Voltage-gated calcium channels: Their discovery, function and importance as drug targets

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Abstract

This review will first describe the importance of Ca^{2+} entry for function of excitable cells, and the subsequent discovery of voltage-activated calcium conductances in these cells. This finding was rapidly followed by the identification of multiple subtypes of calcium conductance in different tissues. These were initially termed low- and high-voltage activated currents, but were then further subdivided into L-, N-, PQ-, R- and T-type calcium currents on the basis of differing pharmacology, voltage-dependent and kinetic properties, and single channel conductance. Purification of skeletal muscle calcium channels allowed the molecular identification of the pore-forming and auxiliary $\alpha_2\delta$, β and ϒ subunits present in these calcium channel complexes. These advances then led to the cloning of the different subunits, which permitted molecular characterisation, to match the cloned channels with physiological function. Studies with knockout and other mutant mice then allowed further investigation of physiological and pathophysiological roles of calcium channels. In terms of pharmacology, cardiovascular L-type channels are targets for the widely used antihypertensive 1,4-dihydropyridines and other calcium channel blockers, N-type channels are a drug target in pain, and $\alpha_2\delta$-1 is the therapeutic target of the gabapentinoid drugs, used in neuropathic pain. Recent structural advances have allowed a deeper understanding of Ca^{2+} permeation through the channel pore and the structure of both the pore-forming and auxiliary subunits. Voltage-gated calcium channels are subject to multiple pathways of modulation by G-protein and second messenger regulation. Furthermore, their trafficking pathways, subcellular localisation and functional specificity are the subjects of active investigation.

Introduction: the importance of Ca^{2+} entry for function of excitable cells

It has been clear from the time of Sydney Ringer, working at University College London, that calcium ions (Ca^{2+}) are essential for heart muscle contraction (Ringer, 1883). However, the paramount importance of Na^+ and K^+ for the activation and inactivation underlying action potential generation led to Ca^{2+} permeation being little studied for many years. In the 1950's Paul Fatt, working at University College London with both Katz and Ginsborg, found that Ca^{2+} supports action potential-like spikes in crustacean muscle (Fatt and Ginsborg, 1958; Fatt and Katz, 1953), and this was also found to be true in barnacle muscle (Hagiwara and Takahashi, 1967). When it was also identified that Ca^{2+} was essential for neurotransmitter release (Katz and Miledi, 1967), it became clear that calcium ion entry through membranes was key to many important processes in nerves as well as muscle. These key players in the field are pictured in Figure 1(a)–(d).

Identification of multiple subtypes of calcium channel

A major contribution to the understanding of calcium channel function then came from Harald Reuter (Figure 1(e)), who showed, using microelectrodes, that calcium currents were present in voltage-clamped cardiac Purkinje fibres (Reuter, 1967). The advent of the gigaseal patch-clamp method for recording currents through the membrane of single cells (Hamill et al., 1981) then allowed single calcium channels to be resolved (Fenwick et al., 1982).

The discovery and use of verapamil, and the 1,4-dihydropyridines (DHPs) including nifedipine, as antihypertensive drugs represented a very important advance (Fleckenstein, 1983) (Figure 1(f)). Their target was found to be inhibition of cardiovascular calcium channels (Lee and Tsien, 1983); thus, the term calcium channel blocker or antagonist was coined. Related drugs were found to have agonist effects (Schramm et al., 1983), to increase cardiac calcium conductance and prolong single channel openings (Hess et al., 1984). Both the agonist and antagonist

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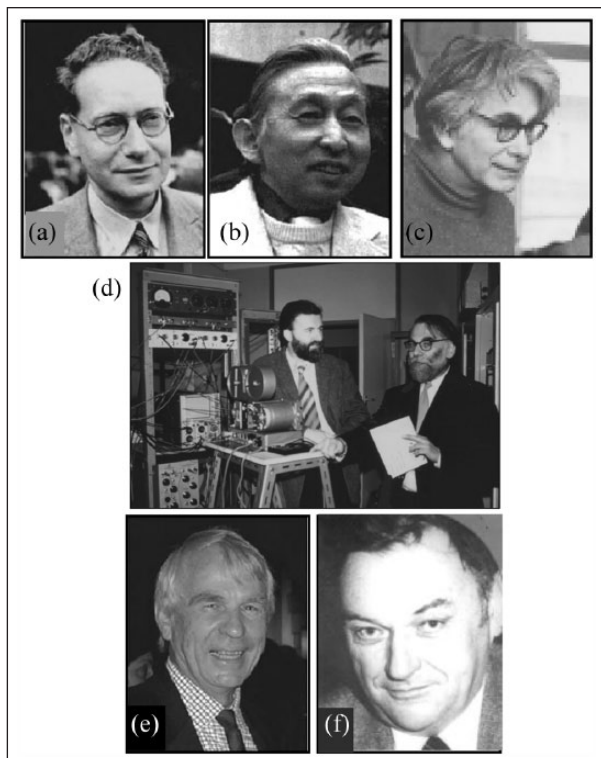Email: a.dolphin@ucl.ac.uk

Figure 1. Some key figures in the early discovery of calcium channels and their pharmacology: (a) Bernard Katz, (b) Susumu Hagiwara, (c) Paul Fatt, (d) Bernard Ginsborg (right) demonstrating equipment similar to that used to record crustacean muscle action potentials, (e) Harald Reuter and (f) Albrecht Fleckenstein. (c) is taken from a photograph (1978) by Martin Rosenberg, the Physiological Society; reproduced with permission; (a), (b) and (f) are reproduced from with permission from Richard W. Tsien (Barrett and Tsien, 2004); (d) is reproduced with permission from Bernard Ginsborg, who died this year (1925–2018).

drugs gave researchers important tools to dissect calcium channel function in a variety of tissues.

The first suggestion that there was more than one component to calcium currents in different tissues came from the group of Hagiwara et al. (1975), followed by evidence of low threshold Ca^{2+} spikes in mammalian central neurons (Llinás and Yarom, 1981), and distinct low voltage-activated currents in peripheral dorsal root ganglion neurons (Carbone and Lux, 1984; Fedulova et al., 1985; Nilius et al., 1985).

Identification of N–, P– and R-type calcium currents as distinct from L-type channels

In dorsal root ganglion (DRG) neurons, it was then found that there were three calcium current components. The DHP-sensitive current was designated L-type (for long-lasting, which also had a large singe channel conductance) and the low-voltage activated component was termed T (for transient, which also had a Tiny single channel conductance). A third component, which was
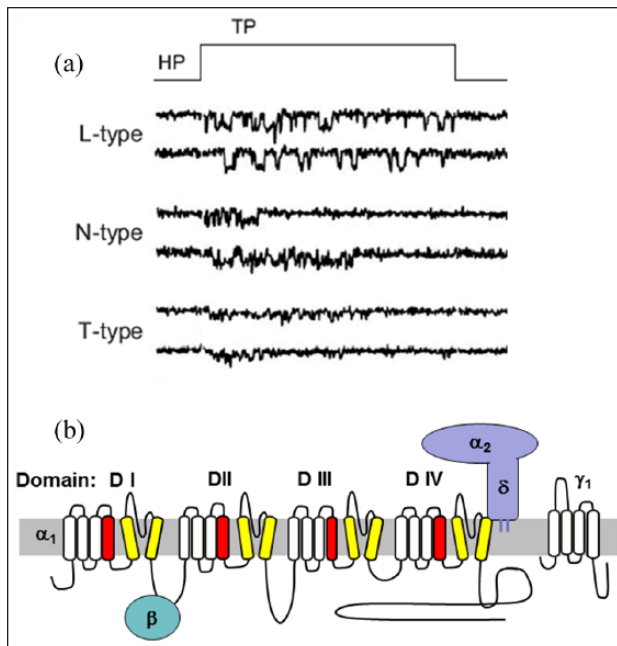

Figure 2. Single calcium channels with different properties, and topology of the channels. (a) Identification of a third component of voltage-gated calcium channels (N-type) from the biophysical properties of single channel currents observed in cell-attached patches on dorsal root ganglion neurons. Redrawn from Nowycky et al. (1985). TP: test potential; HP: holding potential. Reproduced with thanks to Richard W. Tsien. (b) Diagram of α_1 subunit topology and calcium channel subunit structure, also showing $\alpha_2\delta$ (purple) and β (blue). Υ_1 is only present in skeletal muscle calcium channel complexes. S4 voltage sensors in each α_1 domain are represented by red transmembrane segments. Yellow denotes S5 and S6 pore transmembrane segments in each domain.

high-voltage activated but DHP-insensitive, was termed N-type (neither L nor T, and also exclusively Neuronal) (Fox et al., 1987; Nowycky et al., 1985) (Figure 2(a)). A blocker of this component was not long in appearing. A toxin component from the marine snail *Conus geographus*, ω-conotoxin GVIA, first thought to block both neuronal L- and N-type calcium currents (McCleskey et al., 1987), was later found to be highly selective for N-type channels (Boland et al., 1994; Plummer et al., 1989). Using this pharmacological blocker, N-type calcium currents were then shown to play a key role in neurotransmitter release (Hirning et al., 1988).

The importance of pharmacological tools in the discovery of calcium channel subtypes became even more evident when it was found that the calcium current in Purkinje neurons was not blocked by DHPs or by ω-conotoxin GVIA. This current was called P-type (for Purkinje) (Llinás et al., 1989). The same group used a polyamine toxin (FTX) from the American funnel web spider to block Purkinje cell Ca^{2+} currents, but FTX was not particularly selective for P-type channels, whereas a peptide toxin component from the same spider (ω-agatoxin IVA) was more selective, blocking fully the calcium current in Purkinje neurons (Mintz et al., 1992). This toxin also inhibited a component of the calcium current in cerebellar granule cells (Pearson et al., 1995;

Randall and Tsien, 1995), which was initially termed Q-type as it had different biophysical properties from that in Purkinje neurons (Randall and Tsien, 1995); however, these are usually now called PQ currents. That study also identified an additional resistant current component in cerebellar granule cells which was designated R-type (Randall and Tsien, 1995), and a similar novel component was also identified in bullfrog sympathetic neurons (Elmslie et al., 1994). A tarantula toxin, SNX-482, was identified to block this component (Newcomb et al., 1998), but it has subsequently been found also to block other channels (Kimm and Bean, 2014), complicating interpretation of physiological experiments using SNX-482.

Purification and molecular identification of the calcium channel subtypes

Receptors for the DHP calcium antagonists were identified using [^{3}H]-nitrendipine to guide purification. They were found to be highly concentrated in the t-tubules of skeletal muscle (Fosset et al., 1983), where they were shown to be responsible for charge movement and excitation-contraction coupling (Rios and Brum, 1987). Purification studies identified the skeletal muscle DHP receptor to be a complex of five polypeptides in approximately equal amounts, and therefore considered to be subunits. They were termed, in decreasing order of size, the α_1, α_2, β, Υ and δ subunits (Hosey et al., 1987; Takahashi et al., 1987). The 175 kDa α_1 subunit was tentatively identified as the pore-forming subunit of the channel, since it bound radiolabelled DHP. The associated proteins were termed auxiliary or accessory subunits.

Peptide sequence from the purified DHP receptor protein enabled the identification of probes and subsequent cloning of the skeletal muscle calcium channel (Ellis et al., 1988; Tanabe et al., 1987). The hydropathy plot indicated that it was a 24 transmembrane spanning protein, with four homologous repeated domains joined by intracellular linkers, similar to recently cloned voltage-gated Na$^+$ channel (Noda et al., 1984) (Figure 2(b)). This protein was termed α_1S (for skeletal muscle) and was indisputably shown to encode a calcium channel by injection of its cDNA into dysgenic skeletal myotubes which lack the mRNA for α_1S (Tanabe et al., 1988). This restored excitation–contraction coupling, as well as the very slow calcium current observed in native skeletal muscle.

The cardiac L-type calcium channel, termed α_1C, was then cloned by homology with α_1S (Mikami et al., 1989). Prior to this time, the unique permeation selectivity of the voltage-gated calcium channels for Ca^{2+} had already been attributed to high affinity Ca^{2+} binding in the pore of the channel (Hess and Tsien, 1984), and this was borne out by identification of key glutamate residues in the pore 'P loops' (Yang et al., 1993), whose acidic side chains were surmised to participate in Ca (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)$^{2+}$ binding and permeation.

Several brain calcium channels were then cloned and identified to encode P- and N-type channels (Mori et al., 1991; Snutch et al., 1990; Starr et al., 1991). These were termed α_1A and α_1B, respectively. Another channel was cloned and dubbed α_1E (Soong et al., 1993). It was first classified as a low-voltage activated T-type channel, but it soon became clear that it did not have

the expected properties, and it is now considered to encode R-type channels. Genes for three T-type channels were later cloned by Perez-Reyes and colleagues (Cribbs et al., 1998; Lee et al., 1999; Perez-Reyes et al., 1998). These were termed α_1G, H and I. In addition, two further L-type channels were identified. The first, cloned from brain, was called α_1D (Williams et al., 1992) and was shown to have distinctive biophysical properties, being lower voltage-activated than α_1C (Koschak et al., 2001; Xu and Lipscombe, 2001). Finally, a fourth L-type channel was identified because of its role in a genetic form of night blindness (Bech et al., 1998; Strom et al., 1998), and this was also shown to have properties distinguishing it from the other L-type channels (Koschak et al., 2003).

Following the cloning and initial study of all the calcium channel α_1 subunits identified in the mammalian genome, a rationalised nomenclature was adopted in 2000, grouping the α_1 subunits into Ca$_V$1 (L-type), Ca$_V$2 (non-L-type) and Ca$_V$3 (T-type) (Ertel et al., 2000) (Table 1). Since that time the distinctive properties of multiple splice variants of these channels have also been recognised.

Importance of auxiliary subunits

The auxiliary β subunit from skeletal muscle was the first to be cloned (Ruth et al., 1989) (Figure 2(b)). It was subsequently termed β_{1a}, after three further isoforms (β_2, β_3 and β_4) as well as multiple splice variants were identified by homology. β_{1b} is the non-muscle splice variant of β_1 (Pragnell et al., 1991), and β_{2a} is a palmitoylated β_2 splice variant, giving it distinctive properties (Qin et al., 1998). The importance of these β subunits to the expression of the Ca$_V$1 and Ca$_V$2 channels was clear from antisense knockdown studies in native tissues and early expression studies (Berrow et al., 1995; Qin et al., 1998). In contrast, the Ca$_V$3 channels do not appear to have any obligate auxiliary subunits.

When the auxiliary $\alpha_2\delta$ subunit was cloned, it was realised that α_2 and δ are encoded by the same gene and form a pre-protein, which is then proteolytically cleaved, but the α_2 and δ proteins remain associated by pre-formed disulphide bonding (De Jongh et al., 1990; Jay et al., 1991). Its proteolytic cleavage has recently been shown to be essential for $\alpha_2\delta$ function (Kadurin et al., 2016). The skeletal muscle $\alpha_2\delta$ was subsequently termed $\alpha_2\delta$-1, when three further mammalian isoforms were identified: $\alpha_2\delta$-2 (Barclay et al., 2001; Gao et al., 2000), $\alpha_2\delta$-3 and $\alpha_2\delta$-4 (Qin et al., 2002). The muscle $\alpha_2\delta$ subunit was first described as a transmembrane protein, but they have subsequently been shown to be glycosyl-phosphatidylinositol (GPI)-anchored into the outer leaflet of the plasma membrane (Davies et al., 2010) (Figure 2(b)). The $\alpha_2\delta$ subunit was predicted to contain a von Willebrand factor A (VWA) domain, which was found to be essential for trafficking, both of $\alpha_2\delta$ itself, and for its effect on the α_1 subunits (Canti et al., 2005; Cassidy et al., 2014; Hoppa et al., 2012).

The skeletal muscle calcium channel complex also contains a Υ subunit, now called Υ_1 (Takahashi et al., 1987) (Figure 2(b)), but Υ is not associated with other calcium channels, and further members of this 'Υ subunit' family are now known to be trafficking proteins that modulate the function of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) glutamate receptors, rather than voltage-gated calcium channel subunits (Tomita et al., 2003). The roles of the different calcium channel auxiliary

Table 1. Subtypes of calcium channel.

Activation voltage	Functional nomenclature	Channel α_1 subunit	Ca$_V$ nomenclature	Main function
(High)	L	α_1S	1.1	Skeletal muscle voltage sensor
High	L	α_1C	1.2	Cardiac, smooth muscle function
Medium	L	α_1D	1.3	Hearing, sino-atrial node function
Medium	L	α_1F	1.4	Retinal neurotransmission
High	PQ	α_1A	2.1	Synaptic transmission in CNS, motor nerves and elsewhere
High	N	α_1B	2.2	Synaptic transmission in PNS (and CNS, especially early in development)
Medium	R	α_1E	2.3	Present in some neurons and synapses
Low	T	α_1G	3.1	
Low	T	α_1H	3.2	Neuronal excitability, pacemaker activity, subthreshold oscillations
Low	T	α_1I	3.3	

PNS: peripheral nervous system; CNS: central nervous system.

subunits have been more extensively reviewed recently (Dolphin, 2012).

Elucidation of physiological channel function from knockout mouse studies and genetic mutations

Several spontaneously arising mouse loss-of-function mutants were identified which gave important clues as to the function of the channel subunits. This was particularly true for Ca$_V$2.1, β4 and α_2δ-2 which are strongly expressed in cerebellum, and whose mutation produced obvious ataxias (Barclay et al., 2001; Burgess et al., 1997; Fletcher et al., 1996). Subsequent targeted knockouts gave similar phenotypes. A surprise came with the knockout of Ca$_V$1.3, both in mice and in a homozygous human mutation, in whom the main phenotype was deafness and sino-atrial node dysfunction (Baig et al., 2011). Furthermore Ca$_V$1.4 was identified from its role in a retinal disease (Bech-Hansen et al., 1998; Strom et al., 1998), and the knockout mouse has a similar phenotype (Mansergh et al., 2005). Knockout of Ca$_V$2.2 resulted in a diminution of neuropathic pain responses, reinforcing its importance in primary afferent neurotransmission (Saegusa et al., 2001). Similarly, α_2δ-1 knockout delayed the onset of mechanical hyperalgesia following neuropathic injury (Patel et al., 2013) and α_2δ-3 has a role in hearing (Pirone et al., 2014), and in the central control of pain (Neely et al., 2010).

Structural studies

The first components of the calcium channel complex to be amenable to structural studies were the β subunits, which contain two conserved interacting domains (SH3 and guanylate kinase-like), the latter binding to the linker between domains I and II of the channels (Chen et al., 2004; Opatowsky et al., 2004; Pragnell et al., 1994; Richards et al., 2004; Van Petegem et al., 2004).

The first crystal structure for a calcium-selective voltage-gated channel was obtained using a mutant form of a bacterial sodium channel homolog, Na$_V$Ab, a single domain channel which forms homo-tetramers (Payandeh et al., 2011). This was mutated so that the pore became Ca^{2+}-selective, forming Ca$_V$Ab. This structure has provided multiple insights, including

confirmation of the Ca^{2+} permeation process (Tang et al., 2014). Remarkably, this channel was sensitive to calcium channel antagonists, yielding further important insight into the binding and mechanism of action of these drugs (Tang et al., 2016). For mammalian calcium channel complexes, although low-resolution single particle electron microscopic structures were published previously (Serysheva et al., 2002; Walsh et al., 2009; Wolf et al., 2003), major advances in cryo-electron microscopy were needed before a detailed structure of the skeletal muscle calcium channel was produced, very beautifully elucidating details of the pore and the subunit arrangement (Wu et al., 2016). GPI-anchoring of α_2δ (Davies et al., 2010), and interaction of the α1 subunit with the VWA and Cache domains (which have similarity to bacterial chemotaxis domains) of α_2δ (Canti et al., 2005; Cassidy et al., 2014), were confirmed in the structure (Wu et al., 2016).

Calcium channel modulation

Only two canonical second messenger modulation pathways will be considered here, for reasons of space: inhibitory modulation of neuronal calcium channels by G-proteins, and cyclic AMP-dependent phosphorylation, mediating enhancement of L-type channels. Many other pathways also deserve mention, including Ca^{2+}-calmodulin control of Ca^{2+}-dependent inactivation and facilitation of L-type and P-type channels, studied extensively by the late David Yue (Dick et al., 2008; Peterson et al., 1999).

G-protein modulation

Voltage-dependent activation of neuronal calcium channels is required for neurotransmitter release, and this process can be inhibited by a range of modulatory neurotransmitters coupled to seven-transmembrane receptors (Dolphin, 1982; Jessell and Iversen, 1977; Peng and Frank, 1989), leading to the view that inhibitory modulation of the calcium channel-mediated component of the presynaptic action potential underpins receptor-mediated presynaptic inhibition (Dolphin et al., 1986; Dunlap and Fischbach, 1978; Ikeda and Schofield, 1989) (Figure 3(a)). Modulation of neurotransmitter release was found to be mediated by a pertussis toxin-sensitive GTP-binding protein, of the G$_i$/G$_o$ family (Dolphin and Prestwich, 1985). The inhibitory
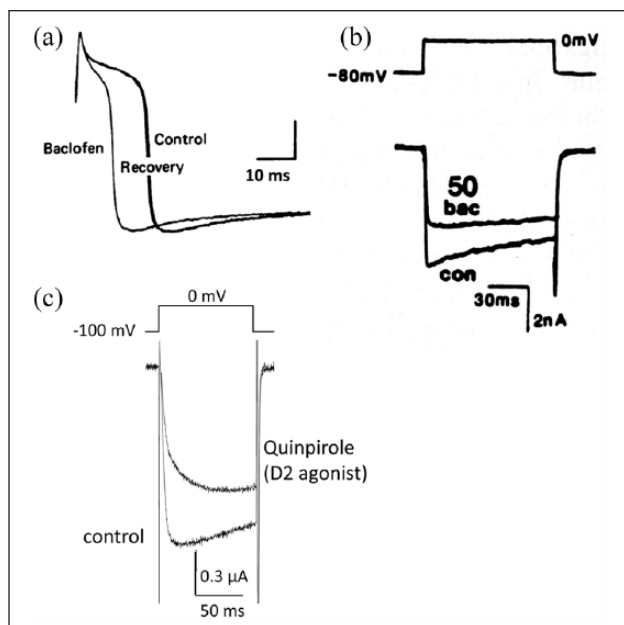
Figure 3. Inhibitory G-protein modulation of neuronal calcium channels. (a) Action potential (prolonged by K+ channel blockade), recorded from dorsal root ganglion neuron, showing the control, inhibition by the GABA-B agonist baclofen (100 μM) and recovery (from Figure 7 of Dolphin et al. (1986)). (b) Calcium channel currents recorded from dorsal root ganglion neuron, showing inhibition by baclofen (bac, 50 μM) (Dolphin et al., 1989). (c) Calcium channel currents recorded from *Xenopus laevis* oocytes injected with $Ca_V2.2/\beta3/\alpha_2\delta$-1 and the dopamine D2 receptor, showing inhibition by the D2 agonist quinpirole (100 nM) (replotted from Figure 2(e) of Canti et al. (2000).

modulation of neuronal calcium currents was subsequently also identified to involve these G-proteins (Scott and Dolphin, 1986; Holz et al., 1986) (Figure 3(c)). Using both native and cloned Ca_V2 channels, the modulation was subsequently shown to be a direct membrane-delimited effect of $G\beta\Upsilon$ subunits (Herlitze et al., 1996; Ikeda, 1996), mediated by the channel I-II linker (Bourinet et al., 1996) and its intracellular N-terminus (Page et al., 1998). The characteristic voltage-dependence of the inhibition, which means that inhibition is lost with large or repeated depolarisations, was shown to require participation of the calcium channel β subunit (Meir et al., 2000).

Cyclic AMP-dependent phosphorylation

Another key example of second messenger modulation is provided by L-type calcium channels, which are potentiated by β-adrenergic receptor activation, via a cyclic AMP-dependent mechanism (Cachelin et al., 1983; Reuter, 1983). In heart, this effect is mediated by β1-adrenergic receptors and forms one of the main components of the fight-or-flight response. However, it has been difficult to reproduce when cloned $Ca_V1.2$ calcium channels are expressed, for example, in HEK-293 cells, suggesting it is more complex than simple channel phosphorylation, and indeed, the role of the several protein kinase A substrate serines in cardiac $Ca_V1.2$ function is still being determined (Lemke et al., 2008; Yang et al., 2016).

Furthermore, the response to β-adrenergic stimulation may involve a proteolytically cleaved C-terminal fragment of the endogenous $Ca_V1.2$ channels (Fu et al., 2013; Fuller et al., 2010). Perhaps surprisingly, there appears to be a somewhat different basis for the spatially restricted stimulation observed in hippocampal neurons following activation by β2-adrenergic receptors of neuronal $Ca_V1.2$ channels (Qian et al., 2017).

Future research

The selective pharmacology that has been so important for dissecting out the functions of different calcium channels is still incomplete. Although a selective inhibitor of the T-type calcium channels exists (Dreyfus et al., 2010), it does not differentiate between the Ca_V3 channels. Similarly, there are currently no selective inhibitors of the different Ca_V1 channels. Such inhibitors that would be able to differentiate between these very similar channels could have important therapeutic possibilities. For example, selective inhibition of $Ca_V3.2$ could be of therapeutic benefit in certain types of pain (Marger et al., 2011), and selective inhibitors of $Ca_V1.3$ have potential for therapeutic use in Parkinson's disease and other disorders (Striessnig et al., 2015). Furthermore, although ω-conotoxin GVIA is a selective blocker of N-type channels and a related compound is licenced for use intrathecally in some chronic pain conditions (Miljanich, 2004), no small molecule inhibitors of N-type channels have yet been shown to be effective in clinical trials for chronic pain.

Future challenges include a full understanding of how particular calcium channels are trafficked into precise subcellular domains, for example, how some channels are targeted to dendrites (Hall et al., 2013), while others are directed to presynaptic active zones to mediate neurotransmitter release (Kaeser et al., 2011). Furthermore, calcium channels have been found to interact, directly or indirectly, with multiple scaffolding proteins, ion channels and second messenger pathways (Müller et al., 2010), but how these are organised and function together remains to be elucidated. Related to this, the pathways for intracellular Ca^{2+} signalling to the nucleus and the selectivity for L-type Ca^{2+} channels in neurons are still being revealed (Cohen et al., 2015; Wheeler et al., 2012).

Declaration of conflicting interests

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References

Baig SM, Koschak A, Lieb A, et al. (2011) Loss of Ca(v)1.3 (CACNA1D) function in a human channelopathy with bradycardia and congenital deafness. *Nature Neuroscience* 14: 77–84.

Barclay J, Balaguero N, Mione M, et al. (2001) Ducky mouse phenotype of epilepsy and ataxia is associated with mutations in the *Cacna2d222* gene and decreased calcium channel current in cerebellar Purkinje cells. *Journal of Neuroscience* 21: 6095–6104.

Barrett CF and Tsien RW (2004) Brief history of calcium channel discovery. In: Zamponi GW (ed.) *Voltage-Gated Calcium Channels*. Dordrecht: Kluwer Academic/Plenum Publishers, pp. 1–21.

Bech-Hansen NT, Naylor MJ, Maybaum TA, et al. (1998) Loss-of-function mutations in a calcium-channel α1-subunit gene in Xp11.23 cause incomplete X-linked congenital stationary night blindness. *Nature Genetics* 19: 264–267.

Berrow NS, Campbell V, Fitzgerald EG, et al. (1995) Antisense depletion of beta-subunits modulates the biophysical and pharmacological properties of neuronal calcium channels. *Journal of Physiology* 482: 481–491.

Boland LM, Morrill JA and Bean BP (1994) Omega-conotoxin block of N-type calcium channels in frog and rat sympathetic neurons. *Journal of Neuroscience* 14: 5011–5027.

Bourinet E, Soong TW, Stea A, et al. (1996) Determinants of the G protein-dependent opioid modulation of neuronal calcium channels. *Proceedings of the National Academy of Sciences of the United States of America* 93: 1486–1491.

Burgess DL, Jones JM, Meisler MH, et al. (1997) Mutation of the Ca^{2+} channel beta subunit gene *Cchb4* is associated with ataxia and seizures in the lethargic (*lh*) mouse. *Cell* 88: 385–392.

Cachelin AB, De Peyer JE, Kokubun S, et al. (1983) Ca^{2+} channel modulation by 8-bromocyclic AMP in cultured heart cells. *Nature* 304: 462–464.

Canti C, Bogdanov Y and Dolphin AC (2000) Interaction between G proteins and accessory subunits in the regulation of α1B calcium channels in Xenopus oocytes. *Journal of Physiology* 527: 419–432.

Canti C, Nieto-Rostro M, Foucault I, et al. (2005) The metal-ion-dependent adhesion site in the Von Willebrand factor-A domain of alpha2delta subunits is key to trafficking voltage-gated Ca^{2+} channels. *Proceedings of the National Academy of Sciences of the United States of America* 102: 11230–11235.

Carbone E and Lux HD (1984) A low voltage-activated fully inactivating Ca channel in vertebrate sensory neurones. *Nature* 310: 501–502.

Cassidy JS, Ferron L, Kadurin I, et al. (2014) Functional exofacially tagged N-type calcium channels elucidate the interaction with auxiliary α2δ-1 subunits. *Proceedings of the National Academy of Sciences of the United States of America* 111: 8979–8984.

Chen YH, Li MH, Zhang Y, et al. (2004) Structural basis of the alpha1-beta subunit interaction of voltage-gated Ca^{2+} channels. *Nature* 429: 675–680.

Cohen SM, Li B, Tsien RW, et al. (2015) Evolutionary and functional perspectives on signaling from neuronal surface to nucleus. *Biochemical and Biophysical Research Communications* 460: 88–99.

Cribbs LL, Lee J-H, Yang J, et al. (1998) Cloning and characterization of α1H from human heart, a member of the T type Ca^{2+} channel gene family. *Circulation Research* 83: 103–109.

Davies A, Kadurin I, Alvarez-Laviada A, et al. (2010) The α2δ subunits of voltage-gated calcium channels form GPI-anchored proteins, a post-translational modification essential for function. *Proceedings of the National Academy of Sciences of the United States of America* 107: 1654–1659.

De Jongh KS, Warner C and Catterall WA (1990) Subunits of purified calcium channels: α2 and δ are encoded by the same gene. *Journal of Biological Chemistry* 265: 14738–14741.

Dick IE, Tadross MR, Liang H, et al. (2008) A modular switch for spatial Ca^{2+} selectivity in the calmodulin regulation of CaV channels. *Nature* 451: 830–834.

Dolphin AC (1982) Noradrenergic modulation of glutamate release in the cerebellum. *Brain Research* 252: 111–116.

Dolphin AC (2012) Calcium channel auxiliary alpha(2)delta and beta subunits: Trafficking and one step beyond. *Nature Reviews Neuroscience* 13: 542–555.

Dolphin AC and Prestwich SA (1985) Pertussis toxin reverses adenosine inhibition of neuronal glutamate release. *Nature* 316: 148–150.

Dolphin AC, Forda SR and Scott RH (1986) Calcium-dependent currents in cultured rat dorsal root ganglion neurones are inhibited by an adenosine analogue. *Journal of Physiology* 373: 47–61.

Dolphin AC, McGuirk SM and Scott RH (1989) An investigation into the mechanisms of inhibition of calcium channel currents in cultured sensory neurones of the rat by guanine nucleotide analogues and (–)–baclofen. *British Journal of Pharmacology* 97: 263–273.

Dreyfus FM, Tscherter A, Errington AC, et al. (2010) Selective T-type calcium channel block in thalamic neurons reveals channel redundancy and physiological impact of I(T)window. *The Journal of Neuroscience* 30: 99–109.

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

Ellis SB, Williams ME, Ways NR, et al. (1988) Sequence and expression of mRNAs encoding the α1 and α2 subunits of a DHP-sensitive calcium channel. *Science* 241: 1661–1664.

Elmslie KS, Kammermeier PJ and Jones SW (1994) Reevaluation of Ca^{2+} channel types and their modulation in bullfrog sympathetic neurons. *Neuron* 13: 217–228.

Ertel EA, Campbell KP, Harpold MM, et al. (2000) Nomenclature of voltage-gated calcium channels. *Neuron* 25: 533–535.

Fatt P and Ginsborg BL (1958) The ionic requirements for the production of action potentials in crustacean muscle fibres. *Journal of Physiology* 142: 516–543.

Fatt P and Katz B (1953) The electrical properties of crustacean muscle fibres. *Journal of Physiology* 120: 171–204.

Fedulova SA, Kostyuk PG and Veselovsky NS (1985) Two types of calcium channels in the somatic membrane of new-born rat dorsal root ganglion neurones. *Journal of Physiology* 359: 431–446.

Fenwick EM, Marty A and Neher E (1982) Sodium and calcium channels in bovine chromaffin cells. *Journal of Physiology* 331: 599–635.

Fleckenstein A (1983) History of calcium antagonists. *Circulation Research* 52: I3–16.

Fletcher CF, Lutz CM, O'Sullivan TN, et al. (1996) Absence epilepsy in tottering mutant mice is associated with calcium channel defects. *Cell* 87: 607–617.

Fosset M, Jaimovich E, Delpont E, et al. (1983) [3H]nitrendipine receptors in skeletal muscle. *Journal of Biological Chemistry* 258: 6086–6092.

Fox AP, Nowycky MC and Tsien RW (1987) Single-channel recordings of three types of calcium channels in chick sensory neurones. *Journal of Physiology* 394: 173–200.

Fu Y, Westenbroek RE, Scheuer T, et al. (2013) Phosphorylation sites required for regulation of cardiac calcium channels in the fight-or-flight response. *Proceedings of the National Academy of Sciences of the United States of America* 110: 19621–19626.

Fuller MD, Emrick MA, Sadilek M, et al. (2010) Molecular mechanism of calcium channel regulation in the fight-or-flight response. *Science signaling* 3: ra70.

Gao B, Sekido Y, Maximov A, et al. (2000) Functional properties of a new voltage-dependent calcium channel α2δ auxiliary subunit gene (CACNA2D2). *Journal of Biological Chemistry* 275: 12237–12242.

Hagiwara S and Takahashi K (1967) Surface density of calcium ions and calcium spikes in the barnacle muscle fiber membrane. *Journal of General Physiology* 50: 583–601.

Hagiwara S, Ozawa S and Sand O (1975) Voltage clamp analysis of two inward current mechanisms in the egg cell membrane of a starfish. *Journal of General Physiology* 65: 617–644.

Hall DD, Dai S, Tseng PY, et al. (2013) Competition between alpha-actinin and Ca(2)(+)-calmodulin controls surface retention of the L-type Ca(2)(+) channel Ca(V)1.2. *Neuron* 78: 483–497.

Hamill OP, Marty A, Neher E, et al. (1981) Improved patch-clamp techniques for high resolution current recording from cells and cell-free

membrane patches. *Pflügers Archiv: European Journal of Physiology* 391: 85–100.

Herlitze S, Garcia DE, Mackie K, et al. (1996) Modulation of Ca²⁺ channels by G-protein β gamma subunits. *Nature* 380: 258–262.

Hess P and Tsien RW (1984) Mechanism of ion permeation through calcium channels. *Nature* 309: 453–456.

Hess P, Lansman JB and Tsien RW (1984) Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca agonists and antagonists. *Nature* 311: 538–544.

Hirning LD, Fox AP, McCleskey EW, et al. (1988) Dominant role of N-type Ca²⁺ channels in evoked release of norepinephrine from sympathetic neurons. *Science* 239: 57–60.

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

Hoppa MB, Lana B, Margas W, et al. (2012) Alpha2delta expression sets presynaptic calcium channel abundance and release probability. *Nature* 486: 122–125.

Hosey MM, Barhanin J, Schmid A, et al. (1987) Photoaffinity labelling and phosphorylation of a 165 kilodalton peptide associated with dihydropyridine and phenylalkylamine-sensitive calcium channels. *Biochemical and Biophysical Research Communications* 147: 1137–1145.

Ikeda SR (1996) Voltage-dependent modulation of N-type calcium channels by G-protein beta gamma subunits. *Nature* 380: 255–258.

Ikeda SR and Schofield GG (1989) Somatostatin blocks a calcium current in rat sympathetic ganglion neurones. *Journal of Physiology* 409: 221–240.

Jay SD, Sharp AH, Kahl SD, et al. (1991) Structural characterization of the dihydropyridine-sensitive calcium channel α₂-subunit and the associated delta peptides. *Journal of Biological Chemistry* 266: 3287–3293.

Jessell TM and Iversen LL (1977) Opiate analgesics inhibit substance P release from rat trigeminal nucleus. *Nature* 268: 549–551.

Kadurin I, Ferron L, Rothwell SW, et al. (2016) Proteolytic maturation of α2δ represents a checkpoint for activation and neuronal trafficking of latent calcium channels. *Elife* 5: e21143.

Kaeser PS, Deng L, Wang Y, et al. (2011) RIM proteins tether Ca(2+) channels to presynaptic active zones via a direct PDZ-domain interaction. *Cell* 144: 282–295.

Katz B and Miledi R (1967) A study of synaptic transmission in the absence of nerve impulses. *The Journal of Physiology* 192: 407–436.

Kimm T and Bean BP (2014) Inhibition of A-type potassium current by the peptide toxin SNX-482. *The Journal of Neuroscience* 34: 9182–9189.

Koschak A, Reimer D, Huber I, et al. (2001) Alpha 1D (Cav1.3) subunits can form L-type Ca²⁺ channels activating at negative voltages. *Journal of Biological Chemistry* 276: 22100–22106.

Koschak A, Reimer D, Walter D, et al. (2003) Cav1.4alpha1 subunits can form slowly inactivating dihydropyridine-sensitive L-type Ca²⁺ channels lacking Ca²⁺-dependent inactivation. *The Journal of Neuroscience* 23: 6041–6049.

Lee J-H, Daud AN, Cribbs LL, et al. (1999) Cloning and expression of a novel member of the low voltage activated T type calcium channel family. *Journal of Neuroscience* 19: 1912–1921.

Lee KS and Tsien RW (1983) Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialysed heart cells. *Nature* 302: 790–794.

Lemke T, Welling A, Christel CJ, et al. (2008) Unchanged beta-adrenergic stimulation of cardiac l-type calcium channels in CAv1.2 phosphorylation site S1928A mutant mice. *Journal of Biological Chemistry* 283: 34738–34744.

Llinás R and Yarom Y (1981) Electrophysiology of mammalian inferior olivary neurones in vitro. Different types of voltage-dependent ionic conductances. *The Journal of Physiology* 315: 549–567.

Llinás R, Sugimori M, Lin J-W, et al. (1989) Blocking and isolation of a calcium channel from neurons in mammals and cephalopods

utilizing a toxin fraction (FTX) from funnel-web spider poison. *Proceedings of the National Academy of Sciences of the United States of America* 86: 1689–1693.

McCleskey EW, Fox AP, Feldman DH, et al. (1987) Omega-conotoxin: Direct and persistent blockade of specific types of calcium channels in neurons but not muscle. *Proceedings of the National Academy of Sciences of the United States of America* 84: 4327–4331.

Mansergh F, Orton NC, Vessey JP, et al. (2005) Mutation of the calcium channel gene Cacna1f disrupts calcium signaling, synaptic transmission and cellular organization in mouse retina. *Human Molecular Genetics* 14: 3035–3046.

Marger F, Gelot A, Alloui A, et al. (2011) T-type calcium channels contribute to colonic hypersensitivity in a rat model of irritable bowel syndrome. *Proceedings of the National Academy of Sciences of the United States of America* 108: 11268–11273.

Meir A, Bell DC, Stephens GJ, et al. (2000) Calcium channel beta subunit promotes voltage-dependent modulation of α1B by Gβγ. *Biophysical Journal* 79: 731–746.

Mikami A, Imoto K, Tanabe T, et al. (1989) Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel. *Nature* 340: 230–233.

Miljanich GP (2004) Ziconotide: Neuronal calcium channel blocker for treating severe chronic pain. *Current Medicinal Chemistry* 11: 3029–3040.

Mintz IM, Venema VJ, Swiderek KM, et al. (1992) P-type calcium channels blocked by the spider toxin w-Aga-IVA. *Nature* 355: 827–829.

Mori Y, Friedrich T, Kim MS, et al. (1991) Primary structure and functional expression from complementary DNA of a brain calcium channel. *Nature* 350: 398–402.

Müller CS, Haupt A, Bildl W, et al. (2010) Quantitative proteomics of the Cav2 channel nano-environments in the mammalian brain. *Proceedings of the National Academy of Sciences of the United States of America* 107: 14950–14957.

Neely GG, Hess A, Costigan M, et al. (2010) A genome-wide Drosophila screen for heat nociception identifies alpha2delta3 as an evolutionarily conserved pain gene. *Cell* 143: 628–638.

Newcomb R, Szoke B, Palma A, et al. (1998) Selective peptide antagonist of the class E calcium channel from the venom of the tarantula *Hysterocrates gigas. Biochemistry* 37: 15353–15362.

Nilius B, Hess P, Lansman JB, et al. (1985) A novel type of cardiac calcium channel in ventricular cells. *Nature* 316: 443–446.

Noda M, Shimizu S, Tanabe T, et al. (1984) Primary structure of electrophorus electricus sodium channel deduced from cDNA sequence. *Nature* 312: 121–127.

Nowycky MC, Fox AP and Tsien RW (1985) Three types of neuronal calcium channel with different calcium agonist sensitivity. *Nature* 316: 440–446.

Opatowsky Y, Chen CC, Campbell KP, et al. (2004) Structural analysis of the voltage-dependent calcium channel beta subunit functional core and its complex with the alpha1 interaction domain. *Neuron* 42: 387–399.

Page KM, Canti C, Stephens GJ, et al. (1998) Identification of the amino terminus of neuronal Ca²⁺ channel α1 subunits α1B and α1E as an essential determinant of G protein modulation. *Journal of Neuroscience* 18: 4815–4824.

Patel R, Bauer CS, Nieto-Rostro M, et al. (2013) alpha2delta-1 gene deletion affects somatosensory neuron function and delays mechanical hypersensitivity in response to peripheral nerve damage. *The Journal of Neuroscience* 33: 16412–16426.

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

Pearson HA, Sutton KG, Scott RH, et al. (1995) Characterization of Ca²⁺ channel currents in cultured rat cerebellar granule neurones. *Journal of Physiology* 482: 493–509.

Peng YY and Frank E (1989) Activation of GABAB receptors causes presynaptic inhibition at synapses between muscle spindle afferents and motoneurons in the spinal cord of bullfrogs. *Journal of Neuroscience* 9: 1502–1515.

Perez-Reyes E, Cribbs LL, Daud A, et al. (1998) Molecular characterization of a neuronal low-voltage-activated T type calcium channel. *Nature* 391: 896–900.

Peterson BZ, DeMaria CD and Yue DT (1999) Calmodulin is the Ca^{2+} sensor for Ca^{2+}-dependent inactivation of 1-type calcium channels. *Neuron* 22: 549–558.

Pirone A, Kurt S, Zuccotti A, et al. (2014) alpha2delta3 is essential for normal structure and function of auditory nerve synapses and is a novel candidate for auditory processing disorders. *The Journal of Neuroscience* 34: 434–445.

Plummer MR, Logothetis DE and Hess P (1989) Elementary properties and pharmacological sensitivities of calcium channels in mammalian peripheral neurons. *Neuron* 2: 1453–1463.

Pragnell M, De Waard M, Mori Y, et al. (1994) Calcium channel β-subunit binds to a conserved motif in the I-II cytoplasmic linker of the α_1-subunit. *Nature* 368: 67–70.

Pragnell M, Sakamoto J, Jay SD, et al. (1991) Cloning and tissue-specific expression of the brain calcium channel β-subunit. *FEBS Letters* 291: 253–258.

Qian H, Patriarchi T, Price JL, et al. (2017) Phosphorylation of Ser1928 mediates the enhanced activity of the L-type Ca^{2+} channel Cav1.2 by the beta2-adrenergic receptor in neurons. *Science Signaling* 10: eaaf9659.

Qin N, Platano D, Olcese R, et al. (1998) Unique regulatory properties of the type 2a Ca^{2+} channel β subunit caused by palmitoylation. *Proceedings of the National Academy of Sciences of the United States of America* 95: 4690–4695.

Qin N, Yagel S, Momplaisir ML, et al. (2002) Molecular cloning and characterization of the human voltage-gated calcium channel $\alpha_2\delta$-4 subunit. *Molecular Pharmacology* 62: 485–496.

Randall A and Tsien RW (1995) Pharmacological dissection of multiple types of Ca^{2+} channel currents in rat cerebellar granule neurons. *Journal of Neuroscience* 15: 2995–3012.

Reuter H (1967) The dependence of slow inward current in Purkinje fibres on the extracellular calcium-concentration. *The Journal of Physiology* 192: 479–492.

Reuter H (1983) Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature* 301: 569–574.

Richards MW, Butcher AJ and Dolphin AC (2004) Calcium channel beta subunits: Structural insights AID our understanding. *Trends in Pharmacological Sciences* 25: 626–632.

Ringer S (1883) A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. *The Journal of Physiology* 4: 29–4223.

Rios E and Brum G (1987) Involvement of dihydropyridine receptors in excitation-contraction coupling in skeletal muscle. *Nature* 325: 717–720.

Ruth P, Röhrkasten A, Biel M, et al. (1989) Primary structure of the β subunit of the DHP-sensitive calcium channel from skeletal muscle. *Science* 245: 1115–1118.

Saegusa H, Kurihara T, Zong S, et al. (2001) Suppression of inflammatory and neuropathic pain symptoms in mice lacking the N-type Ca^{2+} channel. *EMBO Journal* 20: 2349–2356.

Schramm M, Thomas G, Towart R, et al. (1983) Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels. *Nature* 303: 535–537.

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

Serysheva II, Ludtke SJ, Baker MR, et al. (2002) Structure of the voltage-gated L-type Ca^{2+} channel by electron cryomicroscopy. *Proceedings of the National Academy of Sciences of the United States of America* 99: 10370–10375.

Snutch TP, Leonard JP, Gilbert MM, et al. (1990) Rat brain expresses a heterogeneous family of calcium channels. *Proceedings of the National Academy of Sciences of the United States of America* 87: 3391–3395.

Soong TW, Stea A, Hodson CD, et al. (1993) Structure and functional expression of a member of the low voltage-activated calcium channel family. *Science* 260: 1133–1136.

Starr TVB, Prystay W and Snutch TP (1991) Primary structure of a calcium channel that is highly expressed in the rat cerebellum. *Proceedings of the National Academy of Sciences of the United States of America* 88: 5621–5625.

Striessnig J, Ortner NJ and Pinggera A (2015) Pharmacology of L-type calcium channels: Novel drugs for old targets? *Current Molecular Pharmacology* 8: 110–122.

Strom TM, Nyakatura G, Apfelstedt Sylla E, et al. (1998) An L-type calcium-channel gene mutated in incomplete X-linked congenital stationary night blindness. *Nature Genetics* 19: 260–263.

Takahashi M, Seager MJ, Jones JF, et al. (1987) Subunit structure of dihydropyridine-sensitive calcium channels from skeletal muscle. *Proceedings of the National Academy of Sciences of the United States of America* 84: 5478–5482.

Tanabe T, Beam KG, Powell JA, et al. (1988) Restoration of excitation-contraction coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA. *Nature* 336: 134–139.

Tanabe T, Takeshima H, Mikami A, et al. (1987) Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature* 328: 313–318.

Tang L, Gamal El-Din TM, Payandeh J, et al. (2014) Structural basis for Ca^{2+} selectivity of a voltage-gated calcium channel. *Nature* 505: 56–61.

Tang L, Gamal El-Din TM, Swanson TM, et al. (2016) Structural basis for inhibition of a voltage-gated Ca^{2+} channel by Ca^{2+} antagonist drugs. *Nature* 537: 117–121.

Tomita S, Chen L, Kawasaki Y, et al. (2003) Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins. *Journal of Cell Biology* 161: 805–816.

Van Petegem F, Clark KA, Chatelain FC, et al. (2004) Structure of a complex between a voltage-gated calcium channel beta-subunit and an alpha-subunit domain. *Nature* 429: 671–675.

Walsh CP, Davies A, Butcher AJ, et al. (2009) 3D structure of CaV3.1: Comparison with the cardiac L-type voltage-gated calcium channel monomer architecture. *Journal of Biological Chemistry* 284: 22310–22321.

Wheeler DG, Groth RD, Ma H, et al. (2012) Ca(V)1 and Ca(V)2 channels engage distinct modes of Ca(2+) signaling to control CREB-dependent gene expression. *Cell* 149: 1112–1124.

Williams ME, Feldman DH, McCue AF, et al. (1992) Structure and functional expression of α_1, α_2, and β subunits of a novel human neuronal calcium channel subtype. *Neuron* 8: 71–84.

Wolf M, Eberhart A, Glossmann H, et al. (2003) Visualization of the domain structure of an L-type Ca^{2+} channel using electron cryomicroscopy. *Journal of Molecular Biology* 332: 171–182.

Wu J, Yan Z, Li Z, et al. (2016) Structure of the voltage-gated calcium channel Cav1.1 at 3.6 A resolution. *Nature* 537: 191–196.

Xu WF and Lipscombe D (2001) Neuronal $Ca_v1.3\alpha_1$ L-type channels activate at relatively hyperpolarized membrane potentials and are incompletely inhibited by dihydropyridines. *Journal of Neuroscience* 21: 5944–5951.

Yang J, Ellinor PT, Sather WA, et al. (1993) Molecular determinants of Ca^{2+} selectivity and ion permeation in L-type Ca^{2+} channels. *Nature* 366: 158–161.

Yang L, Dai DF, Yuan C, et al. (2016) Loss of beta-adrenergic-stimulated phosphorylation of CaV1.2 channels on Ser1700 leads to heart failure. *Proceedings of the National Academy of Sciences of the United States of America* 113: E7976–E7985.