model for G protein-mediated synaptic facilitation and depression. J Neurophysiol **90:**1643–1653.

Bezprozvanny, I., R. H. Scheller, and R. W. Tsien (1995) Functional impact of syntaxin on gating of N-type and Q-type calcium channels. Nature **378:**623–626.

Boland, L. M., and B. P. Bean (1993) Modulation of N-type calcium channels in bullfrog sympathetic neurons by luteinizing hormone-releasing hormone: kinetics and voltage dependence. J Neurosci **13:**516–533.

Bonacci, T. M., J. L. Mathews, C. Yuan, D. M. Lehmann, S. Malik, D. Wu, J. L. Font, J. M. Bidlack, and A. V. Smrcka (2006) Differential targeting of Gbetagamma-subunit signaling with small molecules. Science **312:**443–446.

Bourinet, E., T. W. Soong, A. Stea, and T. P. Snutch (1996) Determinants of the G protein-dependent opioid modulation of neuronal calcium channels. Proc Natl Acad Sci U S A **93:**1486–1491.

Brody, D. L., P. G. Patil, J. G. Mulle, T. P. Snutch, and D. T. Yue (1997) Bursts of action potential waveforms relieve G-protein inhibition of recombinant P/Q-type Ca2+ channels in HEK 293 cells. J Physiol **499:**637–644.

Brown, C. H., and J. A. Russell (2004) Cellular mechanisms underlying neuronal excitability during morphine withdrawal in physical dependence: lessons from the magnocellular oxytocin system. Stress **7:**97–107.

Brown, D. A., A. K. Filippov, and E. A. Barnard (2000) Inhibition of potassium and calcium

currents in neurones by molecularly-defined P2Y receptors. J Auton Nerv Syst **81:**31–36.

Brunger, A. T. (2001) Structure of proteins involved in synaptic vesicle fusion in neurons. Annu Rev Biophys Biomol Struct **30:**157–171.

Bucci, G., S. Mochida, and G. J. Stephens (2011) Inhibition of synaptic transmission and G protein modulation by synthetic CaV2.2 Ca(2)+ channel peptides. J Physiol **589:**3085–3101.

Burgess, D. L., J. M. Jones, M. H. Meisler, and J. L. Noebels (1997) Mutation of the Ca2+ channel beta subunit gene Cchb4 is associated with ataxia and seizures in the lethargic (lh) mouse. Cell **88:**385–392.

Caddick, S. J., C. Wang, C. F. Fletcher, N. A. Jenkins, N. G. Copeland, and D. A. Hosford (1999) Excitatory but not inhibitory synaptic transmission is reduced in lethargic (Cacnb4(lh)) and tottering (Cacna1atg) mouse thalami. J Neurophysiol **81:**2066–2074.

Campbell, V., N. Berrow, and A. C. Dolphin (1993) GABAB receptor modulation of Ca2+ currents in rat sensory neurones by the G protein G(0): antisense oligonucleotide studies. J Physiol **470:**1–11.

Campbell, V., N. S. Berrow, E. M. Fitzgerald, K. Brickley, and A. C. Dolphin (1995) Inhibition of the interaction of G protein G(o) with calcium channels by the calcium channel beta-subunit in rat neurones. J Physiol **485:**365–372.

Canti, C., Y. Bogdanov, and A. C. Dolphin (2000) Interaction between G proteins and accessory subunits in the regulation of 1B calcium channels in Xenopus oocytes. J Physiol **527 Pt 3:**419–432.

Canti, C., K. M. Page, G. J. Stephens, and A. C. Dolphin (1999) Identification of residues in the N terminus of alpha1B critical for inhibition of the voltage-dependent calcium channel by Gbeta gamma. J Neurosci **19:**6855–6864.

Casey, L. M., A. R. Pistner, S. L. Belmonte, D. Migdalovich, O. Stolpnik, F. E. Nwakanma, G.

Vorobiof, O. Dunaevsky, A. Matavel, C. M. Lopes, A. V. Smrcka, and B. C. Blaxall (2010) Small molecule disruption of G beta gamma signaling inhibits the progression of heart failure. Circ Res **107:**532–539.

Catterall, W. A. (2011) Voltage-gated calcium channels. Cold Spring Harb Perspect Biol **3:**a003947.

Chavis, P., H. Shinozaki, J. Bockaert, and L. Fagni (1994) The metabotropic glutamate receptor types 2/3 inhibit L-type calcium channels via a pertussis toxin-sensitive G-protein in cultured cerebellar granule cells. J Neurosci **14:**7067–7076.

Chee, M. J., K. Morl, D. Lindner, N. Merten, G. W. Zamponi, P. E. Light, A. G. Beck-Sickinger, and W. F. Colmers (2008) The third intracellular loop stabilizes the inactive state of the neuropeptide Y1 receptor. J Biol Chem **283:**33337–33346.

Chen, H., H. L. Puhl, S. L. Niu, D. C. Mitchell, and S. R. Ikeda (2005) Expression of Rem2, an RGK family small GTPase, reduces N-type calcium current without affecting channel surface density. J Neurosci **25:**9762–9772.

Chen, J., M. DeVivo, J. Dingus, A. Harry, J. Li, J. Sui, D. J. Carty, J. L. Blank, J. H. Exton, R. H. Stoffel, and A. et (1995) A region of adenylyl cyclase 2 critical for regulation by G protein beta gamma subunits. Science **268:**1166–1169.

De Waard, M., J. Hering, N. Weiss, and A. Feltz (2005) How do G proteins directly control neuronal Ca2+ channel function? Trends Pharmacol Sci **26:**427–436.

De Waard, M., H. Liu, D. Walker, V. E. Scott, C. A. Gurnett, and K. P. Campbell (1997) Direct binding of G-protein betagamma complex to voltage-dependent calcium channels. Nature **385:**446–450.

De Waard, M., D. R. Witcher, M. Pragnell, H. Liu, and K. P. Campbell (1995) Properties of the alpha 1-beta anchoring site in voltage-dependent Ca2+ channels. J Biol Chem **270:**12056–12064.

Degtiar, V. E., R. H. Scheller, and R. W. Tsien (2000) Syntaxin modulation of slow inactivation of N-type calcium channels. J Neurosci **20:**4355–4367.

Deisz, R. A., and H. D. Lux (1985) gamma-Aminobutyric acid-induced depression of calcium currents of chick sensory neurons. Neurosci Lett **56:**205–210.

Delmas, P., B. Coste, N. Gamper, and M. S. Shapiro (2005) Phosphoinositide lipid second messengers: new paradigms for calcium channel modulation. Neuron **47:**179–182.

DePuy, S. D., J. Yao, C. Hu, W. McIntire, I. Bidaud, P. Lory, F. Rastinejad, C. Gonzalez, J. C. Garrison, and P. Q. Barrett (2006) The molecular basis for T-type Ca2+ channel inhibition by G protein beta2gamma2 subunits. Proc Natl Acad Sci U S A **103:**14590–14595.

Dittman, J. S., and W. G. Regehr (1996) Contributions of calcium-dependent and calcium-independent mechanisms to presynaptic inhibition at a cerebellar synapse. J Neurosci **16:**1623–1633.

Docherty, R. J., and I. McFadzean (1989) Noradrenaline-Induced Inhibition of Voltage-Sensitive Calcium Currents in NG108-15 Hybrid Cells. Eur J Neurosci **1:**132–140.

Dolphin, A. C., and R. H. Scott (1987) Calcium channel currents and their inhibition by (-)-baclofen in rat sensory neurones: modulation by guanine nucleotides. J Physiol **386:**1–17.

Doupnik, C. A., and R. Y. Pun (1994) G-protein activation mediates prepulse facilitation of Ca2+ channel currents in bovine chromaffin cells. J Membr Biol **140:**47–56.

Drolet, P., L. Bilodeau, A. Chorvatova, L. Laflamme, N. Gallo-Payet, and M. D. Payet (1997) Inhibition of the T-type Ca2+ current by the dopamine D1 receptor in rat adrenal glomerulosa cells: requirement of the combined action of the G betagamma protein subunit and cyclic adenosine 3',5'-monophosphate. Mol Endocrinol **11:**503–514.

Ducros, A. (2013) [Genetics of migraine]. Rev Neurol (Paris) **169:**360–371.

Dulubova, I., S. Sugita, S. Hill, M. Hosaka, I. Fernandez, T. C. Sudhof, and J. Rizo (1999) A conformational switch in syntaxin during exocytosis: role of munc18. EMBO J **18:**4372–

4382.

Dunlap, K., and G. D. Fischbach (1978) Neurotransmitters decrease the calcium ocmponent of sensory neurone action potentials. Nature **276:**837–839.

Dunlap, K., and G. D. Fischbach (1981) Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. J Physiol **317:**519–535.

Elmslie, K. S., and S. W. Jones (1994) Concentration dependence of neurotransmitter effects on calcium current kinetics in frog sympathetic neurones. J Physiol **481:**35–46.

Elmslie, K. S., W. Zhou, and S. W. Jones (1990) LHRH and GTP-gamma-S modify calcium current activation in bullfrog sympathetic neurons. Neuron **5:**75–80.

Ertel, E. A., K. P. Campbell, M. M. Harpold, F. Hofmann, Y. Mori, E. Perez-Reyes, A. Schwartz, T. P. Snutch, T. Tanabe, L. Birnbaumer, R. W. Tsien, and W. A. Catterall (2000) Nomenclature of voltage-gated calcium channels. Neuron **25:**533–535.

Evans, R. M., H. You, S. Hameed, C. Altier, A. Mezghrani, E. Bourinet, and G. W. Zamponi (2010) Heterodimerization of ORL1 and opioid receptors and its consequences for N-type calcium channel regulation. J Biol Chem **285:**1032–1040.

Ewald, D. A., I. H. Pang, P. C. Sternweis, and R. J. Miller (1989) Differential G protein-mediated coupling of neurotransmitter receptors to Ca2+ channels in rat dorsal root ganglion neurons in vitro. Neuron **2:**1185–1193.

Filippov, A. K., D. A. Brown, and E. A. Barnard (2000a) The P2Y(1) receptor closes the N-type Ca(2+) channel in neurones, with both adenosine triphosphates and diphosphates as potent agonists. Br J Pharmacol **129:**1063–1066.

Filippov, A. K., A. Couve, M. N. Pangalos, F. S. Walsh, D. A. Brown, and S. J. Moss (2000b) Heteromeric assembly of GABA(B)R1 and GABA(B)R2 receptor subunits inhibits Ca(2+) current in sympathetic neurons. J Neurosci **20:**2867–2874.

Flynn, R., and G. W. Zamponi (2010) Regulation of calcium channels by RGK proteins.

Channels (Austin) **4:**434–439.

Ford, C. E., N. P. Skiba, H. Bae, Y. Daaka, E. Reuveny, L. R. Shekter, R. Rosal, G. Weng, C. S. Yang, R. Iyengar, R. J. Miller, L. Y. Jan, R. J. Lefkowitz, and H. E. Hamm (1998) Molecular basis for interactions of G protein betagamma subunits with effectors. Science **280:**1271–1274.

Forscher, P., G. S. Oxford, and D. Schulz (1986) Noradrenaline modulates calcium channels in avian dorsal root ganglion cells through tight receptor-channel coupling. J Physiol **379:**131–144.

Fredriksson, R., M. C. Lagerstrom, L. G. Lundin, and H. B. Schioth (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol Pharmacol **63:**1256–1272.

Furukawa, T., R. Miura, Y. Mori, M. Strobeck, K. Suzuki, Y. Ogihara, T. Asano, R. Morishita, M. Hashii, H. Higashida, M. Yoshii, and T. Nukada (1998a) Differential interactions of the C terminus and the cytoplasmic I-II loop of neuronal Ca2+ channels with G-protein alpha and beta gamma subunits. II. Evidence for direct binding. J Biol Chem **273:**17595–17603.

Furukawa, T., T. Nukada, Y. Mori, M. Wakamori, Y. Fujita, H. Ishida, K. Fukuda, S. Kato, and M. Yoshii (1998b) Differential interactions of the C terminus and the cytoplasmic I-II loop of neuronal Ca2+ channels with G-protein alpha and beta gamma subunits. I. Molecular determination. J Biol Chem **273:**17585–17594.

Gamper, N., V. Reznikov, Y. Yamada, J. Yang, and M. S. Shapiro (2004) Phosphatidylinositol [correction] 4,5-bisphosphate signals underlie receptor-specific Gq/11-mediated modulation of N-type Ca2+ channels. J Neurosci **24:**10980–10992.

Garcia, D. E., S. Brown, B. Hille, and K. Mackie (1998a) Protein kinase C disrupts cannabinoid actions by phosphorylation of the CB1 cannabinoid receptor. J Neurosci **18:**2834–2841.

Garcia, D. E., B. Li, R. E. Garcia-Ferreiro, E. O. Hernandez-Ochoa, K. Yan, N. Gautam, W. A.

Catterall, K. Mackie, and B. Hille (1998b) G-protein beta-subunit specificity in the fast membrane-delimited inhibition of Ca2+ channels. J Neurosci **18:**9163–9170.

Garza-Lopez, E., R. Gonzalez-Ramirez, M. A. Gandini, A. Sandoval, and R. Felix (2013) The familial hemiplegic migraine type 1 mutation K1336E affects direct G protein-mediated regulation of neuronal P/Q-type Ca2+ channels. Cephalalgia **33:**398–407.

Garza-Lopez, E., A. Sandoval, R. Gonzalez-Ramirez, M. A. Gandini, A. Van den Maagdenberg, M. De Waard, and R. Felix (2012) Familial hemiplegic migraine type 1 mutations W1684R and V1696I alter G protein-mediated regulation of Ca(V)2.1 voltage-gated calcium channels. Biochim Biophys Acta **1822:**1238–1246.

Golard, A., L. Role, and S. A. Siegelbaum (1994) Substance P potentiates calcium channel modulation by somatostatin in chick sympathetic ganglia. J Neurophysiol **72:**2683–2690.

Grassi, F., and H. D. Lux (1989) Voltage-dependent GABA-induced modulation of calcium currents in chick sensory neurons. Neurosci Lett **105:**113–119.

Hamid, J., D. Nelson, R. Spaetgens, S. J. Dubel, T. P. Snutch, and G. W. Zamponi (1999) Identification of an integration center for cross-talk between protein kinase C and G protein modulation of N-type calcium channels. J Biol Chem **274:**6195–6202.

Hans, M., S. Luvisetto, M. E. Williams, M. Spagnolo, A. Urrutia, A. Tottene, P. F. Brust, E. C. Johnson, M. M. Harpold, K. A. Stauderman, and D. Pietrobon (1999) Functional consequences of mutations in the human alpha1A calcium channel subunit linked to familial hemiplegic migraine. J Neurosci **19:**1610–1619.

Harkins, A. B., A. L. Cahill, J. F. Powers, A. S. Tischler, and A. P. Fox (2004) Deletion of the synaptic protein interaction site of the N-type (CaV2.2) calcium channel inhibits secretion in mouse pheochromocytoma cells. Proc Natl Acad Sci U S A **101:**15219–15224.

Haws, C. M., P. A. Slesinger, and J. B. Lansman (1993) Dihydropyridine- and omega-conotoxin-sensitive Ca2+ currents in cerebellar neurons: persistent block of L-type channels by a

pertussis toxin-sensitive G-protein. J Neurosci **13:**1148–1156.

Herlitze, S., D. E. Garcia, K. Mackie, B. Hille, T. Scheuer, and W. A. Catterall (1996) Modulation of Ca2+ channels by G-protein beta gamma subunits. Nature **380:**258–262.

Herlitze, S., G. H. Hockerman, T. Scheuer, and W. A. Catterall (1997) Molecular determinants of inactivation and G protein modulation in the intracellular loop connecting domains I and II of the calcium channel alpha1A subunit. Proc Natl Acad Sci U S A **94:**1512–1516.

Herlitze, S., H. Zhong, T. Scheuer, and W. A. Catterall (2001) Allosteric modulation of Ca2+ channels by G proteins, voltage-dependent facilitation, protein kinase C, and Ca(v)beta subunits. Proc Natl Acad Sci U S A **98:**4699–4704.

Hill, R. H., E. Svensson, Y. Dewael, and S. Grillner (2003) 5-HT inhibits N-type but not L-type Ca(2+) channels via 5-HT1A receptors in lamprey spinal neurons. Eur J Neurosci **18:**2919–2924.

Holz, G. G., S. G. Rane, and K. Dunlap (1986) GTP-binding proteins mediate transmitter inhibition of voltage-dependent calcium channels. Nature **319:**670–672.

Hosford, D. A., F. H. Lin, Y. Wang, S. J. Caddick, M. Rees, N. J. Parkinson, J. Barclay, R. D. Cox, R. M. Gardiner, D. A. Hosford, P. Denton, Y. Wang, M. F. Seldin, and B. Chen (1999) Studies of the lethargic (lh/lh) mouse model of absence seizures: regulatory mechanisms and identification of the lh gene. Adv Neurol **79:**239–252.

Hummer, A., O. Delzeith, S. R. Gomez, R. L. Moreno, M. D. Mark, and S. Herlitze (2003) Competitive and synergistic interactions of G protein beta(2) and Ca(2+) channel beta(1b) subunits with Ca(v)2.1 channels, revealed by mammalian two-hybrid and fluorescence resonance energy transfer measurements. J Biol Chem **278:**49386–49400.

Ikeda, S. R. (1991) Double-pulse calcium channel current facilitation in adult rat sympathetic neurones. J Physiol **439:**181–214.

Ikeda, S. R. (1996) Voltage-dependent modulation of N-type calcium channels by G-protein beta

gamma subunits. Nature **380:**255–258.

Ikeda, S. R., and G. G. Schofield (1989a) Somatostatin blocks a calcium current in rat sympathetic ganglion neurones. J Physiol **409:**221–240.

Ikeda, S. R., and G. G. Schofield (1989b) Somatostatin cyclic octapeptide analogs which preferentially bind to SOMa receptors block a calcium current in rat superior cervical ganglion neurons. Neurosci Lett **96:**283–288.

Jarvis, S. E., W. Barr, Z. P. Feng, J. Hamid, and G. W. Zamponi (2002) Molecular determinants of syntaxin 1 modulation of N-type calcium channels. J Biol Chem **277:**44399–44407.

Jarvis, S. E., J. M. Magga, A. M. Beedle, J. E. Braun, and G. W. Zamponi (2000) G protein modulation of N-type calcium channels is facilitated by physical interactions between syntaxin 1A and Gbetagamma. J Biol Chem **275:**6388–6394.

Jarvis, S. E., and G. W. Zamponi (2001) Distinct molecular determinants govern syntaxin 1A-mediated inactivation and G-protein inhibition of N-type calcium channels. J Neurosci **21:**2939–2948.

Jones, L. P., P. G. Patil, T. P. Snutch, and D. T. Yue (1997) G-protein modulation of N-type calcium channel gating current in human embryonic kidney cells (HEK 293). J Physiol **498:**601–610.

Kammermeier, P. J., and S. R. Ikeda (1999) Expression of RGS2 alters the coupling of metabotropic glutamate receptor 1a to M-type K+ and N-type Ca2+ channels. Neuron **22:**819–829.

Kasai, H. (1992) Voltage- and time-dependent inhibition of neuronal calcium channels by a GTP-binding protein in a mammalian cell line. J Physiol **448:**189–209.

Kasai, H., and T. Aosaki (1989) Modulation of Ca-channel current by an adenosine analog mediated by a GTP-binding protein in chick sensory neurons. Pflugers Arch **414:**145–149.

Keith, R. K., R. E. Poage, C. T. Yokoyama, W. A. Catterall, and S. D. Meriney (2007)

Bidirectional modulation of transmitter release by calcium channel/syntaxin interactions in vivo. J Neurosci **27:**265–269.

Kisilevsky, A. E., S. J. Mulligan, C. Altier, M. C. Iftinca, D. Varela, C. Tai, L. Chen, S. Hameed, J. Hamid, B. A. Macvicar, and G. W. Zamponi (2008) D1 receptors physically interact with N-type calcium channels to regulate channel distribution and dendritic calcium entry. Neuron **58:**557–570.

Kisilevsky, A. E., and G. W. Zamponi (2008) D2 dopamine receptors interact directly with N-type calcium channels and regulate channel surface expression levels. Channels (Austin) **2:**269–277.

Kitano, J., M. Nishida, Y. Itsukaichi, I. Minami, M. Ogawa, T. Hirano, Y. Mori, and S. Nakanishi (2003) Direct interaction and functional coupling between metabotropic glutamate receptor subtype 1 and voltage-sensitive Cav2.1 Ca2+ channel. J Biol Chem **278:**25101–25108.

Kiyonaka, S., M. Wakamori, T. Miki, Y. Uriu, M. Nonaka, H. Bito, A. M. Beedle, E. Mori, Y. Hara, M. De Waard, M. Kanagawa, M. Itakura, M. Takahashi, K. P. Campbell, and Y. Mori (2007) RIM1 confers sustained activity and neurotransmitter vesicle anchoring to presynaptic Ca2+ channels. Nat Neurosci **10:**691–701.

Kraus, R. L., M. J. Sinnegger, H. Glossmann, S. Hering, and J. Striessnig (1998) Familial hemiplegic migraine mutations change alpha1A Ca2+ channel kinetics. J Biol Chem **273:**5586–5590.

Kraus, R. L., M. J. Sinnegger, A. Koschak, H. Glossmann, S. Stenirri, P. Carrera, and J. Striessnig (2000) Three new familial hemiplegic migraine mutants affect P/Q-type Ca(2+) channel kinetics. J Biol Chem **275:**9239–9243.

Kuo, C. C., and B. P. Bean (1993) G-protein modulation of ion permeation through N-type calcium channels. Nature **365:**258–262.

Lee, H. K., and K. S. Elmslie (2000) Reluctant gating of single N-type calcium channels during

neurotransmitter-induced inhibition in bullfrog sympathetic neurons. J Neurosci **20:**3115–3128.

Leveque, C., O. el Far, N. Martin-Moutot, K. Sato, R. Kato, M. Takahashi, and M. J. Seagar (1994) Purification of the N-type calcium channel associated with syntaxin and synaptotagmin. A complex implicated in synaptic vesicle exocytosis. J Biol Chem **269:**6306–6312.

Leveque, C., S. Pupier, B. Marqueze, L. Geslin, M. Kataoka, M. Takahashi, M. De Waard, and M. Seagar (1998) Interaction of cysteine string proteins with the alpha1A subunit of the P/Q-type calcium channel. J Biol Chem **273:**13488–13492.

Li, Q., A. Lau, T. J. Morris, L. Guo, C. B. Fordyce, and E. F. Stanley (2004) A syntaxin 1, Galpha(o), and N-type calcium channel complex at a presynaptic nerve terminal: analysis by quantitative immunocolocalization. J Neurosci **24:**4070–4081.

Li, X., A. Hummer, J. Han, M. Xie, K. Melnik-Martinez, R. L. Moreno, M. Buck, M. D. Mark, and S. Herlitze (2005) G protein beta2 subunit-derived peptides for inhibition and induction of G protein pathways. Examination of voltage-gated Ca2+ and G protein inwardly rectifying K+ channels. J Biol Chem **280:**23945–23959.

Lipscombe, D., S. Kongsamut, and R. W. Tsien (1989) Alpha-adrenergic inhibition of sympathetic neurotransmitter release mediated by modulation of N-type calcium-channel gating. Nature **340:**639–642.

Liu, L., C. F. Barrett, and A. R. Rittenhouse (2001) Arachidonic acid both inhibits and enhances whole cell calcium currents in rat sympathetic neurons. Am J Physiol Cell Physiol **280:**C1293–C1305.

Liu, L., and A. R. Rittenhouse (2003) Arachidonic acid mediates muscarinic inhibition and enhancement of N-type Ca2+ current in sympathetic neurons. Proc Natl Acad Sci U S A **100:**295–300.

Magga, J. M., S. E. Jarvis, M. I. Arnot, G. W. Zamponi, and J. E. Braun (2000) Cysteine string protein regulates G protein modulation of N-type calcium channels. Neuron **28:**195–204.

Maguire, G., B. Maple, P. Lukasiewicz, and F. Werblin (1989) Gamma-aminobutyrate type B receptor modulation of L-type calcium channel current at bipolar cell terminals in the retina of the tiger salamander. Proc Natl Acad Sci U S A **86:**10144–10147.

Marchetti, C., E. Carbone, and H. D. Lux (1986) Effects of dopamine and noradrenaline on Ca channels of cultured sensory and sympathetic neurons of chick. Pflugers Arch **406:**104–111.

Mathews, J. L., A. V. Smrcka, and J. M. Bidlack (2008) A novel Gbetagamma-subunit inhibitor selectively modulates mu-opioid-dependent antinociception and attenuates acute morphine-induced antinociceptive tolerance and dependence. J Neurosci **28:**12183–12189.

McFadzean, I., and R. J. Docherty (1989) Noradrenaline- and Enkephalin-Induced Inhibition of Voltage-Sensitive Calcium Currents in NG108-15 Hybrid Cells. Eur J Neurosci **1:**141–147.

McFadzean, I., I. Mullaney, D. A. Brown, and G. Milligan (1989) Antibodies to the GTP binding protein, Go, antagonize noradrenaline-induced calcium current inhibition in NG108-15 hybrid cells. Neuron **3:**177–182.

Melliti, K., M. Grabner, and G. R. Seabrook (2003) The familial hemiplegic migraine mutation R192Q reduces G-protein-mediated inhibition of P/Q-type (Ca(V)2.1) calcium channels expressed in human embryonic kidney cells. J Physiol **546:**337–347.

Menon-Johansson, A. S., N. Berrow, and A. C. Dolphin (1993) G(o) transduces GABAB-receptor modulation of N-type calcium channels in cultured dorsal root ganglion neurons. Pflugers Arch **425:**335–343.

Meza, U., and B. Adams (1998) G-Protein-dependent facilitation of neuronal alpha1A, alpha1B, and alpha1E Ca channels. J Neurosci **18:**5240–5252.

Michailidis, I. E., Y. Zhang, and J. Yang (2007) The lipid connection-regulation of voltage-gated

Ca(2+) channels by phosphoinositides. Pflugers Arch **455:**147–155.

Mirshahi, T., V. Mittal, H. Zhang, M. E. Linder, and D. E. Logothetis (2002) Distinct sites on G protein beta gamma subunits regulate different effector functions. J Biol Chem **277:**36345–36350.

Mochida, S., Z. H. Sheng, C. Baker, H. Kobayashi, and W. A. Catterall (1996) Inhibition of neurotransmission by peptides containing the synaptic protein interaction site of N-type Ca2+ channels. Neuron **17:**781–788.

Mullner, C., L. A. Broos, A. M. van den Maagdenberg, and J. Striessnig (2004) Familial hemiplegic migraine type 1 mutations K1336E, W1684R, and V1696I alter Cav2.1 Ca2+ channel gating: evidence for beta-subunit isoform-specific effects. J Biol Chem **279:**51844–51850.

Murali, S. S., I. A. Napier, B. K. Rycroft, and M. J. Christie (2012) Opioid-related (ORL1) receptors are enriched in a subpopulation of sensory neurons and prolonged activation produces no functional loss of surface N-type calcium channels. J Physiol **590:**1655–1667.

Natochin, M., T. N. Campbell, B. Barren, L. C. Miller, S. Hameed, N. O. Artemyev, and J. E. Braun (2005) Characterization of the G alpha(s) regulator cysteine string protein. J Biol Chem **280:**30236–30241.

Ophoff, R. A., G. M. Terwindt, M. N. Vergouwe, R. van Eijk, P. J. Oefner, S. M. Hoffman, J. E. Lamerdin, H. W. Mohrenweiser, D. E. Bulman, M. Ferrari, J. Haan, D. Lindhout, G. J. van Ommen, M. H. Hofker, M. D. Ferrari, and R. R. Frants (1996) Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell **87:**543–552.

Page, K. M., C. Canti, G. J. Stephens, N. S. Berrow, and A. C. Dolphin (1998) Identification of the amino terminus of neuronal Ca2+ channel alpha1 subunits alpha1B and alpha1E as an essential determinant of G-protein modulation. J Neurosci **18:**4815–4824.

Page, K. M., G. J. Stephens, N. S. Berrow, and A. C. Dolphin (1997) The intracellular loop between domains I and II of the B-type calcium channel confers aspects of G-protein sensitivity to the E-type calcium channel. J Neurosci **17:**1330–1338.

Patil, P. G., M. de Leon, R. R. Reed, S. Dubel, T. P. Snutch, and D. T. Yue (1996) Elementary events underlying voltage-dependent G-protein inhibition of N-type calcium channels. Biophys J **71:**2509–2521.

Payandeh, J., T. Scheuer, N. Zheng, and W. A. Catterall (2011) The crystal structure of a voltage-gated sodium channel. Nature **475:**353–358.

Penington, N. J., J. S. Kelly, and A. P. Fox (1991) A study of the mechanism of Ca2+ current inhibition produced by serotonin in rat dorsal raphe neurons. J Neurosci **11:**3594–3609.

Pietrobon, D. (2013) Calcium channels and migraine. Biochim Biophys Acta **1828:**1655–1665.

Pietrobon, D., and M. A. Moskowitz (2013) Pathophysiology of migraine. Annu Rev Physiol **75:**365–391.

Plummer, M. R., A. Rittenhouse, M. Kanevsky, and P. Hess (1991) Neurotransmitter modulation of calcium channels in rat sympathetic neurons. J Neurosci **11:**2339–2348.

Pragnell, M., M. De Waard, Y. Mori, T. Tanabe, T. P. Snutch, and K. P. Campbell (1994) Calcium channel beta-subunit binds to a conserved motif in the I-II cytoplasmic linker of the alpha 1-subunit. Nature **368:**67–70.

Qin, N., D. Platano, R. Olcese, E. Stefani, and L. Birnbaumer (1997) Direct interaction of gbetagamma with a C-terminal gbetagamma-binding domain of the Ca2+ channel alpha1 subunit is responsible for channel inhibition by G protein-coupled receptors. Proc Natl Acad Sci U S A **94:**8866–8871.

Rettig, J., C. Heinemann, U. Ashery, Z. H. Sheng, C. T. Yokoyama, W. A. Catterall, and E. Neher (1997) Alteration of Ca2+ dependence of neurotransmitter release by disruption of Ca2+ channel/syntaxin interaction. J Neurosci **17:**6647–6656.

Rettig, J., Z. H. Sheng, D. K. Kim, C. D. Hodson, T. P. Snutch, and W. A. Catterall (1996) Isoform-specific interaction of the alpha1A subunits of brain Ca2+ channels with the presynaptic proteins syntaxin and SNAP-25. Proc Natl Acad Sci U S A **93:**7363–7368.

Roberts-Crowley, M. L., T. Mitra-Ganguli, L. Liu, and A. R. Rittenhouse (2009) Regulation of voltage-gated Ca2+ channels by lipids. Cell Calcium **45:**589–601.

Roche, J. P., V. Anantharam, and S. N. Treistman (1995) Abolition of G protein inhibition of alpha 1A and alpha 1B calcium channels by co-expression of the beta 3 subunit. FEBS Lett **371:**43–46.

Roche, J. P., and S. N. Treistman (1998a) The Ca2+ channel beta3 subunit differentially modulates G-protein sensitivity of alpha1A and alpha1B Ca2+ channels. J Neurosci **18:**878–886.

Roche, J. P., and S. N. Treistman (1998b) Ca2+ channel beta3 subunit enhances voltage-dependent relief of G-protein inhibition induced by muscarinic receptor activation and Gbetagamma. J Neurosci **18:**4883–4890.

Rodriguez-Menchaca, A. A., S. K. Adney, L. Zhou, and D. E. Logothetis (2012) Dual Regulation of Voltage-Sensitive Ion Channels by PIP(2). Front Pharmacol **3:**170.

Rousset, M., T. Cens, A. Gouin-Charnet, F. Scamps, and P. Charnet (2004) Ca2+ and phosphatidylinositol 4,5-bisphosphate stabilize a Gbeta gamma-sensitive state of Ca V2 Ca 2+ channels. J Biol Chem **279:**14619–14630.

Sandoz, G., I. Lopez-Gonzalez, D. Grunwald, D. Bichet, X. Altafaj, N. Weiss, M. Ronjat, A. Dupuis, and M. De Waard (2004) Cavbeta-subunit displacement is a key step to induce the reluctant state of P/Q calcium channels by direct G protein regulation. Proc Natl Acad Sci U S A **101:**6267–6272.

Scott, R. H., and A. C. Dolphin (1986) Regulation of calcium currents by a GTP analogue: potentiation of (-)-baclofen-mediated inhibition. Neurosci Lett **69:**59–64.

Scott, R. H., and A. C. Dolphin (1990) Voltage-dependent modulation of rat sensory neurone calcium channel currents by G protein activation: effect of a dihydropyridine antagonist. Br J Pharmacol **99:**629–630.

Shapiro, M. S., and B. Hille (1993) Substance P and somatostatin inhibit calcium channels in rat sympathetic neurons via different G protein pathways. Neuron **10:**11–20.

Shapiro, M. S., M. D. Loose, S. E. Hamilton, N. M. Nathanson, J. Gomeza, J. Wess, and B. Hille (1999) Assignment of muscarinic receptor subtypes mediating G-protein modulation of Ca(2+) channels by using knockout mice. Proc Natl Acad Sci U S A **96:**10899–10904.

Sheng, Z. H., J. Rettig, M. Takahashi, and W. A. Catterall (1994) Identification of a syntaxin-binding site on N-type calcium channels. Neuron **13:**1303–1313.

Simen, A. A., C. C. Lee, B. B. Simen, V. P. Bindokas, and R. J. Miller (2001) The C terminus of the Ca channel alpha1B subunit mediates selective inhibition by G-protein-coupled receptors. J Neurosci **21:**7587–7597.

Simen, A. A., and R. J. Miller (2000) Involvement of regions in domain I in the opioid receptor sensitivity of alpha1B Ca(2+) channels. Mol Pharmacol **57:**1064–1074.

Somasundaram, B., and R. T. Tregear (1993) Isoproterenol and GTP gamma S inhibit L-type calcium channels of differentiating rat skeletal muscle cells. J Muscle Res Cell Motil **14:**341–346.

Soong, T. W., A. Stea, C. D. Hodson, S. J. Dubel, S. R. Vincent, and T. P. Snutch (1993) Structure and functional expression of a member of the low voltage-activated calcium channel family. Science **260:**1133–1136.

Stanley, E. F., and R. R. Mirotznik (1997) Cleavage of syntaxin prevents G-protein regulation of presynaptic calcium channels. Nature **385:**340–343.

Stephens, G. J., N. L. Brice, N. S. Berrow, and A. C. Dolphin (1998) Facilitation of rabbit alpha1B calcium channels: involvement of endogenous Gbetagamma subunits. J Physiol

509:15–27.

Stephens, G. J., K. M. Page, Y. Bogdanov, and A. C. Dolphin (2000) The alpha1B Ca2+ channel amino terminus contributes determinants for beta subunit-mediated voltage-dependent inactivation properties. J Physiol **525 Pt 2:**377–390.

Suh, B. C., K. Leal, and B. Hille (2010) Modulation of high-voltage activated Ca(2+) channels by membrane phosphatidylinositol 4,5-bisphosphate. Neuron **67:**224–238.

Tedford, H. W., A. E. Kisilevsky, J. B. Peloquin, and G. W. Zamponi (2006) 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 **96:**465–470.

Tonelli, A., M. G. D'Angelo, R. Salati, L. Villa, C. Germinasi, T. Frattini, G. Meola, A. C. Turconi, N. Bresolin, and M. T. Bassi (2006) Early onset, non fluctuating spinocerebellar ataxia and a novel missense mutation in CACNA1A gene. J Neurol Sci **241:**13–17.

Toth, P. T., V. P. Bindokas, D. Bleakman, W. F. Colmers, and R. J. Miller (1993) Mechanism of presynaptic inhibition by neuropeptide Y at sympathetic nerve terminals. Nature **364:**635–639.

Tottene, A., R. Conti, A. Fabbro, D. Vecchia, M. Shapovalova, M. Santello, A. M. van den Maagdenberg, M. D. Ferrari, and D. Pietrobon (2009) Enhanced excitatory transmission at cortical synapses as the basis for facilitated spreading depression in Ca(v)2.1 knockin migraine mice. Neuron **61:**762–773.

Tottene, A., F. Pivotto, T. Fellin, T. Cesetti, A. M. van den Maagdenberg, and D. Pietrobon (2005) Specific kinetic alterations of human CaV2.1 calcium channels produced by mutation S218L causing familial hemiplegic migraine and delayed cerebral edema and coma after minor head trauma. J Biol Chem **280:**17678–17686.

van den Maagdenberg, A. M., D. Pietrobon, T. Pizzorusso, S. Kaja, L. A. Broos, T. Cesetti, R. C. van de Ven, A. Tottene, J. van der Kaa, J. J. Plomp, R. R. Frants, and M. D. Ferrari (2004)

A Cacna1a knockin migraine mouse model with increased susceptibility to cortical spreading depression. Neuron **41:**701–710.

van den Maagdenberg, A. M., T. Pizzorusso, S. Kaja, N. Terpolilli, M. Shapovalova, F. E. Hoebeek, C. F. Barrett, L. Gherardini, R. C. van de Ven, B. Todorov, L. A. Broos, A. Tottene, Z. Gao, M. Fodor, C. I. De Zeeuw, R. R. Frants, N. Plesnila, J. J. Plomp, D. Pietrobon, and M. D. Ferrari (2010) High cortical spreading depression susceptibility and migraine-associated symptoms in Ca(v)2.1 S218L mice. Ann Neurol **67:**85–98.

Vassilatis, D. K., J. G. Hohmann, H. Zeng, F. Li, J. E. Ranchalis, M. T. Mortrud, A. Brown, S. S. Rodriguez, J. R. Weller, A. C. Wright, J. E. Bergmann, and G. A. Gaitanaris (2003) The G protein-coupled receptor repertoires of human and mouse. Proc Natl Acad Sci U S A **100:**4903–4908.

Weiss, N. (2009) Regulation of N-type calcium channels by G-proteins: multiple pathways to control calcium entry into neurons. Channels (Austin) **3:**219–220.

Weiss, N., C. Arnoult, A. Feltz, and M. De Waard (2006) Contribution of the kinetics of G protein dissociation to the characteristic modifications of N-type calcium channel activity. Neurosci Res **56:**332–343.

Weiss, N., C. Legrand, S. Pouvreau, H. Bichraoui, B. Allard, G. W. Zamponi, M. De Waard, and V. Jacquemond (2010) In vivo expression of G-protein beta1gamma2 dimer in adult mouse skeletal muscle alters L-type calcium current and excitation-contraction coupling. J Physiol **588:**2945–2960.

Weiss, N., A. Sandoval, R. Felix, A. Van den Maagdenberg, and M. De Waard (2008) The S218L familial hemiplegic migraine mutation promotes deinhibition of Ca(v)2.1 calcium channels during direct G-protein regulation. Pflugers Arch **457:**315–326.

Weiss, N., A. Sandoval, S. Kyonaka, R. Felix, Y. Mori, and M. De Waard (2011) Rim1 modulates direct G-protein regulation of Ca(v)2.2 channels. Pflugers Arch **461:**447–459.

Weiss, N., A. Tadmouri, M. Mikati, M. Ronjat, and M. De Waard (2007a) Importance of voltage-dependent inactivation in N-type calcium channel regulation by G-proteins. Pflugers Arch **454:**115–129.

Weiss, N., E. Tournier-Lasserve, and M. De Waard (2007b) [Role of P/Q calcium channel in familial hemiplegic migraine]. Med Sci (Paris) **23:**53–63.

Weiss, N., and G. W. Zamponi (2012) Regulation of voltage-gated calcium channels by synaptic proteins. Adv Exp Med Biol **740:**759–775.

Wettschureck, N., and S. Offermanns (2005) Mammalian G proteins and their cell type specific functions. Physiol Rev **85:**1159–1204.

Williams, S., M. Serafin, M. Muhlethaler, and L. Bernheim (1997) Facilitation of N-type calcium current is dependent on the frequency of action potential-like depolarizations in dissociated cholinergic basal forebrain neurons of the guinea pig. J Neurosci **17:**1625–1632.

Wiser, O., M. K. Bennett, and D. Atlas (1996) Functional interaction of syntaxin and SNAP-25 with voltage-sensitive L- and N-type Ca2+ channels. EMBO J **15:**4100–4110.

Wolfe, J. T., H. Wang, J. Howard, J. C. Garrison, and P. Q. Barrett (2003) T-type calcium channel regulation by specific G-protein betagamma subunits. Nature **424:**209–213.

Wu, L. G., and P. Saggau (1994) Adenosine inhibits evoked synaptic transmission primarily by reducing presynaptic calcium influx in area CA1 of hippocampus. Neuron **12:**1139–1148.

Wu, L. G., and P. Saggau (1995) GABAB receptor-mediated presynaptic inhibition in guinea-pig hippocampus is caused by reduction of presynaptic Ca2+ influx. J Physiol **485:**649–657.

Wu, L. G., and P. Saggau (1997) Presynaptic inhibition of elicited neurotransmitter release. Trends Neurosci **20:**204–212.

Yassin, M., S. Zong, and T. Tanabe (1996) G-protein modulation of neuronal class E (alpha 1E) calcium channel expressed in GH3 cells. Biochem Biophys Res Commun **220:**453–458.

Yawo, H., and N. Chuhma (1993) Preferential inhibition of omega-conotoxin-sensitive

presynaptic Ca2+ channels by adenosine autoreceptors. Nature **365:**256–258.

Yoshida, A., C. Oho, A. Omori, R. Kuwahara, T. Ito, and M. Takahashi (1992) HPC-1 is associated with synaptotagmin and omega-conotoxin receptor. J Biol Chem **267:**24925–24928.

Zamponi, G. W. (2001) Determinants of G protein inhibition of presynaptic calcium channels. Cell Biochem Biophys **34:**79–94.

Zamponi, G. W., E. Bourinet, D. Nelson, J. Nargeot, and T. P. Snutch (1997) Crosstalk between G proteins and protein kinase C mediated by the calcium channel alpha1 subunit. Nature **385:**442–446.

Zamponi, G. W., and K. P. Currie (2013) Regulation of Ca(V)2 calcium channels by G protein coupled receptors. Biochim Biophys Acta **1828:**1629–1643.

Zamponi, G. W., and T. P. Snutch (1998) Decay of prepulse facilitation of N type calcium channels during G protein inhibition is consistent with binding of a single Gbeta subunit. Proc Natl Acad Sci U S A **95:**4035–4039.

Zhang, J. F., P. T. Ellinor, R. W. Aldrich, and R. W. Tsien (1996) Multiple structural elements in voltage-dependent Ca2+ channels support their inhibition by G proteins. Neuron **17:**991–1003.

Zhang, X., W. Ren, P. DeCaen, C. Yan, X. Tao, L. Tang, J. Wang, K. Hasegawa, T. Kumasaka, J. He, J. Wang, D. E. Clapham, and N. Yan (2012) Crystal structure of an orthologue of the NaChBac voltage-gated sodium channel. Nature **486:**130–134.

Zhang, Y., M. E. Devries, and J. Skolnick (2006) Structure modeling of all identified G protein-coupled receptors in the human genome. PLoS Comput Biol **2:**e13.

Zhong, H., B. Li, T. Scheuer, and W. A. Catterall (2001) Control of gating mode by a single amino acid residue in transmembrane segment IS3 of the N-type Ca2+ channel. Proc Natl Acad Sci U S A **98:**4705–4709.

Zhong, H., C. T. Yokoyama, T. Scheuer, and W. A. Catterall (1999) Reciprocal regulation of P/Q-type Ca2+ channels by SNAP-25, syntaxin and synaptotagmin. Nat Neurosci **2:**939–941.

Zhu, Y., and S. R. Ikeda (1994) VIP inhibits N-type Ca2+ channels of sympathetic neurons via a pertussis toxin-insensitive but cholera toxin-sensitive pathway. Neuron **13:**657–669.

Footnotes

Research in NW's laboratory is supported by the Czech Science Foundation (grant 15-13556S) and the Institute of Organic Chemistry and Biochemistry (IOCB). JP is supported by a postdoctoral fellowship from IOCB.

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

Legends for figures

Fig. 1. Structural organization and diversity of voltage-gated Ca^{2+} channels. VGCCs are integral membrane proteins composed of a Ca_v pore forming-subunit surrounded by $Ca_v\beta$, $\alpha_2\delta$ and in some cases γ auxiliary subunits. **A.** Putative membrane topology of the Ca_v pore forming-subunit. It consists of four repeat homologous domains (I to IV) linked by large intracellular loops (loops I-II to III-IV), each composed of six transmembrane segments (S1 to S6). The S4 segment of each of the four domains, rich in positively charged residues, presumably constitute the voltage-sensor while the P loops between segments S5 and S6 form the pore selectivity filter. **B.** Schematic representation of the VGCC macromolecular complex embedded in the plasma membrane in association with typical auxiliary subunits. **C.** Overview of the diversity of VGCCs. High-voltage-activated (HVA) channels are composed of Ca_v1 (L-type) and Ca_v2 (P/Q-, N- and R-types) subfamilies, while low-voltage-activated (LVA) channels are only composed by Ca_v3 channels, so-called T-type channels.

Fig. 2. GPCR activation cycle. In the inactive state of the receptor (**A**), the GDP-bound Gα-subunit and the G$\beta\gamma$-dimer are associated together on the receptor. Conformational change of the receptor upon agonist binding (**B**) catalyze the exchange of GDP for GTP on the Gα-subunit and GTP-bound Gα-subunit and G$\beta\gamma$-dimer complex dissociates (**C**) and activates downstream signaling. Intrinsic hydrolysis of GTP to GDP, potentially stimulated by RGS proteins, allows reassociation of the G$\beta\gamma$-dimer with GDP-bound Gα-subunit (**D**), which terminates G-protein signaling.

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 3. Putative kinetic model illustrating landmarks of Gβγ-dependent regulation of Ca_v2 channels. This scheme relies on a single conducting state where dissociation of the Gβγ-dimer has occurred while the channel remains open. The transition from Gβγ-bound closed channel (**B**) to Gβγ-unbound open conducting channel (**D**) requires a transition though an additional transient Gβγ-bound open non-conducting state of the channel (**C**) characterized by whole cell current inhibition (**G**) ("*ON*" landmark). This transition state produce a time-dependent current recovery from Gβγ-dependent inhibition, at the origin of the slowing of activation / inactivation kinetics of the whole cell current (**H**) and an apparent depolarized shift of the voltage-dependence of activation (**I**) ("*OFF*" landmarks). Dissociation of the Gβγ-dimer from the Ca_v channel-subunit is intrinsically independent of the membrane potential and is rather triggered by conformational changes of the Ca_v-subunit upon channel opening.

Fig. 4. Schematic representation of the Ca_v molecular determinants involved in Gβγ-mediated regulation of VGCCs. A. Schematic representation of the location of the important Ca_v channel molecular determinants of Gβγ-dependent regulation identified on the basis of functional evidence. Several Gβγ determinants are present within the I-II loop including the region S2 containing the QXXER motif and the G-protein interaction domain (GID) (in red). The alpha-interaction domain (AID) of the Ca_vβ-subunit on the I-II loop is shown in blue. Furthermore, additional molecular determinants located within the amino- and carboxy-terminal regions of the $Ca_v2.1$ and $Ca_v2.2$ subunits have been functionally documented. The EF-hand, IQ (Ca^{2+}-binding domain) and pre-IQ domains, and Ca^{2+}-binding domain (CBD), which constitute the carboxy-terminal Ca^{2+}-binding domains, are shown in blue. $G_q\alpha$ and $G_o\alpha$ binding sites are shown in purple. **B**. Schematic representation of a

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

presumable G-protein βγ binding pocket within the Ca_v2-subunits involving the different Ca_v molecular determinants functionally and biochemically characterized, and likely responsible of the variable sets of Gβγ-mediated modulation of the channel ("*ON*" and "*OFF*" landmarks). The carboxy-terminal region of the Ca_v2-subunits also contributes in some cases to the biochemical coupling of the channel with the GPCR.

Fig. 5. Functional role of the Ca_vβ-subunit in Gβγ-dependent modulation of VGCCs. In this scheme, the presence of a Ca_vβ-subunit does not affect the binding of the Gβγ-dimer to the channel and thus does not alter the maximal extend of inhibition ("*ON*" regulation). In contrast, Ca_vβ-mediated inactivation of the channel delimitate a temporal window during which the Gβγ-dimer can dissociate from the open channel before it inactivates, letting Ca^{2+} flowing into the cell. Hence, a "slow inactivating" channel produced for example by the $Ca_v\beta_2$-subunit (**A**), presents a large temporal window for Gβγ unbinding (Gβγ-unbound open channel state) and current recovery evidenced by an increased "*OFF*" regulation landmarks (slowing of activation / inactivation kinetics) and apparent decreased "*ON*" regulation (apparent decreased current inhibition). In contrast, a "fast inactivating" channel produced by $Ca_v\beta_3$-subunit (**B**) rather undergoes inactivation, decreasing the occurrence of a Gβγ-unbound open channel state, producing an apparent increased current imbibition ("*ON*" regulation) while preventing "*OFF*" regulation.

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

Tables

Table 1. Neurotransmitters- and receptors-mediated G-protein modulation of Ca_v2 channels. Abbreviations: SCG, Superior Cervical Ganglion; DRG, Dorsal Root Ganglion.

Neurotransmitter	Receptor	Ca_v channel	Tissu / Species	Reference
Ach	M4	$Ca_v2.2$	SCG / Rat	(Bernheim et al., 1991)
	M2	$Ca_v2.1$ & $Ca_v2.2$	SCG / Mouse	(Shapiro et al., 1999)
Adenosine	A1	$Ca_v2.2$	Ciliary ganglion / Chicken	(Yawo and Chuhma, 1993)
		$Ca_v2.2$	DRG / Chicken	(Kasai and Aosaki, 1989)
		$Ca_v2.1$ & $Ca_v2.2$	Cerebellum / Rat	(Dittman and Regehr, 1996)
		$Ca_v2.2$ & $Ca_v2.3$	Hippocampus (CA3–CA1) / Rat	(Wu and Saggau, 1994)
ATP / ADP	P2Y	$Ca_v2.2$	SCG / Rat	(Brown et al., 2000) (Filippov et al., 2000a)
Dopamine	D2	HVA	DRG / Chicken	(Marchetti et al., 1986)
Endocannabinoids	CB1	$Ca_v2.1$	SCG / Rat	(Garcia et al., 1998a)
		$Ca_v2.1$, $Ca_v2.2$ & $Ca_v2.3$	Cerebellum	(Brown and Russell, 2004)
GABA	GABA B	$Ca_v2.1$ & $Ca_v2.2$	DRG / Rat	(Dolphin and Scott, 1987)
			DRG / Chicken	(Deisz and Lux, 1985) (Grassi and Lux, 1989)
			Cerebellum/ Rat	(Dittman and Regehr, 1996)

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

		$Ca_v2.2$ & $Ca_v2.3$	Hippocampus (CA3–CA1) / Guinea pig	(Wu and Saggau, 1995)
		$Ca_v2.2$	SCG / Rat	(Filippov et al., 2000b)
Galanin	GalR1	$Ca_v2.2$	Hypothalamus / Rat	(Simen et al., 2001)
Glutamate	mGluR1	$Ca_v2.2$	SCG / Rat	(Kammermeier and Ikeda, 1999)
LHRH	LHRH-R	$Ca_v2.2$	SCG /Bullfrog	(Elmslie et al., 1990) (Boland and Bean, 1993) (Kuo and Bean, 1993)
Noradrenaline	α_2-adrénergique	$Ca_v2.2$	SCG / Bullfrog	(Bean, 1989)
		$Ca_v2.2$	SCG / Rat	(Garcia et al., 1998b)
		Non-L	NG108-15	(Docherty and McFadzean, 1989, McFadzean and Docherty, 1989)
NPY	Y2	Non-L	SCG / Rat	(Plummer et al., 1991)
		$Ca_v2.2$	SCG / Rat	(Toth et al., 1993)
Opioids - Enkephalins	μ	$Ca_v2.2$	NG108-15	(Kasai, 1992)
Opioids - Dynorphins	κ	Non-L	DRG / Rat	(Bean, 1989)
Serotonin	5HT-1A	Non-L	Spinal neuron / Lamprey	(Hill et al., 2003)
Somatostatin	SS-R	$Ca_v2.2$	DRG / Rat	(Ikeda and Schofield, 1989b)
			SCG /Rat	(Ikeda and Schofield, 1989a)
Substance P	NK1	$Ca_v2.2$	SCG / Rat	(Shapiro and Hille, 1993)
VIP	VIP-R	$Ca_v2.2$	SCG / Rat	(Zhu and Ikeda, 1994)

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

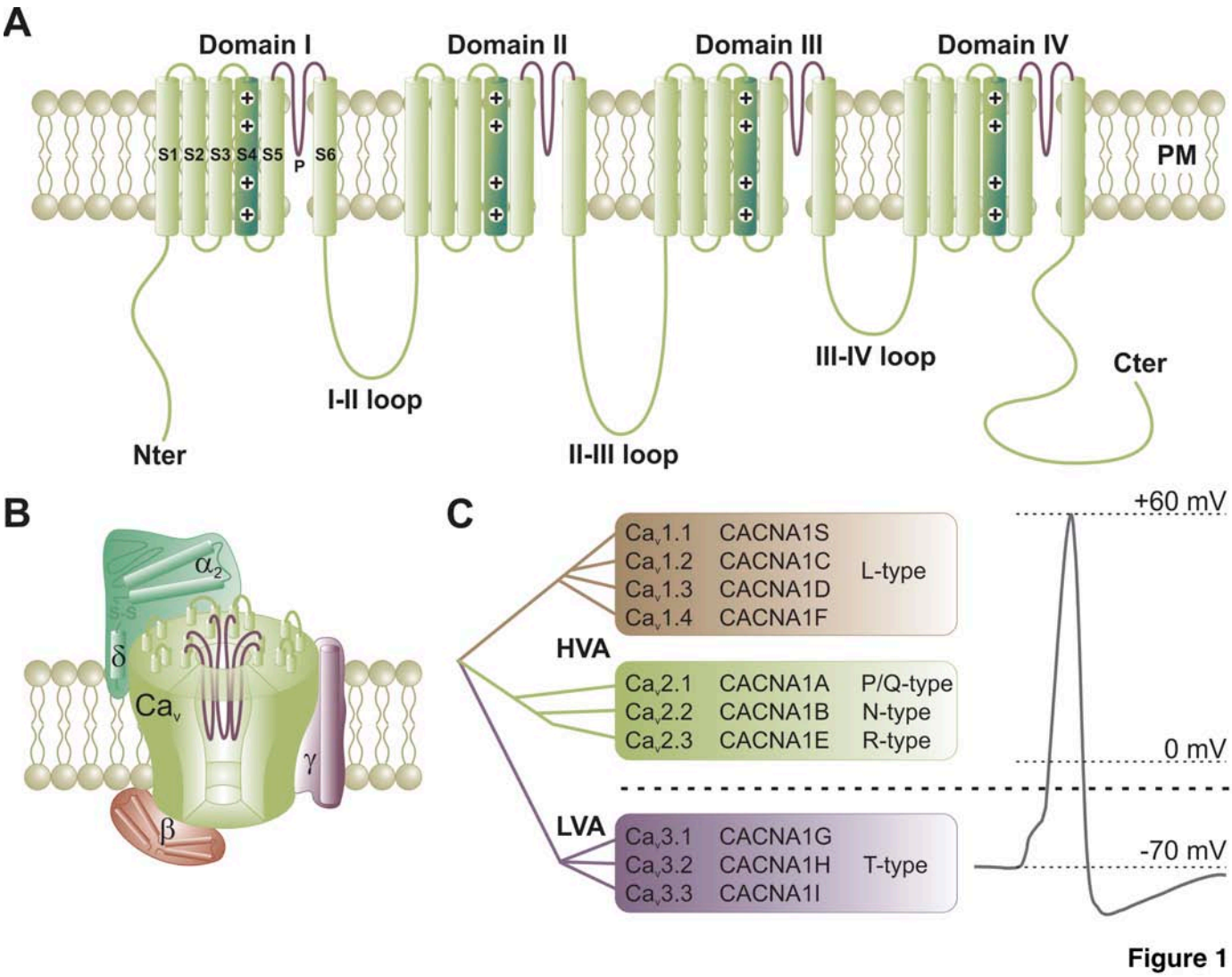


Figure 1

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

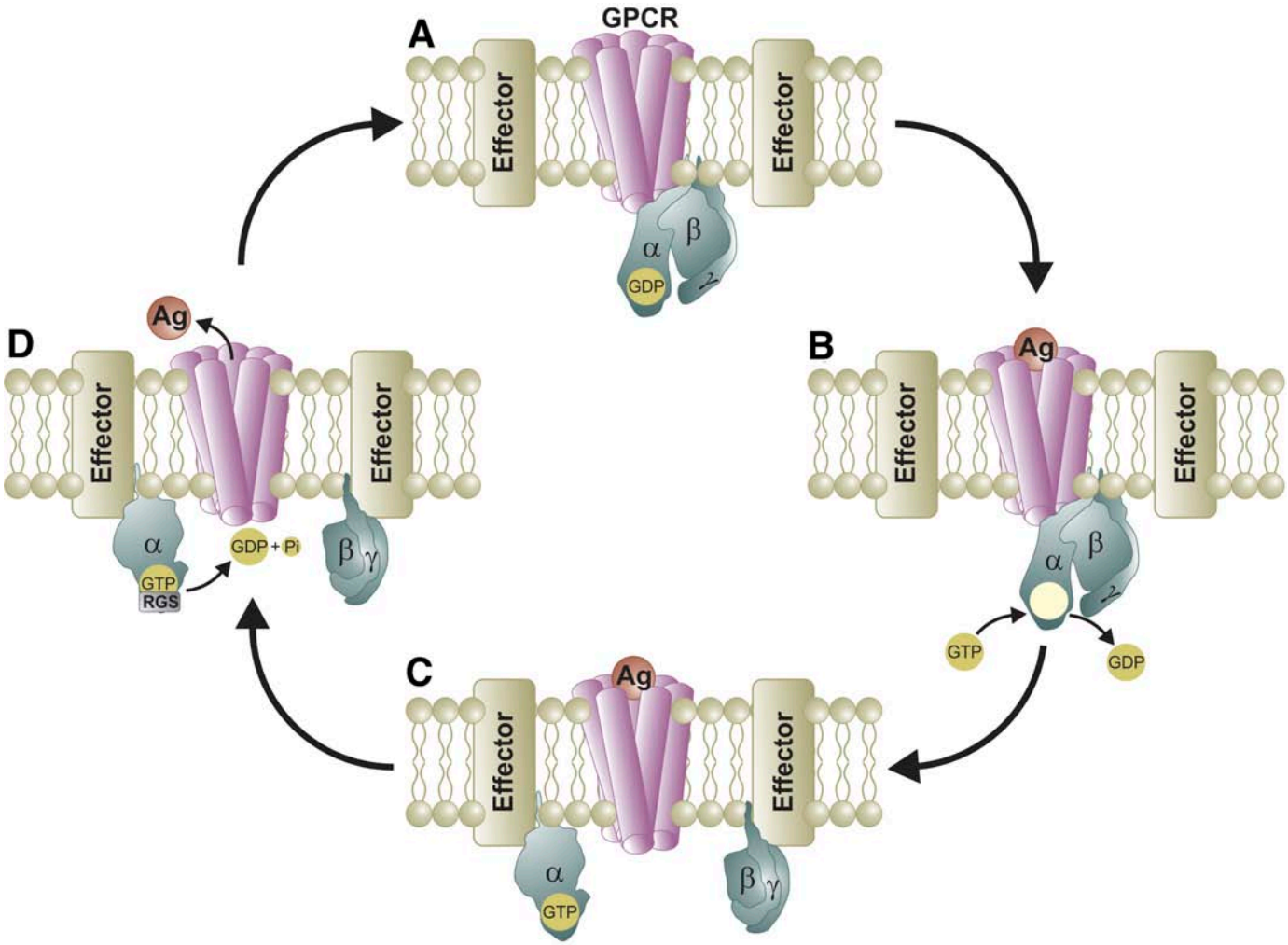
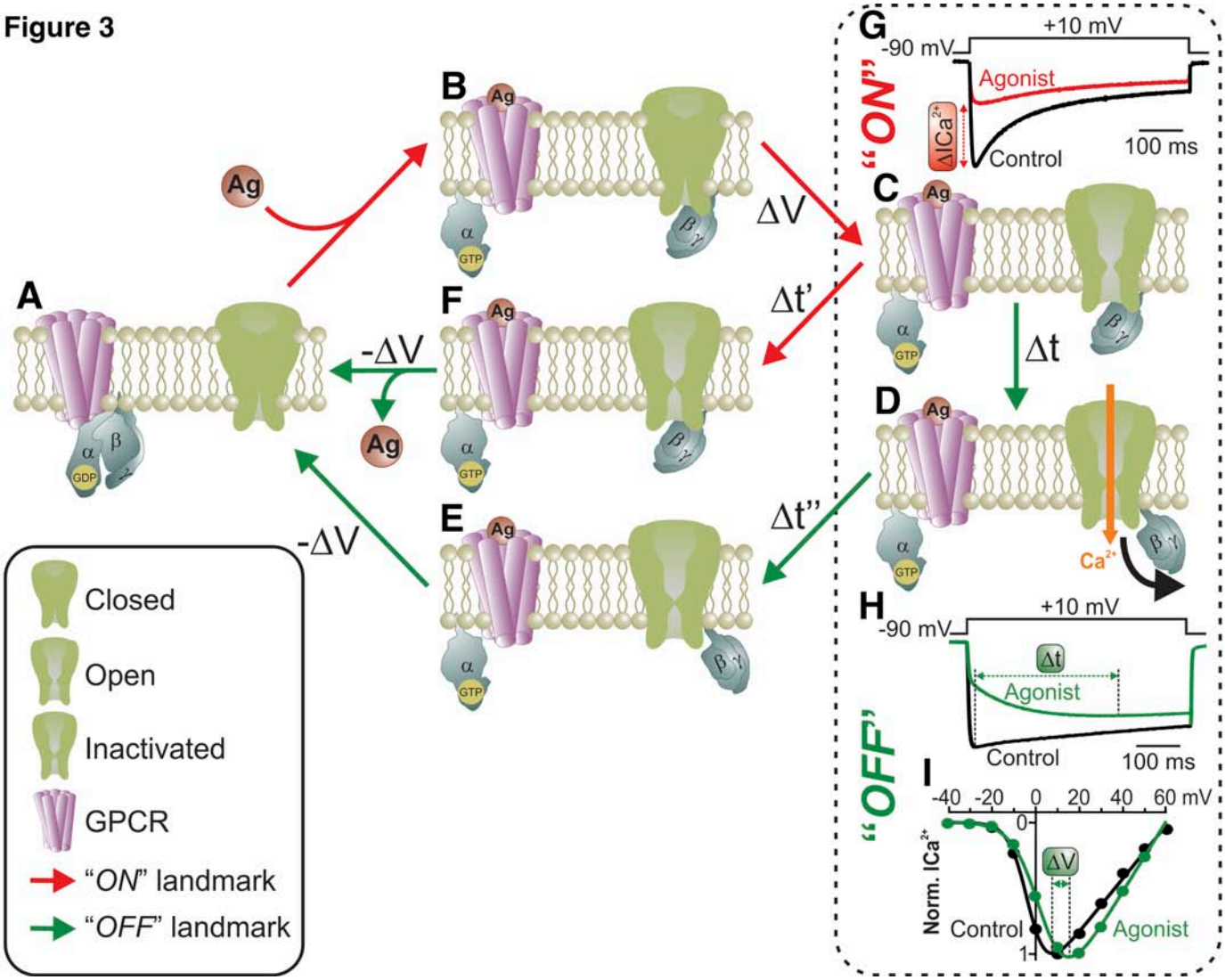


Figure 2

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 3



Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

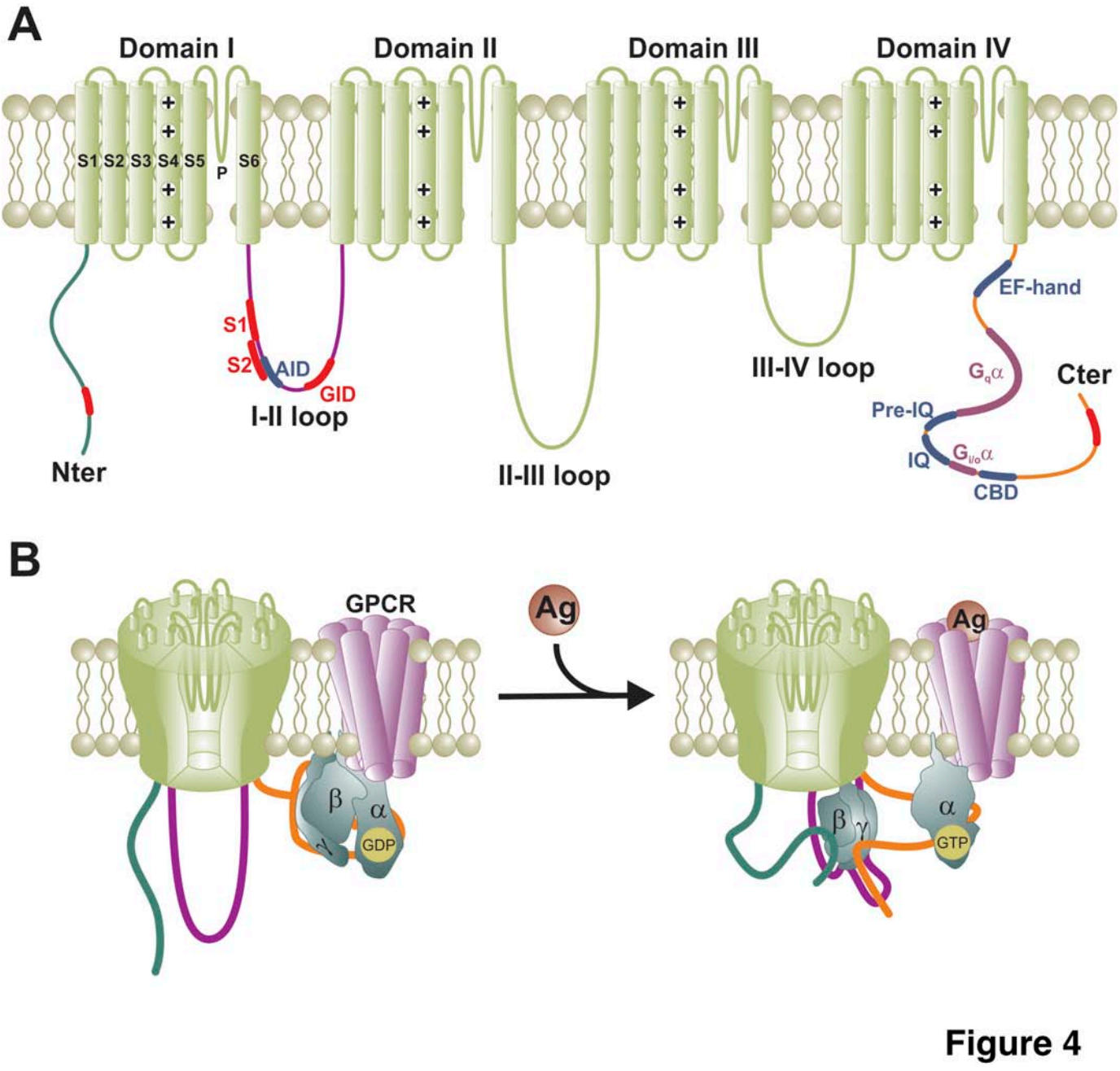


Figure 4

Molecular Pharmacology Fast Forward. Published on December 30, 2014 as DOI: 10.1124/mol.114.096008
This article has not been copyedited and formatted. The final version may differ from this version.

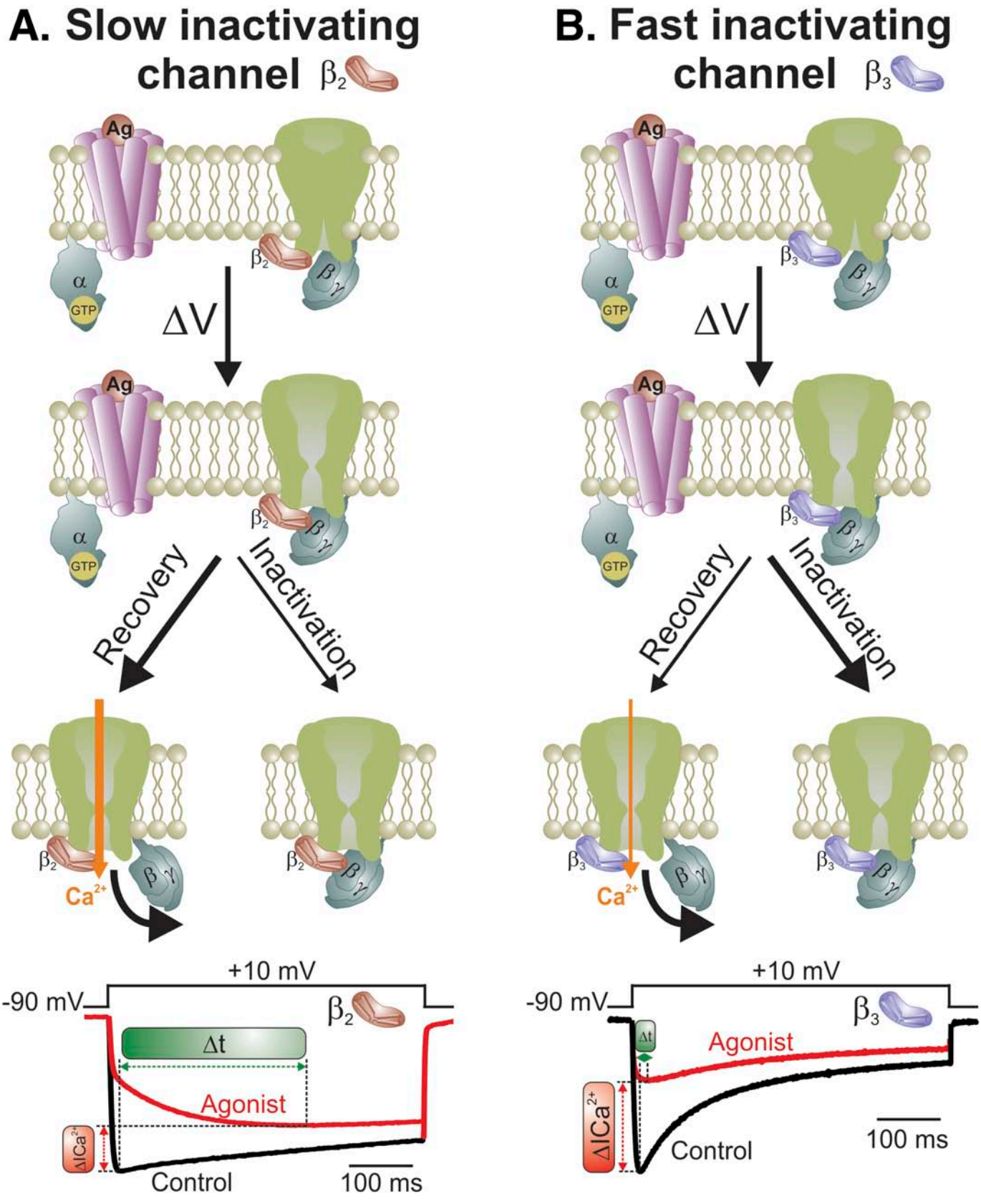


Figure 5