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Review

# Alternative splicing: Functional diversity among voltage-gated calcium channels and behavioral consequences ☆

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ABSTRACT

Neuronal voltage-gated calcium channels generate rapid, transient intracellular calcium signals in response to membrane depolarization. Neuronal $Ca_V$ channels regulate a range of cellular functions and are implicated in a variety of neurological and psychiatric diseases including epilepsy, Parkinson's disease, chronic pain, schizophrenia, and bipolar disorder. Each mammalian *Cacna1* gene has the potential to generate tens to thousands of $Ca_V$ channels by alternative pre-mRNA splicing, a process that adds fine granulation to the pool of $Ca_V$ channel structures and functions. The precise composition of $Ca_V$ channel splice isoform mRNAs expressed in each cell are controlled by cell-specific splicing factors. The activity of splicing factors are in turn regulated by molecules that encode various cellular features, including cell-type, activity, metabolic states, developmental state, and other factors. The cellular and behavioral consequences of individual sites of $Ca_V$ splice isoforms are being elucidated, as are the cell-specific splicing factors that control splice isoform selection. Altered patterns of alternative splicing of $Ca_V$ pre-mRNAs can alter behavior in subtle but measurable ways, with the potential to influence drug efficacy and disease severity. This article is part of a Special Issue entitled: Calcium channels.



**Contents**



## 1. Introduction

Voltage-gated calcium channel ($Ca_V$) currents originate from the activity of multiple classes of channels with different pharmacological sensitivities and unique functional properties. Neuronal $Ca_V$ channels are implicated in a variety of neurological and psychiatric diseases including epilepsy, Parkinson's disease, chronic pain, schizophrenia and bipolar disorder [1–9]. Relatively large differences in the voltage-dependence of channel activation between $Ca_V3$ (T-type) channels on the one hand, and $Ca_V1$ (L-type) and $Ca_V2$ (N, P/Q, R-types) channels on the other offered the first clues that many cells expressed more than one type of voltage-gated calcium channel [10–12]. Genome and transcriptome sequencing showed that mammalian neurons can express up to 9 of 10 different $Ca_V$ $\alpha1$ subunit genes [13,14]. Low threshold activation and slow deactivation characteristics are features of all $Ca_V3$ channels that set them apart from $Ca_V1$ and $Ca_V2$ family members [15].

With the exception of the skeletal muscle $Ca_V1.1$ that functions primarily as a voltage sensor, the unifying function of $Ca_V$ channels is

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rapid control of voltage-dependent calcium entry [16,17]. Calcium entering through different $Ca_V$ channels activates distinct signaling pathways depending on the unique sub-cellular localization, protein associations, and functional properties of $Ca_V$ channels [13]. Thus, certain $Ca_V$ channels are commonly associated with certain cellular functions, but new roles for $Ca_V$ channels continue to be discovered. $Ca_V1$ channels expressed in neurons (encoded by *Cacna1c*, *Cacna1d*, and *Cacna1f* genes) support a range of calcium-dependent processes including modulation of gene expression, long and short-term changes in synaptic plasticity ($Ca_V1.2$, $Ca_V1.3$) [18–20], transmitter release at sensory nerve terminals ($Ca_V1.3$, $Ca_V1.4$) [21,22], and intrinsic spiking ($Ca_V1.3$) [23]. $Ca_V2$ channels (encoded by *Cacna1a*, *Cacna1b*, and *Cacna1e* genes) are primarily located at presynaptic nerve terminals where they control voltage-dependent calcium entry that triggers transmitter release [24,25]. $Ca_V3$ channels (*Cacna1g*, *Cacna1h*, *Cacna1i* genes) underlie pacemaking in many neurons including thalamic relay neurons [4,26].

This review is focused on the even finer granulation of structural and functional diversity among $Ca_V$ channels that originates from each major $Ca_V$ channel gene. Distinct $Ca_V$ channels expressed in a given cell, distinguished by relatively small discrete differences in amino acid sequence, may number in the tens to hundreds depending on the extent of alternative pre-mRNA splicing [15,27]. Several sites of alternative splicing are present in *Cacna1* genes predicting variations in amino acid sequence (*e.g.* see Fig. 1 for *Cacna1b*). Assuming each exon is regulated independent of the others, the number of discrete mRNA isoforms possible from each *Cacna1* gene is potentially staggering ($2^N$ for N sites of alternative splicing). Analyses of different brain regions at different stages of development show that the composition of the pool of $Ca_V$ mRNA splice isoforms varies with cell-type, state of development, and possibly neuronal activity [15,28]. This suggests that anticipated subtle functional differences among splice isoforms within a given $Ca_V$ family are either individually or collectively contributing in important ways to neuronal processes. Pre-mRNA splicing is also implicated in many neurological diseases. Disease-causing mutations can disrupt splicing such as inherited frontotemporal dementia and Parkinsonism linked to chromosome 17, amyotrophic lateral sclerosis, spinocerebellar ataxia 8, and myotonic dystrophy [29]. Alternative splicing can play a role in modifying disease such as in the case of Timothy syndrome [30]. The cellular and behavioral consequences of only a few $Ca_V$ splice isoforms are known (discussed below), but this should change as new methods are developed.

### 1.1. Alternative pre-mRNA splicing

Alternative pre-mRNA splicing is particularly prevalent in the mammalian brain [31,32], consistent with the theory that alternative splicing evolved in parallel with biological complexity [33]. Alternative splicing is essential for normal neuronal development, axon targeting, neuronal excitability, and neural circuit formation [27,34–38]. Several excellent reviews outline the molecular interactions involved in alternative pre-mRNA splicing [39–42]. In brief, this form of pre-mRNA processing occurs in the cell's nucleus and it is controlled by the concerted actions of cell-specific splicing factors (SFs). These cell-specific SFs bind to consensus motifs on pre-mRNAs and influence the action of the spliceosome by promoting or repressing inclusion of alternatively spliced exons, and promoting or repressing the use of alternative splice acceptor or donor sites at intron/exon boundaries. The cell-specific features of alternative pre-mRNA splicing are controlled by the collective action of cell-specific SFs that bind to elements encoded in each gene [40,43–49].

SFs known to control alternative pre-mRNA splicing in neurons include Nova1/2 [45], nPTB [50–52], rbFox1/2/3 [53–55] and SF2/AF [56]. Networks of genes targeted by specific SFs have been generated from genome-wide analyses of pre-mRNAs that bind them. Based on data from these types of studies, certain SFs are shown to associate with genes that control particular aspects of neuronal function. For example, Nova appears to preferentially regulate alternative splicing of pre-mRNAs encoding proteins found at inhibitory synapses

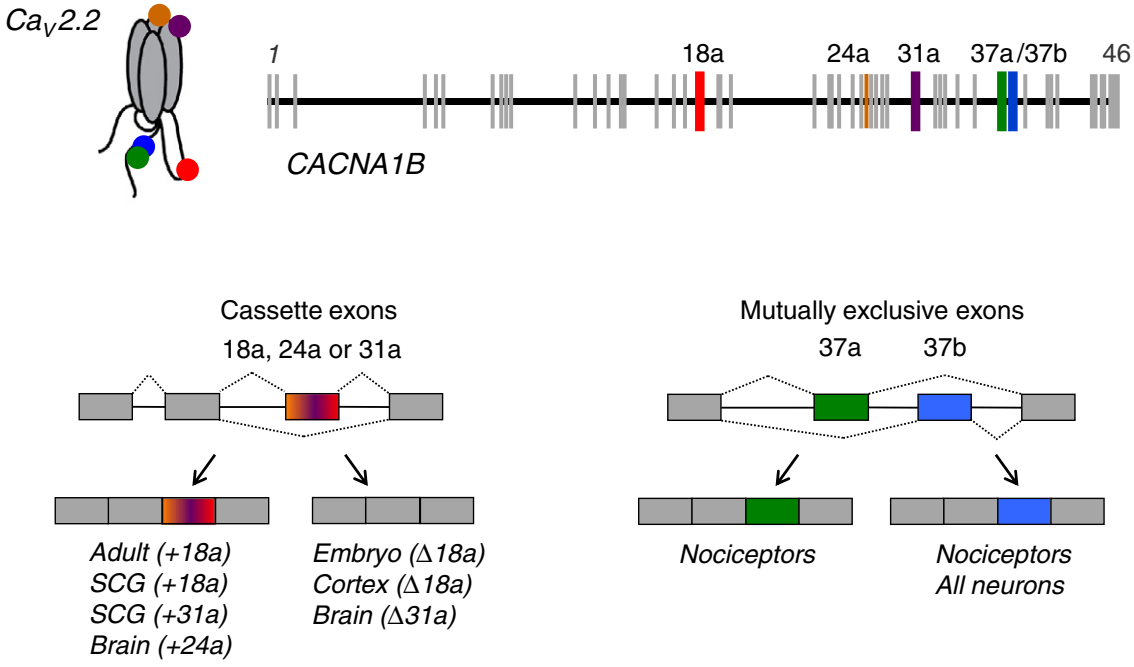


**Fig. 1.** Alternatively spliced exons in *Cacna1b* and patterns of expression. *Cacna1b* pre-mRNA undergoes extensive alternative splicing generating 10 s to 100 s of unique $Ca_V2.2$ proteins in neurons. Alternatively spliced exons e18a, e24a, and e31a are cassette exons. E37a and e37b are mutually exclusive exons. Alternatively spliced exons are expressed in different regions of the nervous system, in different types of cells, and at different stages of development. Examples of different expression patterns of splice isoforms are shown for embryonic and adult brain, superior cervical ganglia (SCG), cortex, and nociceptors. The tissue distribution and functional consequences of these exons on $Ca_V2.2$ channel properties have been described in a series of publications [15,28,85,86,88,119,120].