# Chapter 13
# Splicing and Editing to Customize $Ca_V$ Channel Structures for Optimal Neural Function

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**Abstract** Post-transcriptional modification (PTM) including mechanisms such as alternative splicing and A-to-I RNA editing are powerful and versatile mechanisms that greatly expand the coding potential of the genome, giving rise to a more diverse transcriptome and subsequently a larger proteome. While alternative splicing relies on combinatorial assembly of alternative exons, A-to-I RNA enables pin-point recoding of specific single nucleotides in the transcripts. The primary transcripts of neuronal $Ca_V$ channels undergo extensive alternative splicing, but a restricted A-to-I RNA editing, often in a tissue specific manner to generate distinct channel isoforms that could be optimally customized for different aspects of neuronal activities. Here, we discuss the functional relevance of alternative splicing and RNA editing of $Ca_V$ channels focusing on L-type $Ca_V1.2$ and $Ca_V1.3$, P/Q-type $Ca_V2.1$, N-type $Ca_V2.2$ and R-type $Ca_V2.3$ channels.

**Keywords** Post-transcriptional modification • Alternative splicing • RNA editing • Single Nucleotide Polymorphism • Channelopathy

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## 13.1 Introduction

Rapid influx of $Ca^{2+}$ through the voltage-gated calcium ($Ca_V$) channels (VGCCs) initiates a number of physiological processes such as neurotransmitter release and muscle contraction. VGCCs are a group of hetero-oligomeric trans-membrane proteins that are activated upon sensing membrane depolarization. There are ten members of VGCCs that are broadly categorized into two main groups: high-voltage-activated and low-voltage-activated channels. The high-voltage-activated calcium channels can further be subdivided into L-type ($Ca_V1.1$, 1.2, 1.3, and 1.4), P/Q-type ($Ca_V2.1$), N-type ($Ca_V2.2$), and R-type ($Ca_V2.3$) channels. The low-voltage-activated T-type channels, on the other hand, consist of the $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ channels. Besides the pore-forming $\alpha_1$-subunit, auxiliary $\beta$, $\alpha_2$-$\delta$, and/or $\gamma$ subunits are also required for the formation of functional channels that closely resemble native channels.

Alternative splicing is an exquisite post-transcriptional mechanism to diversify protein structures to expand the range of mammalian physiological processes (Black and Grabowski 2003). The exons of primary RNA transcripts can be assembled in multiple arrays to enable the production of proteomic diversity that possibly confer differences in structure, function, pharmacology, localization and other properties (Black 2003; Matlin et al. 2005). Different mechanisms for alternative splicing exist including utilization of: (i) cassette exon—an alternate exon could either be included or excluded; (ii) mutually exclusive exons—either one of a pair of similar exons is alternatively spliced and retained at a time; (iii) different junctional acceptor or donor splice sites allowing for either the lengthening or shortening of a particular exon; (iv) intron retention where an intron is included in the mature mRNA; and (v) alternative promoter or poly-adenylation sites.

A recent progress in VGCCs is the identification of increasingly more functionally important splice variations of the pore-forming $\alpha_1$ and auxiliary subunits. The phenotypic variations accompanying the proteomic changes arising from alternative splicing could influence the pharmacological and electrophysiological properties of the VGCCs in the presynaptic terminus of neurons. Moreover, mutations in the pore-forming $\alpha_1$-subunit were also found to alter the functional properties of VGCCs in the presynaptic terminus. In this review, we highlight five of the seven HVA $Ca_V$ channels, namely the L-type $Ca_V1.2$ and $Ca_V1.3$ channels and the three channels of the $Ca_V2$ subfamily.

## 13.2 L-Type $Ca_V1.2$

### *13.2.1 Functional Roles of $Ca_V1.2$*

The $Ca_V1.2$ ($\alpha_{1C}$) calcium channels were reported to be expressed widely in the soma and proximal dendrites of many types of neurons throughout the central nervous system (CNS) (Westenbroek et al. 1990, 1998; Hell et al. 1993;

Sinnegger-Brauns et al. 2004) and peripheral nervous system (PNS) (Waka et al. 2003). $Ca_V1.2$ channels expressed in hippocampal neurons were involved in post-tetanic potentiation of the GABAergic synapses (Holmgaard et al. 2009; Frey 2010; Malinina et al. 2010). Presynaptic $Ca_V1.2$ channels located on the GABAergic nerve terminals of the medial preoptic nucleus (MPN) neurons are involved in the control of impulse-evoked release and development of synaptic plasticity, which are likely to play a role in the behavioural functions controlled by the MPN (Malinina et al. 2010). The $Ca_V1.2$ channels also mediate cocaine-induced GluA1 trafficking in the nucleus accumbens (Schierberl et al. 2011).

Mice globally lacking the $Ca_V1.2$ L-type calcium channel die in utero before day 15 post-coitum (Seisenberger et al. 2000). Generation of a mouse line with an inactivation of the *CACNA1C* ($Ca_V1.2$) gene specifically in the hippocampus and neocortex ($Ca_V1.2^{HCKO}$) provided a good model for investigating the role of $Ca_V1.2$ channels in the CNS. The report provided strong evidence to indicate that $Ca_V1.2$ channels have an important role in hippocampal long-term potentiation (LTP), a process implicated in the formation of spatial memory of behaving animal (Moosmang et al. 2005; White et al. 2008). Moreover, $Ca_V1.2$ calcium channels have been shown to regulate the presynaptic mechanism of LTP in the amygdala via enhancing glutamate release (Fourcaudot et al. 2009). In another mouse line, deletion of $Ca_V1.2$ channel expression was limited to the anterior cingulate cortex, and these transgenic mice were found to display impaired observational fear learning and reduced behavioral pain responses, demonstrating the role of $Ca_V1.2$ channels in observational social fear (Jeon et al. 2010).

### *13.2.2 $Ca_V1.2$ Mutation and Single Nucleotide Polymorphism (SNP)*

By genome-wide association study (GWAS), two sex-specific SNPs (rs2370419 and rs2470411) were found in *CACNA1C*, the gene that codes for the $Ca_V1.2$ channel, to be associated with mood disorders (Dao et al. 2010). The SNP rs1006737, located in the third intron of the *CACNA1C* gene, was found to be strongly linked to bipolar disorder (BPD) and schizophrenia (Sklar et al. 2008; Nyegaard et al. 2010). Using other neuroimaging modalities such as fMRI, BPD patients with the *CACNA1C* rs1006737 SNP showed higher brain activities in the prefrontal cortex (executive cognition) and hippocampus (emotional processing) (Bigos et al. 2010) and possibly displayed attention deficits (Thimm et al. 2011). To understand how a SNP in intron 3 could be implicated in BPD, it was shown that the occurrence of rs1006737 SNP resulted in a higher expression of the $Ca_V1.2$ transcripts that is assumed to result in correspondingly higher level of expression of the proteins, with presumably larger $Ca^{2+}$ influx in at least the prefrontal cortex and hippocampus (Bigos et al. 2010). However, the pathomechanisms linking the presence of these *CACNA1C* SNPs in patients to disease phenotypes are still largely unknown.

One *de novo* missense mutation G406R in mutually exclusive exons 8/8a of the *CACNA1C* gene is associated with Timothy syndrome (TS) and autism spectrum disorder (ASD) (Splawski et al. 2004, 2005; Bader et al. 2011). The G406R mutation selectively slowed $Ca_V1.2$ channel inactivation upon co-expression with the brain $\beta_1$-subunit in Chinese hamster ovary cells (Barrett and Tsien 2008). The severity of the G406R mutation upon disease presentation, such as cardiac arrhythmia, is exon-specific and depended largely on the levels of expression of exons 8/8a in the heart. It will be of interest to determine whether the expression of autistic traits or $Ca_V1.2$-dependent LTP associated with G406R mutation in the *CACNA1C* is similarly modulated. A mouse model of TS (more severe TS2) showed some aspects of autistic spectrum disorder only in the heterozygote TS2-neo mice as the TS2-like heterozygous and homozygous mice died before weaning (Bader et al. 2011). Other mutations, A39V, G402S and G490R, of the *CACNA1C* gene, were also shown to be associated with TS (Liao and Soong 2010).

The IQ-domain of the $Ca_V1.2$ channels, encoded by amino acids 1,624–1,635 of the C-terminus, can be bound by calmodulin (CaM), a $Ca^{2+}$ sensor which mediates $Ca^{2+}$-dependent inactivation (CDI) and facilitation of the channel. In particular, I1624 of the isoleucine and glutamine (I-Q) dipeptide is essential for CaM binding. Artificially engineered mutation of I1624 substantially attenuates CDI. (Zuhlke et al. 2000). Interestingly, the $Ca_V1.2$ currents of a transgenic knock-in mouse $Ca_V1.2^{I1642E}$ channels showed a modified steady-state inactivation and recovery from inactivation, and an almost abolished voltage-dependent facilitation, indicating that the I/E mutation abolished $Ca^{2+}$/calmodulin-dependent regulation of the $Ca_V1.2^{I1642E}$ channels (Poomvanicha et al. 2011).

### *13.2.3 Splice Variations of $Ca_V1.2$*

The alternative splicing of $Ca_V1.2$ channels has been followed with interest as their antagonists are used in management of cardiovascular disorders. Previously, it has been reported that the gene coding for the $\alpha_1$-subunit of $Ca_V1.2$ contains at least 55 exons, of which more than 19 exons can be alternatively spliced (Soldatov 1994; Tang et al. 2004) to generate channel variants with altered biophysical and/or pharmacological properties (Liao et al. 2004; Tang et al. 2004; Zhang et al. 2010). However the information regarding the tissue specific expression pattern of the abovementioned splice variants are currently limited. Interestingly, Tang et al. reported that Fox proteins including Fox1 and Fox2 can regulate $Ca_V1.2$ exon 9* and exon 33 expression differentially during neuronal development (Tang et al. 2009). The same group also discovered that the polypyrimidine tract-binding protein mediates a switch from exon 8 to 8a during neuronal differentiation (Tang et al. 2011). What could be of scientific or clinical interests are the examination of factors that regulate or modulate Fox protein function and to assess how any dysregulation may affect physiology and disease.

## 13.3 L-Type $Ca_V1.3$

Among the four L-type channels, $Ca_V1.2$ and $Ca_V1.3$ are ubiquitously expressed in the central nervous system. However, the lack of a highly selective blocker towards the $Ca_V1$ channels has hampered the understanding of their respective physiological roles. Nonetheless, extensive studies have suggested that, as compared to $Ca_V1.2$, $Ca_V1.3$ channels play a more significant role in gating low-threshold-activating $Ca^{2+}$ current that underlies neuronal pacemaking (Pennartz et al. 2002; Chan et al. 2007), excitation-transcription coupling (Zhang et al. 2005, 2006; Wheeler et al. 2008), normal synaptic function (Sinnegger-Brauns et al. 2004; Day et al. 2006), cardiac rhythm (Platzer et al. 2000) and hormone secretion (Marcantoni et al. 2007). Even though the $Ca_V1.3$ channels are also widely expressed in the central nervous system, its expression predominates over $Ca_V1.2$ in certain cells such as the cochlear hair cells, sinoatrial node (SAN) of the heart and neurons in the substantia nigra pars compacta and suprachiasmatic nucleus.

### *13.3.1 The Functional Roles of $Ca_V1.3$ Inferred from $Ca_V1.3^{-/-}$ Knockout Mice*

Much of the knowledge regarding the functional roles of $Ca_V1.3$ has been gained from the characterization of the $Ca_V1.3$ knockout mice (Platzer et al. 2000). The $Ca_V1.3$ channels conduct significant inward current at the operating range of the hair cells of the cochlea and the pacemaking cells in SAN due to their low activation threshold (Koschak et al. 2001; Xu and Lipscombe 2001). Correspondingly, deletion of $Ca_V1.3$ resulted in congenital deafness due to an almost complete absence of $Ca^{2+}$ current in the inner hair cells and degeneration of both the outer and inner hair cells (Platzer et al. 2000). The $Ca_V1.3$ channels are expressed in the ribbon synapse of the hair cells and they play a significant role in triggering glutamate release at the auditory synapse (Brandt et al. 2005). In addition, deletion of $Ca_V1.3$ channels impairs the normal development of the auditory brain stem center. As the phenotype appears even before the onset of hearing (Hirtz et al. 2011; Satheesh et al. 2012), it is therefore suggestive that expression of $Ca_V1.3$ channels is essential for the development of the both peripheral sensory cells and neurons.

Furthermore, $Ca_V1.3^{-/-}$mice exhibit bradycardia as a result of SAN dysfunction (Platzer et al. 2000). More recent reports of the same $Ca_V1.3^{-/-}$mice revealed other subtle phenotypic changes. For example, $Ca_V1.3$ deletion impaired the consolidation of conditioned fear (McKinney and Murphy 2006) due to compromised long term potentiation of the amygdala (McKinney et al. 2009). In line with the findings in $Ca_V1.3^{-/-}$mice, a loss-of-function mutation of human $Ca_V1.3$ was recently characterized in two consanguineous Pakistani families (Baig et al. 2011). The mutation resulted in production of non-conducting $Ca_V1.3$ channels and expectedly

subjects homozygous for such mutations suffered from **s**ino**a**trial **n**ode **d**ysfunction and **d**eafness (SANDD) syndrome (Baig et al. 2011). However, other clinical features in human due to loss of $Ca_V1.3$ current are yet to be characterized.

### *13.3.2 Unique Biophysical Properties of $Ca_V1.3$ Channels and Modulation*

The property of the $Ca_V1.3$ current is defined by its gating mechanisms. While the low activation threshold appears to be an intrinsic property of the $Ca_V1.3$ channels, which is still poorly understood, a variety of feedback mechanisms that inactivate the channel in response to either voltage-induced conformational change (voltage dependent inactivation [VDI]) or elevation of intracellular $[Ca^{2+}]_i$ (CDI) have been well characterized. The process of VDI is initiated by the voltage-dependent conformational rearrangement of voltage-sensing domain comprising S1-to-S4 segments (Swartz 2008) leading to subsequent opening of the S6 gate (Liu et al. 1997; Xie et al. 2005), and finally the occlusion of the gate by the I-II loop in a 'hinge-lid' mechanism. Interestingly, a recently identified "shield' that repels the closure of the channel gate by the I-II loop 'lid' appeared to be a unique feature of the $Ca_V1.3$ channel (Tadross et al. 2008), allowing the channel to remain open despite prolonged activation. In comparison, CDI is a negative feedback mechanism arising from $Ca^{2+}$ influx. $Ca^{2+}$, when bound to the bi-lobe $Ca^{2+}$ sensor, calmodulin (CaM) that is constitutively tethered to the preIQ-IQ domain of the C-terminus of the channel, trigger a series of conformational changes which lead eventually to channel inactivation (Peterson et al. 1999; Zuhlke et al. 1999; Pitt et al. 2001; Erickson et al. 2003; Mori et al. 2004; Dick et al. 2008). Although the intermediate steps leading to CDI remained elusive, a more recent study indicated that the final stage of CDI involved allosteric regulation of the opening of the S6 gate (Tadross et al. 2008).

Fitting with the diverse functional roles of the channel, the gating of $Ca_V1.3$ channel is often differentially modulated in a tissue-specific manner. The native $Ca_V1.3$ current in pancreatic β-cells and SAN displayed substantial inactivation (Plant 1988; Mangoni et al. 2003) matching the profile of $Ca_V1.3$ channels characterized in heterologous systems (Xu and Lipscombe 2001; Song et al. 2003). In contrast, $I_{Ca}$ recorded from hair cells in cochlea showed little inactivation (Platzer et al. 2000; Song et al. 2003) suitably allowing for persistent cellular activity even in the presence of prolonged sound stimulus (Shen et al. 2006; Yang et al. 2006). Several mechanisms have been proposed to explain the tissue-specific specialization of $Ca_V1.3$ channels. Taking cochlea as an example, selective co-localizations of $Ca_V1.3$ channels with various proteins such as syntaxin, CaBP ($Ca^{2+}$-binding protein) and Rab3-interacting molecule (RIM) have been proposed to slow down channel inactivation (Song et al. 2003; Yang et al. 2006; Gebhart et al. 2010), although none of them have been conclusively shown in the native system. Alternatively, study by Shen et al. (2006) identified an outer hair cell

splice variant of $Ca_V1.3$ channels with disrupted IQ domain due to utilization of alternative acceptor splice site and frame-shift of exon 41. As the IQ domain is essential for calmodulin-mediated CDI, dominant expression of such a splice variant selectively in outer hair cell (Shen et al. 2006) therefore partly explained the slow inactivating $Ca^{2+}$ current that was observed. It is thus interesting that tissue selective post-transcriptional modifications, such as alternative splicing and RNA editing could potentially generate channel variants of customized properties to suit different physiological needs.

### *13.3.3 Extensive Alternative Splicing Patterns in $Ca_V1.3$ Transcripts*

The $Ca_V1.3$ channels are subject to extensive alternative splicing and a total of 16 exons have been reported to be alternatively spliced and some of them showed tissue and even species specific distribution. Despite the rich assortment of channel isoforms with possibly different functional characteristics, the functional impact of alternative splicing of the $Ca_V1.3$ transcript is still not fully understood.

Alternative splicing of the amino terminus (N-terminus) was known to affect the current density of $Ca_V1.3$ channels (Klugbauer et al. 2002; Xu et al. 2003). Inclusion of either exon 1a (Hui et al. 1991; Seino et al. 1992; Williams et al. 1992a) or 1b (Klugbauer et al. 2002) has been reported in mouse. Exon 1b appears to be mouse specific, while in rat and human, exon 1a is constitutively expressed. Although both splice variants support functional currents with similar gating properties in heterologous expression system, exon 1a conferred a much larger current density as compared to exon 1b (Klugbauer et al. 2002; Xu et al. 2003).

The IS6, IIIS2 and IVS3 segments of $Ca_V1.3$ are encoded by three pairs of mutually exclusive exons including exons 8/8a, 22/22a and 31/31a respectively. Interestingly, $Ca_V1.2$ channels display the same splicing patterns in the abovementioned regions and relatively high sequence conservation was observed between $Ca_V1.3$ and $Ca_V1.2$ channels in these three pairs of mutually exclusive exons. The alternative splicing in IS6, IIIS2 and IVS3 segments of $Ca_V1.2$ was known to alter the sensitivity of the channels towards DHP inhibition with exons 8, 22 and 31 conferring higher drug sensitivity (Liao et al. 2005). In contrast, the functional impacts these three pairs of mutually exclusive exons have on $Ca_V1.3$ channels are less well known. Interestingly, the insertional mutation that results in loss of function of human $Ca_V1.3$ channel is located in exon 8a (Baig et al. 2011). While dominant in heart tissue, approximately 60 % of the total rat brain $Ca_V1.3$ transcripts contain exon 8a (Huang and Soong, unpublished data). Therefore, understanding the tissue specific expression of exon 8a in different brain tissues could have profound implication for prognosis and possible target treatment of any neurophysiological disorder of patients suffering from SANDD syndrome (Baig et al. 2011). On the other hand, exon 22a of $Ca_V1.3$ appeared to be expressed specifically in the rat organ

of Corti with unknown functional roles (Ramakrishnan et al. 2002). In comparison, exon 22 is constitutively expressed in other tissues. Lastly although exon 31 and 31a in $Ca_V1.3$ are both ubiquitously expressed in the brain, the differences in their properties remain uncharacterized.

The I-II loop region of $Ca_V1.3$ contains three splice variations including alternative exons 9*, 11 and 13. Exon 9* (Ramakrishnan et al. 2002) and 13 (Ihara et al. 1995) were identified in the rat organ of Corti and pancreas, respectively, with uncharacterized functional impact. On the other hand exon 11 is more ubiquitously expressed in brain and pancreas and deletion of exon 11 was found not to affect the channel gating of $Ca_V1.3$ (Xu and Lipscombe 2001). Inclusion of exon 9* introduces 26 amino acids into the I-II loop of the $Ca_V1.3$ channels. Sequence of exon 9* in chicken $Ca_V1.3$ contains a consensus sequence of serine surrounded by four basic amino acid residues and is therefore a potential substrate for protein kinase (Ramakrishnan et al. 2002). In contrast, no such consensus site was found in the exon 9* of rat or human $Ca_V1.3$ (Ramakrishnan et al. 2002).

The alternate exon 32 encodes part of the extracellular loop between IVS3 and IVS4. Inclusion or exclusion of exon 32 in $Ca_V1.3$ channels has no effect on the gating properties of the channel and neither was sensitivity towards nitrendipine significantly changed (Xu and Lipscombe 2001).

The carboxyl-terminus (C-terminus) of $Ca_V1.3$ represents another hotspot of alternative splicing that has been more extensively characterized. Alternative splicing at exon 41 and mutually exclusive exons 42 and 42a has been shown to regulate the CDI of the channel. Truncation of exon 41 (half exon 41) due to the alternative use of splice acceptor site in exon 41 resulted in complete removal of the IQ domain and early termination of the C-terminus (Shen et al. 2006). Although functional current could still be observed, deletion of IQ domain resulted in complete elimination of CDI (Shen et al. 2006). Selective localization of half exon 41 in cochlear outer hair cell (Shen et al. 2006) corroborated the previous observation of slowly inactivating native $Ca_V1.3$ current recorded in hair cells, highlighting the tissue specific role of such splice isoform in supporting the normal function of the cochlea. Moreover, exon 41 could also behave as a cassette exon. The $Ca_V1.3$ transcripts devoid of the entire exon 41 have been reported in both rat and human brain (Tan et al. 2011; Bock et al. 2011). Deletion of exon 41 results in complete elimination of the IQ domain, leading to frame-shifting and early truncation of the C-terminus. Functionally, $Ca_V1.3[\Delta e41]$ shows much lower current density and much attenuated CDI (Tan et al. 2011). Interestingly, a most recent study identified three closely spaced A-to-I RNA editing sites in the mRNA sequence which codes for tetra-peptide 'IQDY' in the IQ domain (Huang et al. 2012) The editing is found to be mediated by ADAR2, a member of the family of enzyme known as **a**denosine **d**eaminase **a**cting on **R**NA (ADAR). Expectedly, codon changes from ATA to ATG, CAG to CGG and TAC to TGC result in corresponding amino acid changes from I to M, Q to R and Y to C, resulting in a total of 8 possible amino acid combinations in the IQ domain. Reassuringly, peptide variants containing different amino acids in the IQ domain were detected using the state-of-art mass spectrometry. Biophysically, amino acid changes in the IQ di-peptide specifically attenuated the kinetic of CDI.

Physiologically, editing in the IQ domain was shown to regulate normal rhythmic firing activity of neurons in suprachiasmatic nucleus, a hypothalamic region well known for its role as the master control of biological clock in the mammalian system. Most importantly, RNA editing in the IQ domain was found selectively in the central nervous system and is conserved across different species from mouse, rat to human (Huang et al. 2012). Taken together, it is amazing that two post-transcriptional mechanisms including alternative splicing and RNA editing converge on a single exon 41 to exert overlapping function of regulating the kinetic of CDI.

Further downstream, alternative use of either exon 42 or 42a gives rise to the long-form (LF) or short-form (SF) $Ca_V1.3$ channels respectively (Singh et al. 2008). The stop codon in exon 42a results in expression of only six amino acids immediately after exon 41 and therefore resulting in the early termination of the C-terminus. Although both variants are ubiquitously expressed in the brain, the LF channels display distinctive properties such as a more depolarized-shift in window current, higher expression, lower current density and significantly diminished CDI (Singh et al. 2008). The attenuated CDI in the long-form was later explained by the presence of the CDI-inhibiting module (ICDI) domain at the distal carboxyl terminal which actively competed with calmodulin for the binding to the IQ domain (Liu et al. 2010). The anchoring of calmodulin to the preIQ-IQ domain is critical for CaM-modulated channel inactivation (Erickson et al. 2003; Van Petegem et al. 2005). The attenuated binding between calmodulin and $Ca_V1.3$ channel therefore results in much slower channel inactivation. Consistently, the absence of ICDI domain in short-form channels due to truncation of the C-terminus leads to fast CDI. Moreover, half truncation of exon 42 due to the alternative use of splice donor site and alternative use of splice acceptor and donor sites within exon 42 both resulted in frame-shifting and pre-mature termination of the C-terminus (Seino et al. 1992; Williams et al. 1992b; Bock et al. 2011; Tan et al. 2011). Expectedly, the exclusion of ICDI domain in such a splice isoform supported rapid CDI that is similar to that observed for the short-form $Ca_V1.3$ channels.

Lastly, deletion of exon 44 and use of splice acceptor site within exon 48 resulted in shortening of the C-terminus but did not result in large truncation of the C-terminus. Interestingly, both $Ca_V1.3[\Delta 44]$ and $Ca_V1.3[48S]$ channels displayed slightly slower CDI as compared to the long-form channel suggesting that inhibition of CDI by the ICDI-domain is length-dependent (Tan et al. 2011).

Apart from regulation of CDI, the truncations of the C-terminus due to half exon 41, inclusion of exon 42a and half exon 42 have additional functional implications. Firstly, early truncation of the C-terminus effectively excludes two consensus sites for PKA activity. The two sites, identified using mass spectrometry, include serine 1,743 and serine 1,816 located in exon 43 (Ramadan et al. 2009). Phosphorylation of $Ca_V1.3$ channels by PKA was known to substantially increase $Ca_V1.3$ current which potentially underlies the sympathetic control of heart rate (Qu et al. 2005). The C-terminal alternative splicing of the $Ca_V1.3$ transcripts, particularly in SAN, could therefore regulate the responsiveness of heart rate to the regulation by activation of β-adrenergic receptors via cAMP-dependent PKA. Secondly, shortening of $Ca_V1.3$ C-terminus omits C-terminal **S**rc **h**omology **3** (SH3)

domain binding motifs and **p**ostsynaptic **d**ensity-95/discs large/**z**ona occludens-1 (PDZ) binding motif which has been shown to be crucial for interaction with the scaffold protein Shank (Zhang et al. 2005). Such interaction results in postsynaptic clustering of long form $Ca_V1.3$ channels and was later found to be important for processes such as $Ca_V1.3$ dependent phosphorylated cAMP response element-binding protein (pCREB) signaling (Zhang et al. 2005) and G-protein modulation of $Ca_V1.3$ channels by D2 dopaminergic and M1 muscarinic receptors (Olson et al. 2005). In addition, the PDZ binding motif of $Ca_V1.3$ channel is also known to interact with PDZ domain containing protein, erbin. The association of erbin or harmonin with long-form $Ca_V1.3$ results in voltage dependent facilitation of the current (Calin-Jageman et al. 2007). However, harmonin reduced significantly the peak $Ca_V1.3$ $I_{Ba}$ currents by reducing surface expression of the channels (Gregory et al. 2011).

## 13.4 P/Q-Type $Ca_V2.1$

### *13.4.1 Functional Roles of $Ca_V2.1$*

P/Q-type $Ca_V2.1$ ($\alpha 1_A$) calcium channels are expressed at high levels in the cerebellum, particularly in Purkinje neurons and granule cells (Stea et al. 1994; Ludwig et al. 1997; Kulik et al. 2004), with high expression at the γ-aminobutyric acid (GABA)ergic nerve terminal (Kulik et al. 2004). These channels constitute the major pathways for $Ca^{2+}$ entry at the presynaptic terminals to initiate synaptic neurotransmitters release (Lonchamp et al. 2009). They are also found at the somatodendritic postsynaptic regions throughout the mammalian brain and spinal cord. The two different knock-out mouse strains lacking the expression of the $Ca_V2.1$ ($\alpha_{1A}$) subunit were characterized to exhibit severe phenotypes, including ataxia and dystonia. In the first knock-out line, the mice died 3–4 weeks after birth after displaying problems in motor coordination associated with cerebellar degeneration and defects in synaptic pruning (Jun et al. 1999; Miyazaki et al. 2004). On the other hand, the second knock-out line permitted observation of late-onset cerebellar degeneration, and the neurological deficits appeared prominently about 10 days after birth (Fletcher et al. 2001). Furthermore, in mice lacking the $Ca_V2.1$ subunit, the cerebella were smaller in size than that of wild-type (WT) littermates (Jun et al. 1999; Fletcher et al. 2001). Interestingly, the N-type channels ($Ca_V2.2$) functionally compensated for the absence of P/Q subunits at the calyx of Held and evoked giant synaptic currents in the calyx of Held and medial nucleus of the trapezoid body (MNTB) neurons in the $Ca_V2.1^{-/-}$null mice (Inchauspe et al. 2004). It has also been reported that presynaptic $Ca_V2.1$ calcium channels mediate short-term synaptic plasticity when interrogated in the superior cervical ganglion (SCG) neurons, and this function was regulated by the neuronal $Ca^{2+}$ sensor proteins (Mochida et al. 2008).

### 13.4.2 Mutations in $Ca_V2.1$

Mutations of the *CACNA1A* gene coding for the $Ca_V2.1$ channel have been identified in humans to be associated with several autosomal dominant neurological defects, such as familial hemiplegic migraine (FHM), episodic ataxia type 2 (EA2), and spinocerebellar ataxia type 6, SCA-6 (Pietrobon 2005; Melzer et al. 2010). Approximately 20 missense mutations (loss-of-function) associated with type-1 familial hemiplegic migraine (FHM-1) have been identified in the *CACNA1A* gene (Ophoff et al. 1996) and these FHM-1 mutations altered the voltage-dependent properties of the neuronal $Ca_V2.1$ channels (Hans et al. 1999b; Adams et al. 2009, 2010). It was found that a knock-in transgenic mouse harbouring the most common FHM-1 mutation R192Q has increased neuronal P/Q-type current and facilitation of induction and propagation of cortical spreading depression (CSD) (Tottene et al. 2009). The R192Q mutation also allowed for faster recovery from synaptic depression in the calyx of Held (Inchauspe et al. 2012). Another mutation located at the first intracellular loop of *CACNA1A* (A454T) does not cause FHM but is associated with the absence of sensorimotor symptoms in a migraine with aura pedigree as these mutant channels showed weakened regulation of VDI by $Ca_V\beta$ subunits and impaired modulation by syntaxin 1A or SNAP-25 (Serra et al. 2010).

Episodic ataxia type 2 (EA2) is an autosomal dominant neurological disorder arising from loss-of-function mutations in the *CACNA1A* gene. A clearly dominant negative effect of EA2 mutations was revealed by co-expression of several EA2 missense and truncation mutants with WT human $Ca_V2.1$ channels in mammalian cells. The co-expression of mutant $Ca_V2.1$ channels led to the retention of the WT $Ca_V2.1$ channels in the endoplasmic reticulum and the reduction of membrane expression of the WT $Ca_V2.1$ channels, resulting in reduced $Ca^{2+}$ currents (Jeng et al. 2008; Mezghrani et al. 2008). The *rolling* mouse Nagoya (RMN) is an ataxic mutant mouse, first described by Oda (Oda 1973), that carries a loss-of-function mutation in the gene encoding the $Ca_V2.1$ channels (Mori et al. 2000). Four other mutant mice exhibiting similar phenotypes are the *tottering*, *leaner*, *rocker* and *tottering-4j* mice (Fletcher et al. 1996; Pietrobon 2010). These homozygous mutant mice exhibited ataxia and increased noradrenaline, dopamine and serotonin concentrations in the RMN cerebellum (Oda 1973; Nakamura et al. 2005), but the 22 month-old heterozygous mice showed age-related emotional changes such as reduced anxiety or reduced depression due to alterations in the serotonin synaptic transmission (Takahashi et al. 2011). It has also been reported that the amplitude of the parallel fiber-mediated EPSC was drastically reduced in adult ataxic *tottering* mice of 28–35 days old (Matsushita et al. 2002). Moreover, in these *tottering* mice the feed-forward inhibition from the thalamus to layer IV neurons of the somatosensory cortex was severely impaired and the impairment of the inhibitory synaptic transmission was correlated with the onset of absence epilepsy (Sasaki et al. 2006).

Spinocerebellar ataxia type-6 (SCA-6) is caused by expansion of polyglutamine (polyQ) repeats in the cytoplasmic C-terminus of the $Ca_V2.1$ channel (Zhuchenko et al. 1997) and in human, this repeat is only present in the terminal alternative exon 47 (Soong et al. 2002). Unaltered intrinsic electrophysiological properties of $Ca_V2.1$ channels were recently confirmed in SCA-6 knock-in mice carrying expanded CAG repeats in the C-terminus of the $Ca_V2.1$ channels, and this mouse with the $Sca6^{84Q}$ mutation developed progressive motor impairment and aggregation because of the accumulation of mutant $Ca_V2.1$ channels in the Purkinje neurons (Watase et al. 2008). However, it is thought the possibility of a direct toxic effect of the polyglutamine repeat on the Purkinje neurons mediated possibly via the aberrant activation of the inositol 1,4,5-trisphosphate receptor type 1 (ITPR1). The binding of the $Ca_V2.1$ polyglutamine repeat to ITPR1 might disrupt the timing of ITPR1-dependent plasticity in cerebellar Purkinje neurons (Matsuyama et al. 1999; Restituito et al. 2000; Schorge et al. 2010). Similarly, knowing the distribution of splice variants and the combinatorial patterns of alternative exons in the $Ca_V2.1$ channels have been helpful in explaining why spinocerebellar ataxia-6 (SCA-6) pathology and phenotypic expression is mainly confined to the cerebellum and not the prefrontal cortex (Tsunemi et al. 2008).

### *13.4.3 Splice Variations of $Ca_V2.1$*

The P- (Llinas et al. 1989) and Q-type (Randall and Tsien 1995) calcium channels were identified as two different currents owing to their distinct gating, pharmacological and modulatory characteristics. However it was later shown that the different properties were actually attributed to alternative splicing at distinct sites within the $\alpha_{1A}$ subunit gene (Bourinet et al. 1999). So far, a total of seven exonic loci of the $Ca_V2.1$ gene have been shown to undergo alternative splicing as revealed by the "transcript-scanning" method (Soong et al. 2002). Notably, part of the F helix of the EF-hand domain is encoded by a pair of mutually exclusive exons 37a/37b. Alternative inclusion of either exon 37a or 37b gives rise to two channel splice variants that differ in sequence within the EF-hand-like domain (commonly known as EFa or EFb respectively) in the $\alpha_{1A}$ subunit (Zhuchenko et al. 1997; Bourinet et al. 1999; Krovetz et al. 2000; Soong et al. 2002). Functionally, the $Ca_V2.1_{EFb}$ channels displayed calcium dependent facilitation (CDF) only in combination with the exclusion of exon 47 and in response to a global rise in $Ca^{2+}$ concentration (Chaudhuri et al. 2004). However, the $Ca_V2.1_{EFa}$ channels supported robust CDF in the presence or absence of exon 47 (Chaudhuri et al. 2004). Moreover, exons 37a/37b were observed to display a developmental switch after 1–2 weeks from a high level of EFb expression to a high level of EFa expression in rodent brains. Unexpectedly, in human, there was a biphasic switch of EFb and EFa expression over development and in adult life. Besides, age and gender bias were also observed in human brain tissues, suggestive of a possible role of these EF-hand splice variants in neurophysiological specialization (Chang et al. 2007). Our unpublished data has

also demonstrated a compartmentalization of the subcellular expression of the EFa and EFb in neurons raising the question of the role of CDF of $Ca_V2.1$channels in short-term synaptic plasticity. In addition, two novel splice sites were discovered within the II-III loop of rat $Ca_V2.1$ channel that encode for the loop region that overlaps with the **syn**aptic **pr**otein **int**eraction (synprint) sites (Spafford and Zamponi 2003). Both of these splice variants lacked substantial portion of the synprint sites and in particular, the splice variant $Ca_V2.1_{\Delta 1}$ has a much lower current density and a marked depolarizing shift in the voltage dependence of inactivation (Rajapaksha et al. 2008).

By cross-linking and immunoprecipitation (CLIP) screening technique (Ule et al. 2003), it was found that binding of splicing factor Nova protein to YCAY motifs in pre-mRNA determines the outcome of splicing (Ule et al. 2006), Specifically, Nova-2 was found to regulate the alternative splicing of $Ca_V2.1$ channel by repressing inclusion of alternative exon 31a, but in contrast enhancing exon 24a inclusion (Allen et al. 2010). Functionally, the inclusion of exon 31a in $Ca_V2.1$ decreases the affinity of ω-agatoxin IVA for the channel ∼10-fold, and slowed channel activation and deactivation kinetics (Bourinet et al. 1999; Hans et al. 1999a). On the other hand it is speculated that the extracellular location of exon 24a might play a role in mediating interactions with extracellular proteins (Allen et al. 2010).

## 13.5 N-Type $Ca_V2.2$

The neuron-specific N-type calcium channels ($Ca_V2.2$, $\alpha_{1B}$) play the role to couple action potential excitation with neurotransmitter release (Takahashi and Momiyama 1993; Dunlap et al. 1995; Reuter 1995). The N-type current was identified by its irreversible inhibition by ω-conotoxin GVIA (Catterall et al. 2005) and the extensive expression pattern of the channels in the central nervous system highlighted its importance in neurophysiology (Tanaka et al. 1995).

### *13.5.1 The Physiological Functions of $Ca_V2.2$ Channels as Indicated by $Ca_V2.2^{-/-}$ Mice*

$Ca_V2.2$ knock-out mice displayed hyperactivity and prolonged vigilance state in novel environment and in darkness (Beuckmann et al. 2003). Furthermore, deletion of $Ca_V2.2$ channels results in more aggressive behavior in mice possibly due to increased firing activity of serotonin neurons in the dorsal raphe nucleus as a result of reduced upstream inhibitory neurotransmission (Kim et al. 2009). In addition, deletion of $Ca_V2.2$ channels enhanced ethanol reward while paradoxically reduced excessive ethanol consumption (Newton et al. 2004). Moreover, the channel is known to be important for pain transmission as supported by several previous studies. Firstly, these channels are extensively expressed in the superficial layer of

the dorsal horn and dorsal root ganglion (DRG) which are the main nociceptive areas at the spinal level (Altier and Zamponi 2004; Bell et al. 2004). Secondly, blocker of the N-type current diminishes the release of neuropeptide such as substance P which is intimately involved in nociception (Smith et al. 2002). More directly, knockout of $Ca_V2.2$ in mice model showed reduced threshold for mechanical and thermal pain, attenuated nociceptive response in phase II of formalin test, visceral inflammation pain model and also attenuated nociceptive symptoms in neuropathic pain model (Hatakeyama et al. 2001; Kim et al. 2001; Saegusa et al. 2001).

### *13.5.2 Alternative Splicing Pattern in $Ca_V2.2$ Transcripts and Related Functions*

The $Ca_V2.2$ channel undergoes extensive alternative splicing in at least ten exons giving rise to a large number of possible combinations. Alternative splicing affects many aspects of channel functions including the biophysical properties, synaptic trafficking, surface expression and G-protein mediated inhibition.

In the I-II loop region, the alternative use of 3' splice accepter site allows for inclusion or exclusion of $Ala^{415}$ (Genbank accession no. M92905). Inclusion of $Ala^{415}$ in rat $Ca_V2.2$ channels resulted in a positive shift of activation potential by $\sim$19 mV while the voltage dependent profile of steady-state inactivation was unchanged (Stea et al. 1999).

The II-III loop region of rat $Ca_V2.2$ channel contains over 400 amino acids and a synprint site that plays a role in synaptic targeting of the channel via interaction with synaptic proteins such as syntaxin and SNAP-25 in a $Ca^{2+}$-dependent manner (Sheng et al. 1994, 1996). Alternative splicing in this region generated channel isoforms with altered biophysical properties and different synaptic targeting patterns. Firstly, cassette exon 18a encodes 21 amino acids at the N-terminal portion of the II-III loop (Pan and Lipscombe 2000). Functionally, inclusion of exon 18a slowed down the inactivation kinetic of the N-type current in response to a train of action potential stimuli (Thaler et al. 2004). Prolonged channel opening could potentially elevate residual pre-synaptic $Ca^{2+}$ concentration that could contribute to some aspects of synaptic enhancement such as facilitation, augmentation and potentiation (Zucker and Regehr 2002). In addition, exon 18a inclusion shifted the voltage-dependent steady-state inactivation profile to more depolarizing potential specifically in the presence of $\beta_{1b}$ or $\beta_4$ subunit (Pan and Lipscombe 2000). However, although overlapping with the synprint site, it is not known if addition of 21 amino acids could affect the synaptic protein interaction. While dominating in the SCG, the expression of transcripts containing exon 18a ($Ca_V2.2$[e8a]) is reduced to around 50 % in DRG, spinal cord and caudal region of the brain and to only 20 % in rostral brain regions such as neocortex, hippocampus and cerebellum (Pan and Lipscombe 2000).

Secondly, two human $Ca_V2.2$ splice variants Δ1 (ΔArg756-Leu1139) and Δ2 (ΔLys737-Ala1001) (refer to GenBank accession number M94172 for numbering) were discovered that lack large part of the II-III loop domain including the synprint site (Kaneko et al. 2002). Biophysically, shortening of II-III loop domain positively shifted the steady-state inactivation profile and led to a faster rate of recovery from inactivation. In addition, $Ca_V2.2[\Delta1]$ variant displayed reduced sensitivity towards inhibition by ω-conotoxin MVIIA and GVIA (Kaneko et al. 2002). More importantly, deletion of the synprint site correlated directly with a drastically reduced normal synaptic targeting of both splice variants (Szabo et al. 2006). The expression of the two splice variants could be observed significantly in fetal brain and various regions of adult brain including thalamus, hippocampus, amygdala and cerebellum (Kaneko et al. 2002).

The IIIS3-IIIS4 region contained cassette exon 24a which encodes the tetra-peptide serine-phenylalanine-methionine-glycine. However inclusion or exclusion of the alternative exon did not appear to affect the activation or inactivation kinetics. Nor did it change the current-voltage (*I-V*) profile of the channel (Stea et al. 1999; Pan and Lipscombe 2000). The $Ca_V2.2$ splice variant containing exon 24a was observed in both rat brain and sympathetic ganglion (Lin et al. 1997).

Exon 31a encodes a di-peptide glutamate-threonine (ET) in the IVS3-IVS4 loop domain. Inclusion of exon 31a slowed down channel activation and potentially resulted in reduced $Ca^{2+}$ influx in response to action potential stimulation, as predicted by *in silico* modeling (Lin et al. 1999). Exon 31a is only selectively expressed in the peripheral nervous system in the DRG and SCG (Lin et al. 1999), suggesting that excitation-secretion coupling in postganglionic synapses expressing $Ca_V2.2[e31a]$ may be less efficient as compared to synapses in the CNS.

The C-terminus of the $Ca_V2.2$ channel is another region that is extensively alternatively spliced. The F-helix of the EF hand domain of rat $Ca_V2.2$ channel is encoded by a pair of mutually exclusive exons 37a and 37b. Although both $Ca_V2.2[e37a]$ and $Ca_V2.2[e37b]$ channels have the same unitary conductance, selective inclusion of e37a enhanced the expression of $Ca_V2.2$ channels and prolonged the channel open duration as revealed by single channel recording (Castiglioni et al. 2006). The higher expression of $Ca_V2.2[e37a]$ channels would be explained by a more recent discovery that $Ca_V2.2[e37a]$ isoform is more resistant towards ubiquitination and subsequent degradation by the proteasome system (Marangoudakis et al. 2012).

As compared to the $Ca_V2.2[e37b]$ which is ubiquitously expressed throughout the nervous system, the transcripts containing exon 37a is only selectively enriched in a subset of capsaicin responsive nociceptive neurons in DRG that mediates pain response to heat stimuli (Bell et al. 2004). Following selective down-regulation of $Ca_V2.2[e37a]$ by small interfering RNA (siRNA) in cultured rat DRG neurons the release of neurotransmitter substance P from the nociceptor was reduced (Altier et al. 2007). Furthermore, in vivo down-regulation of $Ca_V2.2[e37a]$ by siRNA attenuated inflammation or neuropathy induced thermal and mechanical

hyperalgesia (Altier et al. 2007). However, $Ca_V2.2$[e37a] mRNA was also found to be selectively down-regulated in rat model of neuropathic pain induced by spinal nerve ligation (Altier et al. 2007). Adding to the existing complexity, selective inclusion of exon 37a sensitizes the channel towards a novel form of $G_{i/o}$ protein-mediated voltage independent inhibition induced by activation of G-protein coupled $GABA_B$- or μ-opioid receptors (Raingo et al. 2007).

Therefore, alternative inclusion of exon 37a seems to impose opposing effects in regulating $Ca_V2.2$ channel function in pain pathway; on one hand, prolonged $Ca^{2+}$ influx through $Ca_V2.2$[e37a] would enhance neurotransmitter release allowing for effective nociception, while on the other hand, selective down-regulation of e37a containing $Ca_V2.2$ transcripts in the presence of persistent pain stimuli could result in overall reduction in expression level of the channel and during intense neuronal activity, $Ca_V2.2$e[37a] channel is susceptible to $G_{i/o}$-mediated activity independent inhibition following activation of $GABA_B$- or μ-opioid receptors, leading to the net reduction of N-type currents.

Therefore, to directly elucidate the role of $Ca_V2.2$[e37a] isoform in the pain pathway, a mouse model was developed whereby exon 37a was selectively knocked out (Andrade et al. 2010). Surprisingly, as compared to the wildtype mice, elimination of exon 37a did not result in any significant change of N-type current density in the capsaicin responsive DRG neurons, in contrast to the previous observation in transfected cell line or native nociceptors (Bell et al. 2004; Castiglioni et al. 2006), nor was basal thermal nociception affected, indicating that expression of $Ca_V2.2$[e37b] alone could compensate for the loss of $Ca_V2.2$[e37a] in mediating normal pain pathway. However, the extent of voltage independent inhibition of N-type current upon G protein activation was indeed found to be significantly reduced in the absence of exon 37a, correlating directly with reduced efficiency of morphine induced spine level analgesia in response to noxious thermal stimuli (Andrade et al. 2010). Hence, rather than a molecular target to be inhibited for pain management, the expression $Ca_V2.2$[e37a] isoform is required for effective relief of thermal pain by morphine.

The distal C-terminus of $Ca_V2.2$ channel contains PDZ and SH3 domain binding motifs that interact with the modular adaptor protein Mint-1 and CASK respectively (Maximov et al. 1999). The PDZ domain binding sequence was found to be the last four amino acids ‘DHWC’ of the C-terminus and the SH3 binding sequence is a proline rich sequence ‘PQTPLTPRP’ located at a short distance upstream of the PDZ binding motif. Both sequences are encoded by the exon 46; the last exon of $Ca_V2.2$ channel (Lipscombe et al. 2002). Coincidentally, a human $Ca_V2.2$ splice isoform (Williams et al. 1992b) was observed which utilizes an alternative 3’-splice accepter site within exon 46 (Genbank accession no. M94173.1). This type of splicing event resulted in truncation of exon 46 leading to a frame-shift and premature termination of the channel and thus effectively removing the SH3 and PDZ binding motifs. Upon transfection in matured hippocampus neurons cultured at high density, hemagglutinin-tagged $Ca_V2.2$ channels with intact C-terminus were found to be co-localized in axonal synaptic cluster with synapsin, a presynaptic marker and PSD-95, a excitatory postsynaptic marker (Maximov

and Bezprozvanny 2002). In comparison, a splice isoform of $Ca_V2.2$ channel with a truncated C-terminus showed restricted expression in the soma and proximal dendrites (Maximov and Bezprozvanny 2002). Specifically, mutating either the proline rich or the PDZ binding motif significantly reduced the number of axonal synaptic clusterings of $Ca_V2.2$ channels and mutating both sites almost completely abolished the co-localization of the channel with synapsin, which is suggestive that both the SH3 and PDZ binding sites encoded by exon 46 worked synergistically to promote synaptic targeting of the channel (Maximov and Bezprozvanny 2002). Furthermore, expression of a distal C-terminus peptide containing both motifs in cultured hippocampal neurons not only dominantly suppressed the synaptic localization of the channel, but also reduced the efficiency of depolarization induced exocytosis, emphasizing the importance of correct presynaptic targeting of the $Ca_V2.2$ channel in maintaining normal synaptic function.

## 13.6 R-Type $Ca_V2.3$

The $Ca_V2.3$ ($\alpha_{1E}$) transcript encodes the R-type calcium channel that has been shown to be insensitive to blockade by the typical antagonists against L-, P/Q- and N-type channels (Soong et al. 1993; Piedras-Renteria and Tsien 1998; Tottene et al. 2000; Wilson et al. 2000). The $Ca_V2.3$ channels were first reported in rabbit and rat brains (Niidome et al. 1992; Soong et al. 1993) and later described in human and mice brains (Schneider et al. 1994; Williams et al. 1994). These channels are widely expressed throughout central nervous system (Soong et al. 1993; Williams et al. 1994). Analysis of $Ca_V2.3$ deficient mice revealed that the $Ca_V2.3$ current accounted for the majority of R-type current in CA1 hippocampal and cortical neurons (Sochivko et al. 2002), amygdala (Lee et al. 2002) and DRG neurons (Yang and Stephens 2009), while only 47 % of R-type current in dentate granule neurons is attributed to $Ca_V2.3$ current (Sochivko et al. 2002).

### *13.6.1 Diverse Physiological Functions of $Ca_V2.3$ Channels*

$Ca_V2.3$ is identified by its specific sensitivity to spider toxin SNX-482 (Newcomb et al. 1998) which has been widely used for determining the physiological roles of the channel. Some studies have suggested that R-type current plays minor roles in mediating fast neurotransmission, pair-pulse facilitation or frequency facilitation as compared to P/Q-type current, possibly due to the more distant localization of the $Ca_V2.3$ channels from the release sites (Wu et al. 1998, 1999; Dietrich et al. 2003). Rather, the $Ca_V2.3$ current is important for accumulation of presynaptic $Ca^{2+}$ that led to a form of presynaptic LTP that is independent of N-methyl-D aspartate-receptor in the mossy fiber synapse in the mouse hippocampus (Breustedt et al. 2003; Dietrich et al. 2003). Secondly, $Ca_V2.3$ channels are implicated in

mediating pain response as they are highly expressed in DRG and dorsal horn of spinal cord and consistently, $Ca_V2.3$ knockout mice displayed attenuated response toward formalin induced somatic nociception (Saegusa et al. 2000). In addition, $Ca_V2.3$ channels have been shown to play a role in nociception during neuropathy caused by partial sciatic nerve ligation in mice (Yang and Stephens 2009). However, it has also been suggested that expression of $Ca_V2.3$ in the periaqueductal gray could mediate the descending anti-nociception pathway (Saegusa et al. 2000). Inhibiting $Ca_V2.3$ channels in different tissues could therefore result in contrasting effects in pain management. Thirdly, $Ca_V2.3$ knockout mice exhibited enhanced fear in open field tests (Saegusa et al. 2000; Lee et al. 2002), emphasizing the important role of $Ca_V2.3$ currents for some aspects of processing of emotional stimuli in brain regions such as amygdala. Most recently, $Ca_V2.3$ channels were found to be important for oscillatory burst firing activity of neurons of the reticular thalamus (RT) that is associated with absence epilepsy (Zaman et al. 2011). Outside the CNS, $Ca_V2.3$ currents played significant roles in hormonal secretion from neuroendocrine cells such as beta cells in the islets of Langerhans (Grabsch et al. 1999; Vajna et al. 2001) and chromaffin cells in the adrenal gland (Albillos et al. 2000). Down-regulation and deletion of $Ca_V2.3$ gene disrupted the glucose induced insulin release and stress induced hyperglycemia (Pereverzev et al. 2002a, c).

### *13.6.2 Alternative Splicing Pattern in $Ca_V2.3$ Transcripts and Related Functions*

The $Ca_V2.3$ transcripts have been shown to be alternatively spliced at three different exon loci, namely exon 19 and exon 20 in the II-III loop and exon 45 in the C-terminus, giving rise to a total of six channel splice variants (Pereverzev et al. 2002b). Alternative inclusion of cassette exon 19 results in addition of 19 amino acids in the II-III loop region (Soong et al. 1993; Schneider et al. 1994; Williams et al. 1994; Mitchell et al. 2002). The selective use of splice donor and receptor sites within exon 20 results in deletion of seven amino acids and such splice variant is only detected in the rabbit (Niidome et al. 1992). Lastly, expression of cassette exon 45 results in inclusion of 43 amino acids in the C-terminus (Soong et al. 1993; Schneider et al. 1994; Williams et al. 1994; Mitchell et al. 2002).

Patch clamp electrophysiological study subsequently revealed that expression of exon 19 slowed down channel inactivation, correlating with faster recovery from inactivation in the presence of extracellular $Ca^{2+}$ as charge carriers, while other properties such as current density, *I-V* relationship, voltage dependent activation and inactivation profiles of the channel remained unchanged (Pereverzev et al. 2002b). Interestingly, a consensus casein kinase I1 phosphorylation site 'SMWE' was detected within exon 19 (Williams et al. 1994) but its functional role has yet to be determined. On the other hand, the presence or absence of seven amino acids in exon 20 and exon 45 did not result in significant change in the biophysical properties

of the channel (Pereverzev et al. 2002b). Lastly, although both $Ca_V2.3$[e45] and $Ca_V2.3$[Δe45] are expressed equally in the mouse brain, $Ca_V2.3$[e45] transcripts were found to be dominant in human cerebellum (Pereverzev et al. 1998). A protein kinase C consensus site has been identified in exon 45 but yet to be verified (Schneider et al. 1994).

More recently, it was found that the two splice variants $Ca_V2.3$[Δe19, e45] and $Ca_V2.3$[Δe19, Δe45] make up all the $Ca_V2.3$ channels in both trigeminal ganglion and DRG neurons, with $Ca_V2.3$[Δe19, e45] being the dominant form in both tissues (Fang et al. 2007, 2010). Specifically, $Ca_V2.3$[Δe19, e45] is preferentially expressed in small nociceptive neurons that are also positive for tyrosine-kinase A (trkA), isolectin B4 (IB4)-negative and transient receptor potential vanilloid 1 (TRPV1)-positive (Fang et al. 2007, 2010). Interestingly, IB4-negative neurons are known to secrete calcitonin gene-related neuropeptide and substance P (Snider and McMahon 1998) and (TRPV1)-positive neurons mediate thermal nociception and inflammatory hyperalgesia (Szallasi and Blumberg 1999). Overlapping expression of channel variants such as $Ca_V2.3$[Δe19, e45] and $Ca_V2.2$[e37a] in TRPV1-positive neurons (Bell et al. 2004) could have similar function in mediating nociception and indeed deletion of $Ca_V2.3$ attenuated somatic inflammatory pain (Saegusa et al. 2000) and similarly, targeting specific splice variant of $Ca_V2.3$ in nociceptors could be a potential therapeutic target in pain management.

## 13.7 Conclusion

VGCCs are indispensible in many aspects of neuronal activity ranging from neural development, cell excitability, synaptic plasticity, neurotransmitter release to excitation-transcription coupling. It would be unimaginable that to complete such a daunting list of tasks requires only a handful of VGCCs. However, the cellular machinery utilizes powerful post-transcriptional mechanisms including alternative splicing and RNA editing to vastly expand the transcriptome. Here we highlighted how such mechanisms when applied to $Ca_V$ channels generated alternatively spliced or edited variants with overt or subtle alterations in channel properties that are optimized or adapted for different biological niches. Information regarding distribution of patho-physiological specific channel variants not only allows for discovery of useful biomarker but also development of new therapeutic targets. On the other hand, the phenotypic expression of $Ca_V$ channel mutations could be influenced by the backbone combinatorial assortment of alternatively spliced exons within the channels and by where such splice combinations are expressed selectively in different brain regions or neuronal types. In the long-term, the acquisition of knowledge of the dynamic regulation of the inclusion or exclusion of alternatively spliced exons via activation of intrinsic or external stimuli will be a major thrust in the field. Such knowledge will contribute to spatial-temporal expression of splice

variants and will also provide another means to modulate channel function to adapt to pathological conditions. Harnessing next-generation RNA sequencing technology will certainly help towards the better understanding of the extent and physiological and pathological significance of alternative splicing and RNA editing, and hopefully also at the level of the single neuron.

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