FUNCTION american physiological society

FUNCTION, 2021, 2(1): zqaa027

doi: 10.1093/function/zqaa027
Advance Access Publication Date: 23 October 2020
Evidence Review

EVIDENCE REVIEW

Functions of Presynaptic Voltage-gated Calcium Channels

Annette C. Dolphin *

Department of Neuroscience, Physiology and Pharmacology, University College London, WC1E 6BT, UK

*Address correspondence to A.C.D. (e-mail: a.dolphin@ucl.ac.uk)

Abstract

Voltage-gated calcium channels are the principal conduits for depolarization-mediated Ca^{2+} entry into excitable cells. In this review, the biophysical properties of the relevant members of this family of channels, those that are present in presynaptic terminals, will be discussed in relation to their function in mediating neurotransmitter release. Voltage-gated calcium channels have properties that ensure they are specialized for particular roles, for example, differences in their activation voltage threshold, their various kinetic properties, and their voltage-dependence of inactivation. All these attributes play into the ability of the various voltage-gated calcium channels to participate in different patterns of presynaptic vesicular release. These include synaptic transmission resulting from single action potentials, and longer-term changes mediated by bursts or trains of action potentials, as well as release resulting from graded changes in membrane potential in specialized sensory synapses.

Key words: calcium channel; biophysical properties; molecular properties; auxiliary subunit; presynaptic terminal; synapse; voltage-gated; second messenger

Introduction

Voltage-gated calcium (Ca_V) channels are well understood to function as the route for Ca^{2+} entry into cells, particularly excitable cells, in response to depolarization. However, they represent a family of channels with a variety of biophysical properties that are exploited differentially to perform particular functions in presynaptic terminals. These varied roles will be explored in relation to different types of synaptic boutons. It is important to understand how the membrane potential of the presynaptic terminal, which is dictated in part by other channels present, as well as the intracellular free Ca^{2+}, affects the dynamics of the Ca_V channel activity. Their properties, in addition to the positional anchoring of the particular channels, dictate their ability to trigger and sustain vesicular release.

Molecular properties of Ca_V channels

Distinct voltage-dependent Ca^{2+} conductances were first characterized by electrophysiological and pharmacological means, involving both whole-cell and single-channel recording. A number of different currents were identified,[1–3] and termed L-type,[4] T-type, or low voltage-activated,[2,4] N-type,[4] P-type,[5] and R-type[6] (Table 1). Subsequent molecular cloning identified three subfamilies of mammalian Ca_V channels: Ca_V1 with four members (all of them giving rise to L-type currents), Ca_V2 with three members (forming P/Q-, N-, and R-type currents), and Ca_V3 with three members, all producing T-type currents (Table 1).

The pore-forming Ca_V α_1 subunits all have very similar structures with 24 transmembrane segments separated into four domains, each with a voltage-sensing and a pore module.[16,17] The domains are joined by intracellular loops, and a long

Submitted: 25 August 2020; **Revised:** 16 October 2020; **Accepted:** 20 October 2020

Table 1. Properties and Voltage-Dependent Activation of Ca_V Channels

	Gene	Name When Cloned	Systematic Protein Name	Physiological Name	$V_{50,\ activation}$ Using 1–4 mM Divalent Cation (except $Ca_V1.4$: 15–20 mM)	Physiological Function	Function in Synaptic Transmission
HVA	*CACNA1S*	α_1S	$Ca_V1.1$	L		Mechanical coupling with SR; skeletal muscle contraction	None known
	CACNA1C	α_1C	$Ca_V1.2$		−18 mV (mouse) in 2 mM Ca^{2+}.[7]	Cardiac/smooth muscle contraction; hormone secretion	Long term processes e.g. LTP in hippocampal mossy fibers[8]
	CACNA1D	α_1D	$Ca_V1.3$		−39 mV (rat) in 2mM Ca^{2+}.[7] −9.4 mV (human) in 2 mM Ca^{2+}.[9]	Secretion of hormones, sinoatrial node function	Auditory hair cell synaptic transmission
	CACNA1F	α_1F	$Ca_V1.4$		−4 mV (human, full-length) and −18 mV (Δ exon 47) in 20 mM Ba^{2+}.[10] +0.6 mV (human) in 15 mM Ca^{2+}.[11]	Retinal transmission in photoreceptors and bipolar neurons	
	CACNA1A	α_1A	$Ca_V2.1$	P/Q	−5.7 mV (rat) in 1 mM Ba^{2+}.[12] +4.9 mV (zebrafish) in 2 mM Ca^{2+}.[13]	Neuronal, mainly presynaptic	
	CACNA1B	α_1B	$Ca_V2.2$	N	−5.7 mV (rabbit) in 1 mM Ba^{2+}.[12] −1.3 mV (zebrafish) in 2 mM Ca^{2+}.[13] −13 mV (rat) in 2mM Ca^{2+}.[7]	Neuronal, mainly presynaptic	
	CACNA1E	α_1E	$Ca_V2.3$	R	−29 mV (rat) in 4 mM Ba^{2+}.[14]	Involved presynaptically, particularly in asynchronous release	
LVA	*CACNA1G*	α_1G	$Ca_V3.1$	T	−47 mV (rat) in 2mM Ca^{2+}.[7] −45.5 mV (rat) in 1.25 mM Ca^{2+}.[15]	Subthreshold and oscillatory behavior in neurons and other excitable cells	
	CACNA1H	α_1H	$Ca_V3.2$		−45.8 mV (human) in 1.25 mM Ca^{2+}.[15]		Present in some synapses
	CACNA1I	α_1I	$Ca_V3.3$		−43.8 mV (rat) in 1.25 mM Ca^{2+}.[15]		

The table describes the 10 mammalian Ca_V genes products and collects data on $V_{50,\ activation}$ from a number of original sources. It is necessary to refer to the papers for details of the auxiliary subunits, splice variants, and other conditions used, which can further affect the biophysical properties of the channels. LVA and HVA refer to the original nomenclature defining two types of calcium current (low- and high-voltage-activated).[2]

C-terminal tail. The Ca_V1 and Ca_V2 channel α_1 subunits are each associated with an auxiliary ß and $\alpha_2\delta$ subunit. There are four ß and four $\alpha_2\delta$ subunits, which have divergent cellular expression patterns, and confer some differing properties on the channels with which they associate (see below). The Ca_V2 channels, particularly $Ca_V2.1$ and $Ca_V2.2$ are the main channels involved in presynaptic function.

How the Biophysical Properties of Ca_V Channels Can Shape Their Function

Ca_V channels have a variety of characteristics that will be considered in this review, including voltage-dependent, kinetic and Ca^{2+}-dependent properties (Table 1, Figure 1). The complex interplay between these elements determines the amount and timing of Ca^{2+} entry that occurs during depolarization, for example, during an action potential.

Voltage-dependent activation dictates the range of voltages over which the channels will activate when depolarized, which, for presynaptic terminals, is key to their excitability. Although Ca_V channels were originally divided into low (Ca_V3) and high (Ca_V1 and Ca_V2) voltage-activated channels, it is clear that there is actually a continuum of activation ranges between these channels, when they are compared under more physiological conditions (Table 1). Such comparisons are nevertheless difficult to equate with physiological activation of these channels in neurons, and more specifically in presynaptic terminals. This is in part because the specific mix of channel splice isoforms[10,20] and the associated auxiliary subunits associated with each channel, which can strongly affect their biophysical properties (Table 1), are rarely known. It is also the case that studies of the biophysical properties of Ca_V channels necessarily use non-physiological conditions to isolate the calcium currents, together with a variety of divalent cation concentrations (Table 1), which affect membrane charge screening to differing extents, and therefore influence the voltage drop across the membrane experienced by the channels.

Since voltage-dependent inactivation also occurs for most Ca_V channels over a range of physiological voltages, which are for the most part more negative than their activation range, the resting potential will determine the proportion of channels available to open. This availability will be different for each channel type; furthermore, in the resting membrane potential range of most neurons, or during small subthreshold depolarizations, only T-type currents will have the ability to exhibit any significant Ca^{2+} entry, termed the window current (Figure 1A, B).

Some Ca_V channels exhibit full voltage-dependent inactivation (Figure 1A, B), whereas for others it is incomplete (Figure 1C), meaning that a small proportion of the channels remain available for extended periods at depolarized potentials. This is particularly relevant to the functioning of the slowly

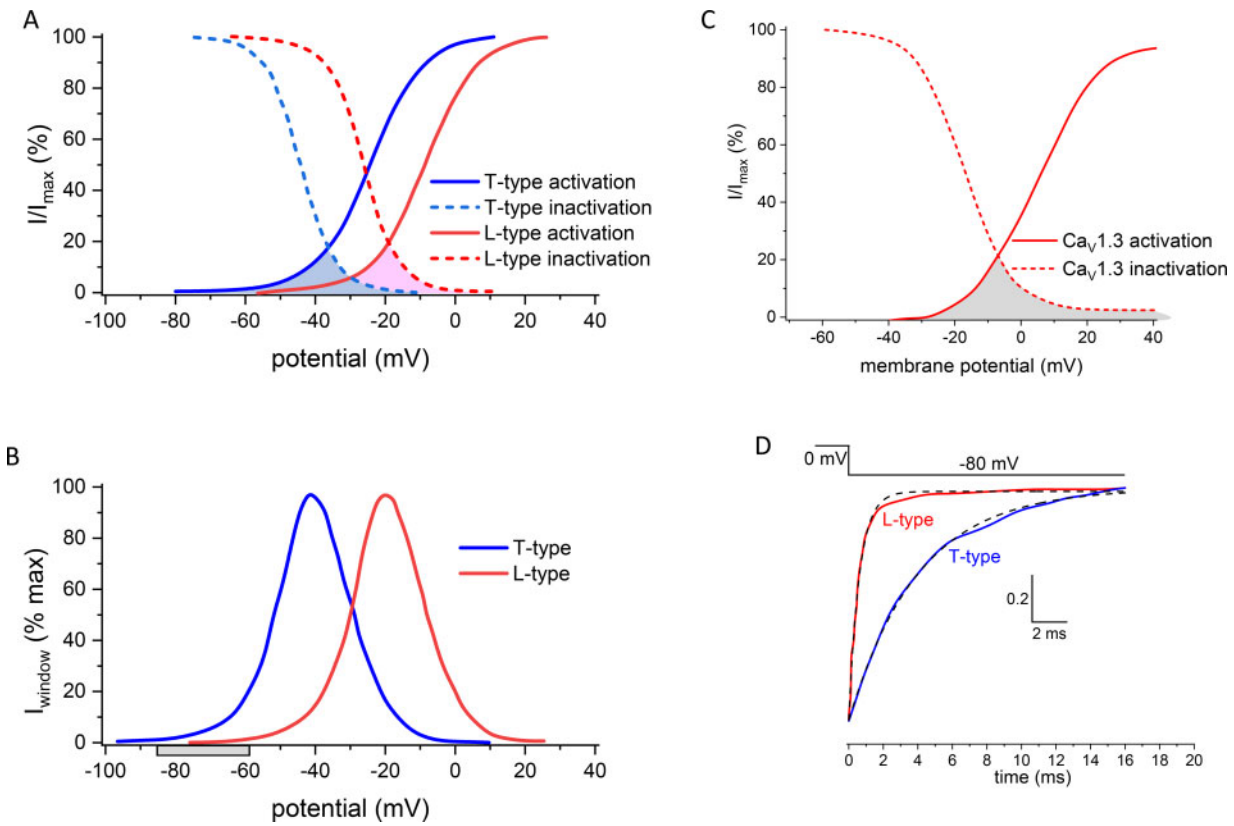

Figure 1. Idealized Voltage-Dependence of Activation and Inactivation for Selected Ca_V Channels. **(A, B)** Voltage-dependence of normalized activation (solid line) and inactivation (dotted line) of approximated T ($Ca_V3.1/2$, blue) and L-type ($Ca_V1.2$, red) currents, with window currents shaded in A, and replotted in B. Gray bar in B shows range of resting membrane potentials. Adapted from Fig 1b in Rossier.[18] **(C)** Data for $Ca_V1.3$ digitized and replotted from Fig 5a,[9] in which 15 mM Ca^{2+} was used as charge carrier, which shifts activation about +14 mV, compared to 2 mM Ca^{2+} (see Supplementary Table 3 in Azizan et al.[9]). **(D)** Normalized tail current data digitized and replotted from Fig. 2d in Carbone and Lux,[19] showing the relative inactivation rate of L-type and T-type Ca^{2+} currents recorded from embryonic chick sensory neurons on repolarization to −80 mV in 5 mM Ca^{2+}. The time constants of the tail currents, fitted by single exponentials (dotted lines) were ~4 ms (T-type) and ~0.6 ms (L-type).

inactivating L-type channels, $Ca_V1.3$ and $Ca_V1.4$, in specific presynaptic terminals in the inner ear and retina, respectively (see below). In addition to voltage-dependent inactivation, a second Ca^{2+}-dependent inactivation process is important for some channels, and this may be triggered by global Ca^{2+} levels or local Ca^{2+} entry.[21] The activation and inactivation of particular channels, as well as other properties, can be influenced by differential splicing,[10,22–24] by auxiliary subunit composition,[22,25,26] and by Ca^{2+}-binding protein interaction.[21,27–29] Although the inactivation processes may be too slow to affect Ca^{2+} entry during most presynaptic single action potentials, they can strongly influence Ca^{2+} entry over the course of action potential trains or bursts, and at specialized retinal and auditory synapses in which continuous Ca^{2+} entry occurs, which is modulated in a graded manner by membrane potential (see, for example, Ohn et al.[30]).

An important point that is infrequently considered is the deactivation rate of channels in response to repolarization of the membrane potential, since, together with activation rate, this can dictate the amount of Ca^{2+} entering a presynaptic terminal, as the extent of Ca^{2+} entry, particularly during a brief action potential, will be strongly affected by the rate of Ca_V channel closing. T-type channels have a slower deactivation rate, which is also voltage-dependent, being longer at more depolarized potentials,[19] whereas for Ca_V1 and Ca_V2 channels, the deactivation rate is much more rapid (Figure 1D). Another key feature is the driving force for Ca^{2+} entry, dictated both by the Ca^{2+} concentration gradient and the membrane potential of the terminal.

Skeletal muscle calcium channels ($Ca_V1.1$ or α_1S) are unusual in that they act primarily as voltage sensors via mechanical coupling to open ryanodine receptors on the sarcoplasmic reticulum, a direct process not involving Ca^{2+} entry.[31,32] Activation of the $Ca_V1.1$ ionic conductance is very slow, relative to movement of its voltage sensors,[32] and therefore Ca^{2+} entry is negligible during a single action potential. However, there is no clear evidence for significant functional expression of $Ca_V1.1$ in neuronal tissue or for any presynaptic function.

Multiple Roles of Ca_V Auxiliary Subunits

The ß and $\alpha_2\delta$ auxiliary subunits of calcium channels increase the transport of Ca_V channels to the plasma membrane, and this is particularly relevant to ß subunits, which prevent endoplasmic reticulum-associated proteasomal degradation of the Ca_V α_1 subunits.[33,34] Subsequently, there is an additional trafficking effect of $\alpha_2\delta$ subunits.[35] The auxiliary subunits also confer a variety of properties on Ca_V1 and Ca_V2 channels; for example, certain splice variants of ß2 (ß2a and ß2e) slow the inactivation of Ca_V1 and Ca_V2 channels and are themselves membrane-associated.[36–38] The $\alpha_2\delta$ subunits generally increase Ca_V channel activation and inactivation rates,[39,40] but also reduce long-closed states.[40] Our work has shown that proteolytic cleavage of the pro-form of $\alpha_2\delta$ into mature $\alpha_2\delta$ acts as a permissive molecular switch for the function of Ca_V1 and 2 channels.[41] It should also be noted that although $\alpha_2\delta$ proteins increase the trafficking of Ca_V channels, they may also be able to traffic to the plasma membrane and to presynaptic terminals alone[41] in the absence of calcium channels,[42] and can have additional roles on synapse morphology.[43–45]

Some Distinct Membrane Properties of Presynaptic Terminals

Presynaptic terminals generally have lower membrane excitability than axons, since voltage-gated Na^+ channels are often more sparse than at nodes of Ranvier.[46] In the presynaptic calyx of Held, Na^+ channels are absent from the calyx terminal region, but concentrated in the final unmyelinated segment of axon (heminode) leading up to the calyx.[47] The concentration of specific voltage-gated K^+ channels, particularly inactivating K^+ channels, controls presynaptic excitability,[46–49] such that presynaptic action potentials are generally either brief,[47] or attenuated.[46] Other channels that may be present presynaptically, such as hyperpolarization-activated HCN channels, also have the ability to affect resting membrane potential.[50] Although a recent study has highlighted that rapid Ca^{2+} entry can occur through tetrodotoxin-sensitive Na^+ channels, which are highly concentrated in the axon initial segment,[51] the sparsity of presynaptic Na^+ channels means it is unlikely that this route contributes significantly to presynaptic Ca^{2+} entry.

The presynaptic membrane potential has been directly measured in several types of accessible terminals. For example, in the calyx of Held excitatory terminal, it was about −80 mV, and in the same study the resting intracellular Ca^{2+} was estimated to be about 50 nM.[52] In hippocampal mossy fiber boutons, the resting membrane potential was between −60 and −85 mV,[48] and in inhibitory Purkinje cell terminals in culture, the membrane potential was −69 mV.[46] At these potentials even Ca_V3 channels, if present, would show little tonic activity (Figure 1A).

Implications of Different Presynaptic Ca_V Channel Compositions for Neurotransmitter Release

From the foregoing discussion, it is clear that the membrane potential of most presynaptic terminals is sufficiently negative that the vast majority of Ca_V2 channels are closed, rather than inactivated in the absence of ongoing activity. Thus, Ca_V2 channels are available to open upon action potential arrival. $Ca_V2.1$ channels generally activate at similar potentials to $Ca_V2.2$ in cell lines (Table 1), but activate more rapidly.[13] However in calyx of Held synapses, presynaptic N-type I_{Ca} was found to activate ~ 8 mV more depolarized than P/Q type current,[53] and this was also seen in chromaffin cells.[54] The third subtype of Ca_V2 channel ($Ca_V2.3$) also known as R-type has a somewhat more hyperpolarized membrane potential[14] (Table 1), potentially pointing to differences in function.

For most synapses, $Ca_V2.1$ (P/Q)- and $Ca_V2.2$ (N)-type channels are involved in varying proportions in synaptic transmission, depending on the synapse in question and the developmental stage. Broadly, $Ca_V2.1$ channels become of increasing importance in many synapses as they develop, such that they predominate in some mature neurons,[53,55] and are also more tightly associated with the release machinery[55] (see below). At some synapses, $Ca_V2.3$ channels, activated by smaller depolarizations, play an important role, rarely as the main channel involved in vesicular release, although this is the case in habenula cholinergic neuron terminals in the interpeduncular nucleus.[56] More often $Ca_V2.3$ has been found to underlie other processes such as delayed or asynchronous release, for example from small hippocampal boutons,[57] and it also plays a role in long-term potentiation.[58]

A key factor to consider is action potential duration, relative to the rate of deactivation of the calcium channels, as much of the Ca^{2+} entry mediating synchronous release will occur on the repolarization phase of each brief action potential-mediated presynaptic depolarization, which has the effect of increasing the driving force for Ca^{2+}. In contrast, asynchronous release is the term for release resulting from stochastic opening of individual channels near the membrane potential, often after a burst of action potentials,[57,59] resulting in long-duration presynaptic Ca^{2+} transients. Although it has been suggested that spontaneous openings of $Ca_V2.3$ channels may be in part responsible for asynchronous release occurring after action potentials at some synapses,[57] $Ca_V2.1$ and $Ca_V2.2$ channels, particularly when associated with the ß2a subunit which reduces their inactivation, may also play a role.[59] For example, at synapses formed by different subtypes of hippocampal GABA-ergic interneuron, $Ca_V2.1$ is involved in the mainly synchronous release from fast-spiking parvalbumin interneurons, whereas $Ca_V2.2$ channels predominantly mediate GABA release from cholecystokinin-containing interneurons, of which a much greater fraction is asynchronous release.[60]

At some specialized sensory synapses, L-type channels, particularly $Ca_V1.3$ and $Ca_V1.4$, are critical for function. These mainly concern the auditory inner hair cells ($Ca_V1.3$)[61,62] and retinal photoreceptors and bipolar neurons ($Ca_V1.4$),[11,63,64] in which the presynaptic responses are graded. These particular Ca_V1 channels have properties suited to this function, in that they remain available at depolarized potentials (Figure 1C).

Concerted Calcium Channel Involvement in Release from Individual Synapses

As described above, both $Ca_V2.1$ and $Ca_V2.2$ calcium channels are involved, to varying extents, in vesicular release at most individual central nervous system terminals, as judged by ω-agatoxin IVA and ω-conotoxin GVIA inhibition, respectively.[65–67] However, the relative amount of block by each toxin cannot be used directly to determine the prevalence of these channels, because of the nonlinear, approximately fourth power, relationship between intracellular Ca^{2+} levels and neurotransmitter release.[68–72] There are several related forms of Ca^{2+} cooperativity that have been described, that between multiple Ca_V channels required to release a single vesicle[73] and the number of Ca^{2+} ions that must bind cooperatively to Ca^{2+} sensors, and the cooperative action of those sensors, to trigger release of a vesicle.[74]

Thus, there is generally found to be synergy between the opening of multiple channels to reach the μM levels of Ca^{2+} at the Ca^{2+} sensors whose occupancy mediates release of each vesicle in an active zone. The numbers of channels involved have been estimated to be very small in some synapses[67,75–77], to over 60 in immature calyx of Held synapses.[78] In a few cases a single channel has been found to be sufficient,[75,76] although the probability of release will be low.[77] The number of channels present in each active zone is much greater than those that open in response to each action potential, because of the low probability of opening of each channel and the stochastic nature of channel openings, meaning they occur with a variable delay following a depolarizing stimulus, which can also lead to failure of exocytosis.

Anchoring of Calcium Channels in Presynaptic Active Zones is Key to Their Differing Roles in Synaptic Transmission

The proximity of the presynaptic Ca_V channels to the vesicular release site is an extremely important factor in determining the properties and speed of neurotransmitter release. In order to study this, knowledge of the relative locations of the channel subtypes, as well as modeling studies are required, in addition to an understanding of the biophysical and biochemical distinctions between $Ca_V2.1$ and $Ca_V2.2$ channels.[77,79] There are well-studied differences in the anchoring of the two main Ca_V2 channels in presynaptic active zones. Both $Ca_V2.1$ and $Ca_V2.2$ channels are tethered in active zones by the RAB3A-interacting molecule (RIM),[80] and $Ca_V2.3$ channels may also associate with RIM proteins.[80] Furthermore, RIM-binding protein interacts with $Ca_V2.1$, $Ca_V2.2$ and $Ca_V1.2$ channels, but recruits only the former two channels via interaction with RIM specifically to the active zone.[80] However, $Ca_V2.1$ is selectively associated with certain Munc13 isoforms potentially leading it to be localized closer to docked vesicles than $Ca_V2.2$[55] (Figure 2). In contrast to the obvious central phenotype of $Ca_V2.1$ knockout mice,[82] the lack of marked phenotype in $Ca_V2.2$ knockout mice suggests that their role is less crucial, and other types of Ca_V channel (particularly $Ca_V2.1$) are able to compensate for the loss of $Ca_V2.2$ at most synapses. However, $Ca_V2.2$ channels have a predominant role at primary afferent synapses in the pain pathway,[83,84] and this pathway is indeed disrupted in $Ca_V2.2$ knockout mice.[85]

Thus, both the properties and distribution of $Ca_V2.1$ channels result in greater activation and Ca^{2+} entry for a brief action potential through these channels than for $Ca_V2.2$.[13] This has been observed, for example, in mossy fiber boutons, where a single terminal was estimated to contain about 2000 channels, and brief presynaptic action potentials activated a presynaptic Ca^{2+} current that was found with pharmacological blockers to be dependent on P/Q (~66%), N (~26%), and R (~8%)-type channels.[86]

Other proteins have also been found to interact with Ca_V2 channels,[87] and some of these proteins affect the properties of the channels, such as the CRMP-2 interaction with $Ca_V2.2$.[88] Another presynaptic protein, Syntaxin 1A has been found to interact with part of the II-III linker of $Ca_V2.2$ channels (synprint site), increasing both slow inactivation and steady-state inactivation, and thus reducing channel availability.[89,90] By contrast, an analogous effect on $Ca_V2.1$ channels may depend on channel splice variant.[91] In presynaptic terminals, this could affect the relative availability of $Ca_V2.1$ and $Ca_V2.2$ channels. However, this synprint site is not essential for presynaptic targeting[92] or neurotransmission.[93]

Ca_V2 Channel Modulation Dramatically Affects Their Presynaptic Function

Since Ca_V2 channels are subject to inhibition by several second messenger pathways, this will affect their availability. Thus, the integral of Ca^{2+} entry at any synapse depends on a multitude of factors that are unique to each condition and to the pattern of action potentials arriving at the terminal. In particular, G-protein-mediated inhibition is an important property of Ca_V2 channels. This can result from stimulation of many presynaptic G-protein coupled receptors linked to $G_{i/o}$, such as GABA-B

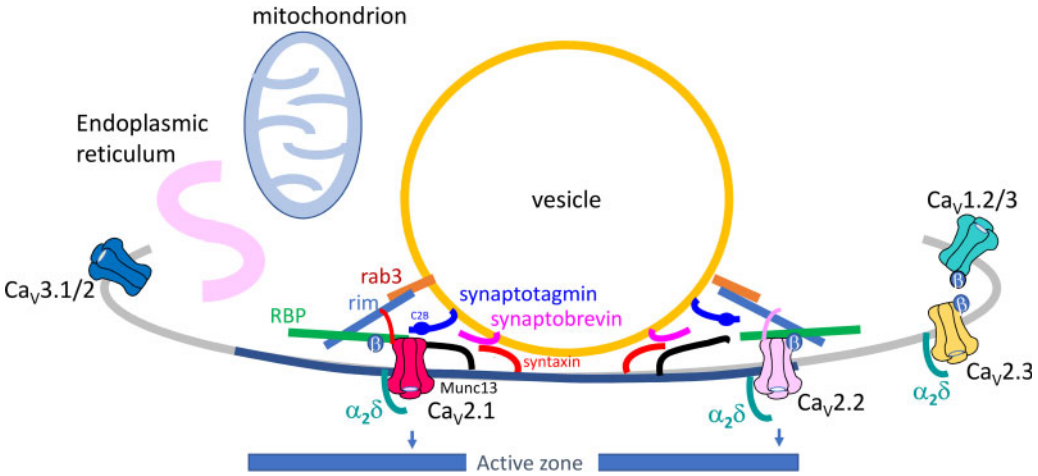

Figure 2. Diagram of Ca_V Channels in Relation to Other Pre-Synaptic Proteins and Organelles. Some of the proteins involved in anchoring Ca_V channels near to synaptic vesicles forming a nanodomain within the presynaptic active zone (dark blue membrane). These include Rab3 (orange), synaptotagmin (purple), and synaptobrevin (pink) associated with the vesicular membrane. Rim (blue) and RBP (green) are cytosolic; Munc13 (black) and syntaxin (orange) are associated with the plasma membrane. $Ca_V2.1$ (red) and $Ca_V2.2$ (light pink) are likely to be differentially localized within active zones, whereas the other Ca_V channels, if present, are thought to be located elsewhere in the presynaptic membrane. Figure based on Fig. 4a in Dolphin and Lee.[81]

receptors, opioid receptors, and others whose activation leads to the release of Gßγ subunits.[94–99] This inhibition, which may have a tonic component, shifts the voltage-dependence of Ca_V2 channel activation to more positive potentials, and slows activation kinetics,[100,101] which can be overcome by prior depolarization, including in some cases an action potential train.[102] This macroscopic current slowing is mediated at the single-channel level by a prolongation of the latency to first opening both of native N-type single-channel currents[103] and of cloned $Ca_V2.2$ channels,[104,105] with no change in single-channel conductance.

Gßγ binding mediates the inhibition, and voltage-dependent Gßγ unbinding underlies the slow activation of the Ca_V2 channels, and triggers the depolarization-mediated reversal of inhibition.[96,98] Here it should be noted that $Ca_V2.1$ channels are less subject to G-protein modulation than $Ca_V2.2$, since the Gßγ off-rate from these channels is more rapid.[98]

Given that, as described above, only a few Ca_V channels may open in response to a single action potential at individual synapses, and Gßγ-mediated inhibition involves slowing of their activation, the effect on synaptic transmission has the potential to be profound, particularly where a high proportion of $Ca_V2.2$ channels is present, such as primary afferent terminals.[6]

T-type Channels Are Partially Inactivated at Resting Membrane Potentials

T-type channels are present in certain presynaptic terminals, and they may play an important role in influencing resting Ca^{2+} levels, or in providing Ca^{2+} for downstream events. Although Ca_V3 channels do not normally supply significant amounts of Ca^{2+} for neurotransmitter release resulting from action potentials arriving at the terminal, nevertheless their availability can be affected by the interplay of other channels such as HCN channels and Ca^{2+}-activated K^+ channels, which affect membrane potential.[50] Functional HCN1 channels are present on particular glutamatergic synaptic terminals, for example onto entorhinal cortical layer III pyramidal neurons, where they depolarize the membrane potential and reduce neurotransmitter release. These effects at least partly result from reduced availability of $Ca_V3.2$ channels.[50] Furthermore, Ca_V3 channels were also found to play an important part in asynchronous dendrodendritic release of glutamate from olfactory bulb mitral cells.[107] In another study GABA release from interneurons could be promoted by activation of presynaptic nicotinic receptors and subsequent activation of presynaptic $Ca_V3.1$ channels, together with release of Ca^{2+} from ryanodine-sensitive intracellular stores.[108] Thus, there is evidence from numerous studies for a variety of presynaptic roles for T-type channels.

A Role for Ca^{2+}-induced Ca^{2+} Release in Presynaptic Terminals

Although Ca^{2+}-induced Ca^{2+} release (CICR) is mainly associated with $Ca_V1.2$ channel function, for example in cardiac muscle cells, nevertheless smooth endoplasmic reticulum is present in presynaptic terminals,[109] and there is evidence that CICR occurs from this endoplasmic reticulum which can affect neurotransmitter release.[110,111] The channels involved in presynaptic CICR are mainly ryanodine receptors,[112,113] and the initial source of Ca^{2+} for CICR could be Ca_V channels, particularly T-type or R-type, which are activated by small depolarizations,[113] or other presynaptic Ca^{2+}-permeable channels such as α7 nicotinic receptors.[108,114] It was further suggested that clustering of the endoplasmic reticulum sensor of Ca^{2+} depletion, STIM1, may directly inhibit Ca_V channels.[110] The importance of CICR in neurotransmitter release is more evident following prolonged activation rather than single action potential-induced responses,[111,113] although single action potentials can also result in CICR.[111,115]

The Roles of Mitochondria in Controlling Intracellular Ca^{2+} in Presynaptic Terminals

Mitochondria are present in about half of all presynaptic terminals,[116] and they can sequester presynaptic Ca^{2+} entry resulting from trains of action potentials.[117,118] Presynaptic mitochondria are found to have a low threshold for Ca^{2+} uptake, relative to

those in other tissues, which is conferred by a brain-specific protein MICU3, allowing mitochondria to take up Ca^{2+} directly from the cytoplasm near to sites of Ca^{2+} entry through the plasma membrane.[119] Indeed, mitochondria have been visualized to be tethered to presynaptic terminal membranes in the calyx of Held.[120] Furthermore, Ca^{2+} is required for optimal ATP levels, and presynaptic mitochondria promote synaptic transmission in active synapses by supplying the essential ATP. Maintenance of the voltage and ionic gradients related to presynaptic function is also a major consumer of ATP,[119] and thus mitochondria fulfill multiple presynaptic roles.

Conclusions

The molecular and biophysical properties of Ca_V channels are finely tuned to their roles in presynaptic terminals to mediate neurotransmitter release. Although there are many types and geometries of synapse, the channels in these terminals function in broadly similar ways to mediate Ca^{2+} entry that triggers vesicular release. Since the opening of a few channels, or even a single channel, is able to mediate release at discrete small excitatory and inhibitory synapses, it is extremely important to understand the individual and distinct properties of these channels, in order to appreciate how this process of release is constrained by the localization, tethering, properties, and modulation of the channels. Similarly, the different mix of types of channels present, and their relative active zone distribution, is tuned to the functions of individual synapses and to changes during development and synaptic activity.

Acknowledgments

The work of the author was supported by a Wellcome Trust Investigator award 206279/Z/17/Z. The author apologizes for not being able to cite all relevant original papers in this short review, and acknowledges the commendable journal rules requiring citation of primary sources, rather than reviews.

Conflict of Interest Statement

The author declares no conflict of interest.

References

1. Reuter H, Beeler, GW, Jr. Calcium current and activation of contraction in ventricular myocardial fibers. *Science* 1969; 163:399–401.
2. Carbone E, Lux HD. A low voltage-activated fully inactivating Ca channel in vertebrate sensory neurones. *Nature* 1984; 310:501–502.
3. Hess P, Lansman JB, Tsien RW. Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca agonists and antagonists. *Nature* 1984;311:538–544.
4. Nowycky MC, Fox AP, Tsien RW. Three types of neuronal calcium channel with different calcium agonist sensitivity. *Nature* 1985;316:440–446.
5. Mintz IM, Venema VJ, Swiderek KM. P-type calcium channels blocked by the spider toxin **w**-Aga-IVA. *Nature* 1992;355: 827–829.
6. Randall A, Tsien RW. Pharmacological dissection of multiple types of Ca^{2+} channel currents in rat cerebellar granule neurons. *J Neurosci* 1995;15:2995–3012.
7. Helton TD, Xu W, Lipscombe D. Neuronal L-type calcium channels open quickly and are inhibited slowly. *J Neurosci* 2005;25:10247–10251.
8. Lauri SE, Bortolotto ZA, Nistico R, et al. A role for Ca2+ stores in kainate receptor-dependent synaptic facilitation and LTP at mossy fiber synapses in the hippocampus. *Neuron* 2003; 39:327–341.
9. Azizan EA, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet* 2013;45:1055–1060.
10. Haeseleer F, Williams B, Lee A. Characterization of C-terminal splice variants of Cav1.4 Ca2+ channels in human retina. *J Biol Chem* 2016;291:15663–15673.
11. Koschak A, Reimer D, Walter D, et al. Cav1.4alpha1 subunits can form slowly inactivating dihydropyridine-sensitive L-type Ca2+ channels lacking Ca2+-dependent inactivation. *J Neurosci* 2003;23:6041–6049.
12. Meyer JO, Dahimene S, Page KM, et al. Disruption of the key Ca^{2+} binding site in the selectivity filter of neuronal voltage-gated calcium channels inhibits channel trafficking. *Cell Rep* 2019;29:22–33.
13. Naranjo D, Wen H, Brehm P. Zebrafish CaV2.1 calcium channels are tailored for fast synchronous neuromuscular transmission. *Biophys J* 2015;108:578–584.
14. Soong TW, Stea A, Hodson CD, et al. Structure and functional expression of a member of the low voltage-activated calcium channel family. *Science* 1993;260:1133–1136.
15. Klockner U, Lee JH, Cribbs LL, et al. Comparison of the Ca2+ currents induced by expression of three cloned alpha 1 subunits, alpha 1G, alpha 1H and alpha 1I, of low-voltage-activated T-type Ca2+ channels. *Eur J Neurosci* 1999;11:4171–4178.
16. Wu J, Yan Z, Li Z. Structure of the voltage-gated calcium channel Cav1.1 at 3.6 A resolution. *Nature* 2016;537:191–196.
17. Tanabe T, Takeshima H, Mikami A. Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature* 1987;328:313–318.
18. Rossier MF. T-Type Calcium channel: A privileged gate for calcium entry and control of adrenal steroidogenesis. *Front Endocrinol (Lausanne)* 2016;7:43.
19. Carbone E, Lux HD. A low voltage-activated calcium conductance in embryonic chick sensory neurons. *Biophys J* 1984;46: 413–418.
20. Gray AC, Raingo J, Lipscombe D. Neuronal calcium channels: splicing for optimal performance. *Cell Calcium* 2007;42: 409–417.
21. DeMaria CD, Soong TW, Alseikhan BA. Calmodulin bifurcates the local Ca2+ signal that modulates P/Q- type Ca2+ channels. *Nature* 2001;411:484–489.
22. Lin Y, McDonough SI, Lipscombe D. Alternative splicing in the voltage-sensing region of N-Type CaV2.2 channels modulates channel kinetics. *J Neurophysiol* 2004;92:2820–2830.
23. Singh A, Hamedinger D, Hoda JC. C-terminal modulator controls Ca2+-dependent gating of Ca(v)1.4 L-type Ca2+ channels. *Nat Neurosci* 2006;9:1108–1116.
24. Bell TJ, Thaler C, Castiglioni AJ. Cell-specific alternative splicing increases calcium channel current density in the pain pathway. *Neuron* 2004;41:127–138.
25. Qin N, Platano D, Olcese R. Unique regulatory properties of the type 2a Ca^{2+} channel b subunit caused by palmitoylation. *Proc Natl Acad Sci U S A* 1998;95:4690–4695.

26. Canti C, Nieto-Rostro M, Foucault I. The metal-ion-dependent adhesion site in the Von Willebrand factor-A domain of alpha2delta subunits is key to trafficking voltage-gated Ca2+ channels. *Proc Natl Acad Sci U S A* 2005;102: 11230–11235.
27. Cui G, Meyer AC, Calin-Jageman I. Ca2+-binding proteins tune Ca2+-feedback to Cav1.3 channels in mouse auditory hair cells. *J Physiol* 2007;585:791–803.
28. Haeseleer F, Imanishi Y, Maeda T. Essential role of Ca2+-binding protein 4, a Cav1.4 channel regulator, in photoreceptor synaptic function. *Nat Neurosci* 2004;7:1079–1087.
29. Schrauwen I, Helfmann S, Inagaki A.. A mutation in CABP2, expressed in cochlear hair cells, causes autosomal-recessive hearing impairment. *Am J Hum Genet* 2012;91:636–645.
30. Ohn TL, Rutherford MA, Jing Z. Hair cells use active zones with different voltage dependence of Ca2+ influx to decompose sounds into complementary neural codes. *Proc Natl Acad Sci U S A* 2016;113:E4716–4725.
31. Rios E, Brum G. Involvement of dihydropyridine receptors in excitation-contraction coupling in skeletal muscle. *Nature* 1987;325:717–720.
32. Tanabe T, Beam KG, Powell JA, et al. Restoration of excitation-contraction coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA. *Nature* 1988;336:134–139.
33. Altier C, Garcia-Caballero A, Simms B, et al. The Cavβ subunit prevents RFP2-mediated ubiquitination and proteasomal degradation of L-type channels. *Nature Neurosci* 2010;14: 173–180.
34. Waithe D, Ferron L, Page KM, et al. b-Subunits promote the expression Of Cav2.2 channels by reducing their proteasomal degradation. *J Biol Chem* 2011;286:9598–9611.
35. Cassidy JS, Ferron L, Kadurin I, et al. Functional exofacially tagged N-type calcium channels elucidate the interaction with auxiliary alpha2delta-1 subunits. *Proc Natl Acad Sci U S A* 2014;111:8979–8984.
36. Takahashi SX, Mittman S, Colecraft HM. Distinctive modulatory effects of five human auxiliary b2 subunit splice variants on L type calcium channel gating. *Biophys J* 2003;84: 3007–3021.
37. Chien AJ, Gao TY, Perez-Reyes E. Membrane targeting of L-type calcium channels - Role of palmitoylation in the subcellular localization of the b_{2a} subunit. *J Biol Chem* 1998;273: 23590–23597.
38. Miranda-Laferte E, Ewers D, Guzman RE, et al. The N-terminal domain tethers the voltage-gated calcium channel beta2e-subunit to the plasma membrane via electrostatic and hydrophobic interactions. *J Biol Chem* 2014;289: 10387–10398.
39. Felix R, Gurnett CA, De Waard M, et al. Dissection of functional domains of the voltage-dependent Ca2+ channel alpha2delta subunit. *J Neurosci* 1997;17:6884–6891.
40. Wakamori M, Mikala G, Mori Y. Auxiliary subunits operate as a molecular switch in determining gating behaviour of the unitary N-type Ca2+ channel current in Xenopus oocytes. *J Physiol-Lond* 1999;517:659–672.
41. Kadurin I, Ferron L, Rothwell SW, et al. Proteolytic maturation of $\alpha 2\delta$ represents a checkpoint for activation and neuronal trafficking of latent calcium channels *ELife* 2016;5: e21143.
42. Held RG, Liu C, Ma K, et al. Synapse and active zone assembly in the absence of presynaptic Ca(2+) channels and Ca(2+) entry. *Neuron* 2020;107:667–683 e669.
43. Eroglu C, et al. Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell* 2009;139:380–392.
44. Pirone A, Kurt S, Zuccotti A, et al. alpha2delta3 is essential for normal structure and function of auditory nerve synapses and is a novel candidate for auditory processing disorders. *J Neurosci* 2014;34:434–445.
45. Geisler S, Schopf CL, Stanika R, et al. Presynaptic alpha2delta-2 calcium channel subunits regulate postsynaptic GABAA receptor abundance and axonal wiring. *J Neurosci* 2019;39:2581–2605.
46. Kawaguchi SY, Sakaba T. Control of inhibitory synaptic outputs by low excitability of axon terminals revealed by direct recording. *Neuron* 2015;85:1273–1288.
47. Leao RM, Kushmerick C, Pinaud R et al. Presynaptic Na+ channels: locus, development, and recovery from inactivation at a high-fidelity synapse. *J Neurosci* 2005;25:3724–3738.
48. Geiger JR, Jonas P. Dynamic control of presynaptic Ca(2+) inflow by fast-inactivating K(+) channels in hippocampal mossy fiber boutons. *Neuron* 2000;28:927–939.
49. Dodson PD, Billups B, Rusznak Z, et al. Presynaptic rat Kv1.2 channels suppress synaptic terminal hyperexcitability following action potential invasion. *J Physiol* 2003;550:27–33.
50. Huang Z, Lujan R, Kadurin I, et al. Presynaptic HCN1 channels regulate Ca(V)3.2 activity and neurotransmission at select cortical synapses. *Nat Neurosci* 2011;14:478–486.
51. Hanemaaijer NA, Popovic MA, Wilders X, et al. Ca(2+) entry through NaV channels generates submillisecond axonal Ca(2+) signaling. *Elife* 2020;9.
52. Helmchen F, Borst JG, Sakmann B. Calcium dynamics associated with a single action potential in a CNS presynaptic terminal. *Biophys J* 1997;72:1458–1471.
53. Inchauspe CG, Martini FJ, Forsythe ID, et al. Functional compensation of P/Q by N-type channels blocks short-term plasticity at the calyx of held presynaptic terminal. *J Neurosci* 2004;24:10379–10383.
54. Currie KPM, Fox AP. Comparison of N and P/Q type voltage-gated calcium channel current inhibition. *J Neurosci* 1997;17: 4570–4579.
55. Kusch V, Bornschein G, Loreth D, et al. Munc13-3 Is required for the developmental localization of Ca(2+) channels to active zones and the nanopositioning of Cav2.1 near release sensors. *Cell Rep* 2018;22:1965–1973.
56. Zhang J, et al. Presynaptic excitation via GABAB receptors in habenula cholinergic neurons regulates fear memory expression. *Cell* 2016;166:716–728.
57. Ermolyuk YS, Alder FG, Surges R, et al. Differential triggering of spontaneous glutamate release by P/Q-, N- and R-type Ca2+ channels. *Nat Neurosci* 2013;16:1754–1763.
58. Dietrich D, Kirschstein T, Kukley M, et al. Functional specialization of presynaptic Cav2.3 Ca2+ channels. *Neuron* 2003; 39:483–496.
59. Few AP, Nanou E, Watari H, et al. Asynchronous Ca2+ current conducted by voltage-gated Ca2+ (CaV)-2.1 and CaV2.2 channels and its implications for asynchronous neurotransmitter release. *Proc Natl Acad Sci U S A* 2012; 109:E452–460.
60. Hefft S, Jonas P. Asynchronous GABA release generates long-lasting inhibition at a hippocampal interneuron-principal neuron synapse. *Nat Neurosci* 2005;8:1319–1328.
61. Platzer J, Engel J, Schrott-Fischer A, et al. Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type Ca2+ channels. *Cell* 2000;102, 89–97.

62. Baig SM, et al. Loss of Ca(v)1.3 (CACNA1D) function in a human channelopathy with bradycardia and congenital deafness. *Nat Neurosci* 2011;14:77–84.
63. Lee A, Wang S, Williams B, et al. Characterization of Cav1.4 complexes (alpha11.4, beta2, and alpha2delta4) in HEK293T cells and in the retina. *J Biol Chem* 2015;290, 1505–1521.
64. McRory JE, Hamid J, Doering CJ,. et al. The CACNA1F gene encodes an L-type calcium channel with unique biophysical properties and tissue distribution. *J Neurosci* 2004;24: 1707–1718.
65. Wheeler DB, Randall A, Tsien RW. Roles of N-type and Q-type Ca^{2+} channels in supporting hippocampal synaptic transmission. *Science* 1994;264:107–111.
66. Wu L-G, Saggau P. Pharmacological identification of two types of presynaptic voltage-dependent calcium channels at CA3-CA1 synapses of the hippocampus. *J Neurosci* 1994;14: 5613–5622.
67. Scimemi A, Diamond JS. The number and organization of Ca2+ channels in the active zone shapes neurotransmitter release from Schaffer collateral synapses. *J Neurosci* 2012;32: 18157–18176.
68. Llinas R, Steinberg IZ, Walton K. Presynaptic calcium currents and their relation to synaptic transmission: voltage clamp study in squid giant synapse and theoretical model for the calcium gate. *Proc Natl Acad Sci U S A* 1976;73: 2918–2922.
69. Ariel P, Ryan TA. Optical mapping of release properties in synapses. *Front Neural Circuits* 2010;4.
70. Borst JGG, Sakmann B. Calcium current during a single action potential in a large presynaptic terminal of the rat brainstem. *J Physiol (Lond)* 1998;506:143–157.
71. Dodge FA, Jr., Rahamimoff R. Co-operative action a calcium ions in transmitter release at the neuromuscular junction. *J Physiol* 1967;193:419–432.
72. Schneggenburger R, Neher E. Intracellular calcium dependence of transmitter release rates at a fast central synapse. *Nature* 2000;406:889–893.
73. Takahashi T, Momiyama A. Different types of calcium channels mediate central synaptic transmission. *Nature* 1993;366: 156–158.
74. Reid CA, Bekkers JM, Clements JD. N- and P/Q-type Ca^{2+} channels mediate transmitter release with a similar cooperativity at rat hippocampal autapses. *J Neurosci* 1998;18: 2849–2855.
75. Bucurenciu I, Bischofberger J, Jonas P. A small number of open Ca2+ channels trigger transmitter release at a central GABAergic synapse. *Nat Neurosci* 2010;13:19–21.
76. Stanley EF. Single calcium channels and acetylcholine release at a presynaptic nerve terminal. *Neuron* 1993;11: 1007–1011.
77. Nakamura Y, Harada H, Kamasawa N. et al. Nanoscale distribution of presynaptic Ca(2+) channels and its impact on vesicular release during development. *Neuron* 2015;85:145–158.
78. Borst JG, Sakmann, B. Calcium influx and transmitter release in a fast CNS synapse. *Nature* 1996;383:431–434.
79. Rebola N, Reva M, Kirizs T, et al. Distinct nanoscale calcium channel and synaptic vesicle topographies contribute to the diversity of synaptic function. *Neuron* 2019;104:693–710 e699.
80. Kaeser PS, Deng L, Wang Y, et al. RIM proteins tether Ca(2+) channels to presynaptic active zones via a direct PDZ-domain interaction. *Cell* 2011;144:282–295.
81. Dolphin AC, Lee A. Presynaptic calcium channels: specialized control of synaptic neurotransmitter release. *Nat Rev Neurosci* 2020.
82. Jun K, Piedras-Renteria ES, Smith SM, et al. Ablation of P/Q-type Ca2+ channel currents, altered synaptic transmission, and progressive ataxia in mice lacking the alpha(1A)-subunit. *Proc Natl Acad Sci U S A* 1999;96:15245–15250.
83. Heinke B, Balzer E, Sandkuhler J. Pre- and postsynaptic contributions of voltage-dependent Ca2+ channels to nociceptive transmission in rat spinal lamina I neurons. *Eur J Neurosci* 2004;19:103–111.
84. Nieto-Rostro M, Ramgoolam K, Pratt WS, et al. Ablation of alpha2delta-1 inhibits cell-surface trafficking of endogenous N-type calcium channels in the pain pathway in vivo. *Proc Natl Acad Sci U S A* 2018;115:E12043–E12052.
85. Saegusa H, Kurihara T, Zong S, et al. Suppression of inflammatory and neuropathic pain symptoms in mice lacking the N-type Ca^{2+} channel. *EMBO J* 2001;20: 2349–2356.
86. Li L, Bischofberger J, Jonas P. Differential gating and recruitment of P/Q-, N-, and R-type Ca2+ channels in hippocampal mossy fiber boutons. *J Neurosci* 2007;27: 13420–13429.
87. Muller CS, Haupt A, Bildl W, et al. Quantitative proteomics of the Cav2 channel nano-environments in the mammalian brain. *Proc Natl Acad Sci U S A* 2010;107:14950–14957.
88. Brittain JM, et al. Suppression of inflammatory and neuropathic pain by uncoupling CRMP-2 from the presynaptic Ca(2+) channel complex. *Nat Med* 2011;17:822–829.
89. Bezprozvanny I, Scheller RH, Tsien RW. Functional impact of syntaxin on gating of N-type and Q- type calcium channels. *Nature* 1995;378:623–626.
90. Yokoyama CT, Myers SJ, Fu J, et al. Mechanism of SNARE protein binding and regulation of Cav2 channels by phosphorylation of the synaptic protein interaction site. *Mol Cell Neurosci* 2005;28:1–17.
91. Rettig J, Sheng ZH, Kim DK, et al. Isoform-specific interaction of the a_{1A} subunits of brain Ca^{2+} channels with the presynaptic proteins syntaxin and SNAP-25. *Proc Natl Acad Sci U S A* 1996;93:7363–7368.
92. Szabo Z, Obermair GJ, Cooper CB, et al. Role of the synprint site in presynaptic targeting of the calcium channel CaV2.2 in hippocampal neurons. *Eur J Neurosci* 2006;24:709–718.
93. Spafford JD, Munno DW, Van NP, et al. Calcium channel structural determinants of synaptic transmission between identified invertebrate neurons. *J Biol Chem* 2003;278: 4258–4267.
94. Holz GGI, Rane SG, Dunlap K. GTP-binding proteins mediate transmitter inhibition of voltage- dependent calcium channels. *Nature* 1986;319:670–672.
95. Scott RH, Dolphin AC. Regulation of calcium currents by a GTP analogue: potentiation of (-)-baclofen-mediated inhibition. *Neurosci Lett* 1986;69:59–64.
96. Herlitze S, Garcia DE, Mackie K, et al. Modulation of Ca^{2+} channels by G-protein bgamma subunits. *Nature* 1996;380: 258–262.
97. Page KM, Canti C, Stephens GJ, et al. Identification of the amino terminus of neuronal Ca^{2+} channel a1 subunits a1B and a1E as an essential determinant of G protein modulation. *J Neurosci* 1998;18:4815–4824.
98. Agler HL, Evans J, Colecraft HM, et al. Custom distinctions in the interaction of G-protein beta subunits with N-type (CaV2.2) versus P/Q-type (CaV2.1) calcium channels. *J Gen Physiol* 2003;121:495–510.
99. Takahashi T, Kajikawa Y, Tsujimoto TG. Protein-coupled modulation of presynaptic calcium currents and transmitter release by a GABAB receptor. *J Neurosci* 1998;18:3138–3146.

100. Bean BP. Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage-dependence. *Nature* 1989;340:153–155.
101. Dolphin AC, Wootton JF, Scott RH, et al. Photoactivation of intracellular guanosine triphosphate analogues reduces the amplitude and slows the kinetics of voltage-activated calcium channel currents in sensory neurones. *Pflügers Archiv* 1988;411:628–636.
102. Currie KPM, Fox AP. Differential facilitation of N- and P/Q-type calcium channels during trains of action potential-like waveforms. *J Physiol* 2002;539:419–431.
103. Carabelli V, Lovallo M, Magnelli V, et al. Voltage-dependent modulation of single N-type Ca^{2+} channel kinetics by receptor agonists in IMR32 cells. *Biophys J* 1996;70:2144–2154.
104. Patil PG, De Leon M, Reed RR, et al. Elementary events underlying voltage-dependent G-protein inhibition of N-type calcium channels. *Biophys J* 1996;71:2509–2521.
105. Meir A, Bell DC, Stephens GJ, et al. Calcium channel b subunit promotes voltage-dependent modulation of a1B by Gbg. *Biophys J* 2000;79:731–746.
106. Heinke B, Gingl E, Sandkuhler, J. Multiple targets of mu-opioid receptor-mediated presynaptic inhibition at primary afferent Adelta- and C-fibers. *J Neurosci* 2011;31:1313–1322.
107. Fekete A, Johnston J, Delaney KR. Presynaptic T-type Ca2+ channels modulate dendrodendritic mitral-mitral and mitral-periglomerular connections in mouse olfactory bulb. *J Neurosci* 2014;34:14032–14045.
108. Tang AH, Karson MA, Nagode DA, et al. Nerve terminal nicotinic acetylcholine receptors initiate quantal GABA release from perisomatic interneurons by activating axonal T-type (Cav3) Ca(2)(+) channels and Ca(2)(+) release from stores. *J Neurosci* 2011;31:13546–13561.
109. Wu Y, Whiteus C, Xu CS, et al. Contacts between the endoplasmic reticulum and other membranes in neurons. *Proc Natl Acad Sci U S A* 2017;114:E4859–E4867.
110. de Juan-Sanz J, Holt GT, Schreiter ER. et al. Axonal endoplasmic reticulum Ca2+ content controls release probability in CNS nerve terminals. *Neuron* 2017;93:867–881 e866.
111. Emptage NJ, Reid CA, Fine A. Calcium stores in hippocampal synaptic boutons mediate short-term plasticity, store-operated Ca2+ entry, and spontaneous transmitter release. *Neuron* 2001;29:197–208.
112. Sharp AH, McPherson PS, Dawson TM. et al. Differential immunohistochemical localization of inositol 1,4,5-trisphosphate- and ryanodine-sensitive Ca2+ release channels in rat brain. *J Neurosci* 1993;13:3051–3063.
113. Unni VK, Zakharenko SS, Zablow L,. et al. Calcium release from presynaptic ryanodine-sensitive stores is required for long-term depression at hippocampal CA3-CA3 pyramidal neuron synapses. *J Neurosci* 2004;24:9612–9622.
114. Sharma G, Vijayaraghavan S. Modulation of presynaptic store calcium induces release of glutamate and postsynaptic firing. *Neuron* 2003;38:929–939.
115. Cabezas C, Buno W. Distinct transmitter release properties determine differences in short-term plasticity at functional and silent synapses. *J Neurophysiol* 2006;95:3024–3034.
116. Kang JS, Tian JH, Pan PY,. et al. Docking of axonal mitochondria by syntaphilin controls their mobility and affects short-term facilitation. *Cell* 2008;132:137–148.
117. Tang Y-G, Zucker RS. Mitochondrial involvement in post-tetanic potentiation of synaptic transmission. *Neuron* 1997;18:483–491.
118. Billups B, Forsythe ID. Presynaptic mitochondrial calcium sequestration influences transmission at mammalian central synapses. *J Neurosci* 2002;22:5840–5847.
119. Ashrafi G, de Juan-Sanz J, Farrell RJ, et al. Molecular Tuning of the Axonal Mitochondrial Ca(2+) Uniporter Ensures Metabolic Flexibility of Neurotransmission. *Neuron* 2020;105:678–687 e675.
120. Perkins GA, Tjong J, Brown, JM, et al. The micro-architecture of mitochondria at active zones: electron tomography reveals novel anchoring scaffolds and cristae structured for high-rate metabolism. *J Neurosci* 2010;30:1015–1026.