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Voltage-gated Na⁺ channels: Potential for β subunits as therapeutic targets

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Abstract

Background—Voltage gated Na⁺ channels (VGSCs) contain a pore-forming α subunit and one or more β subunits. VGSCs are involved in a wide variety of pathophysiologies, including epilepsy, cardiac arrhythmia, Multiple Sclerosis, periodic paralysis, migraine, neuropathic and inflammatory pain, Huntington's disease, and cancer. Increasing evidence implicates the β subunits as key players in these disorders.

Objective—The purpose of this report is to review the recent literature describing the multifunctional roles of VGSC β subunits in the context of their role(s) in disease.

Methods—An extensive review of the literature on β subunits was performed.

Results/conclusion— β subunits are multifunctional. As components of VGSC complexes, β subunits mediate signaling processes regulating electrical excitability, adhesion, migration, pathfinding, and transcription. β subunits may prove useful in disease diagnosis and therapy.

Keywords

β subunit; Cancer; Cell adhesion; Development; Epilepsy; Migration; Voltage-gated Na⁺ channel

1. Introduction

Voltage-gated Na⁺ channels (VGSCs) are required for action potential initiation and propagation in excitable cells (Hodgkin and Huxley, 1952; Hille, 1992). Their expression has also been reported in cell types that are traditionally thought of as non-excitabile, including glia, human vascular endothelial cells, human epidermal keratinocytes, and carcinoma cells, where their role is less clear (Barres et al., 1990; Gautron et al., 1992; Gosling et al., 1998; Diss et al., 2004; Karadottir et al., 2008; Zhao et al., 2008). VGSCs exist as heteromeric membrane-bound protein complexes that typically consist of a single pore-forming α subunit in association with one or more β subunits (Figure 1) (Catterall, 1992b; 2000). There are 10 mammalian α subunits, Na_v1.1–Na_v1.9 and Na_x, encoded by genes *SCN1A-SCN11A* (Goldin et al., 2000). The α subunits differ from one another in terms of subtly different tissue-specific expression, voltage-dependent and kinetic properties, and pharmacological sensitivity (Clare et al., 2000). Aberrant VGSC functional expression/activity has been identified as a major contributing factor in a number of human pathologies, including cardiac arrhythmia, epilepsy, pain, periodic paralysis, migraine, Multiple Sclerosis, and cancer (Fontaine et al., 1990; Wallace et al., 1998; Audenaert et al., 2003; Dichgans et al., 2005; Fraser et al., 2005; George, 2005; Lopez-Santiago et al., 2006; Waxman, 2006; Cummins et al., 2007; Lopez-Santiago et al., 2007; Waxman, 2007). The differential pharmacological sensitivity of the VGSC α subunits to activity-modulating

drugs provides the basis for several VGSC therapies currently in use in the clinic (Clare et al., 2000; George, 2005).

The mammalian VGSC β subunit family consists of four members, $\beta 1$ through $\beta 4$, encoded by genes *SCN1B-SCN4B* (Table 1). Species-specific alternative splicing has been reported for *SCN1B*. Alternative splicing within exon 5 of the zebrafish *SCN1B* ortholog *scn1ba* results in two splice variants, *scn1ba_tv1* and *scn1ba_tv2*, each with novel C-terminal domains and tissue-specific expression patterns (Fein et al., 2007). In rat, the alternative splice product $\beta 1.2$ contains a novel 3' untranslated region generated by retention of intron 5 (Dib-Hajj and Waxman, 1995). Another rat splice variant, $\beta 1A$, contains a novel transmembrane domain and intracellular C-terminus, encoded by retention of intron 3 (Kazen-Gillespie et al., 2000). Similarly, the human *SCN1B* alternative splice product, $\beta 1B$, is produced by retention of intron 3 (Qin et al., 2003). However, the C-terminus of $\beta 1B$ shares less than 33 % sequence identity with rat $\beta 1A$, and has a significantly different expression pattern *in vivo* (Qin et al., 2003). $\beta 1$ and $\beta 3$ are non-covalently associated with the α subunit, whereas $\beta 2$ and $\beta 4$ are covalently linked to the α subunit by a disulfide bond (Isom et al., 1992; Isom et al., 1995; Morgan et al., 2000; Yu et al., 2003). All four β subunit proteins share a similar structure, consisting of one N-terminal extracellular immunoglobulin domain, one transmembrane segment, and a small intracellular domain. All four β subunits shift voltage-dependent gating and alter Na^+ current kinetics expressed by various VGSC α subunits in heterologous expression systems, although effects are varied among reports. These results are reviewed in detail elsewhere (Catterall, 1992b; Isom et al., 1994; Adelman, 1995; Catterall, 2000). Early studies demonstrated that, when co-expressed in *Xenopus* oocytes with $Na_v1.2$, $\beta 1$ and $\beta 2$ both increase the peak Na^+ current, accelerate inactivation, and shift the voltage-dependence of activation and inactivation to more negative potentials (Isom et al., 1992; Isom et al., 1995). These effects are similar, but more subtle, when the β subunits are co-expressed in mammalian expression cell lines (Isom et al., 1994). The roles of $\beta 1$ and $\beta 2$ in modulating Na^+ current *in vivo* are less clear. For example, in hippocampal neurons acutely isolated from *Scn2b* null mice there is a negative shift in the voltage-dependence of VGSC inactivation and a reduction in Na^+ current density (Chen et al., 2002). However, no changes in Na^+ current were observed in hippocampal neurons from *Scn1b* null mice (Chen et al., 2004). The multiplicity of VGSC α and β subunits expressed in neurons suggests that subtle modulation of channel gating and kinetics may be masked or compensated for by other α or β subunit gene products, and/or may be neuron cell type-specific. We have proposed that, in neurons *in vivo*, the β subunits may play major functional roles aside of, or in addition to, kinetic modulation of Na^+ currents (Isom et al., 1994). In agreement with this notion, VGSC β subunits are classified as members of the immunoglobulin superfamily of cell adhesion molecules, and as such, play significant non-conducting roles, including directing VGSC insertion into the plasma membrane, participating in homophilic and heterophilic cell adhesion, regulating cell migration and axonal pathfinding, and modulating transcription of VGSC α subunit genes (Isom et al., 1994; Isom and Catterall, 1996; Malhotra et al., 2000; McEwen and Isom, 2004; Kim et al., 2007; Brackenbury et al., 2008a).

In this review, we will present the recent evidence demonstrating that, as part of VGSC macromolecular signaling complexes, β subunits play key roles in regulating cellular excitability, aspects of central nervous system development and plasticity, and metastatic activity in cancer (Chen et al., 2002; Chen et al., 2004; Chioni et al., 2006; Lopez-Santiago et al., 2007; Brackenbury et al., 2008a). As such, they may present a novel therapeutic target in a variety of pathophysiological disorders.

2. Excitability

Several human mutations have been reported in *SCN1B* that result in generalized epilepsy with febrile seizures plus, temporal lobe epilepsy, idiopathic ventricular fibrillation (Brugada syndrome), and heart block, providing a role for $\beta 1$ in regulation of cellular excitability (Table 2) (Wallace et al., 1998; Meadows et al., 2002; Wallace et al., 2002; Audenaert et al., 2003; Scheffer et al., 2007; Watanabe et al., 2008). In addition, a mutation (L179F) has been reported in $\beta 4$ that results in long-QT syndrome (Medeiros-Domingo et al., 2007). The generalized epilepsy with febrile seizures plus $\beta 1$ C121W mutation increases VGSC availability at hyperpolarized potentials, and reduces rundown during high frequency activity when co-expressed with α subunits in mammalian cell lines, which may explain the hyperexcitable phenotype *in vivo* (Meadows et al., 2002). In contrast, the generalized epilepsy with febrile seizures plus mutations R85C and R85H appear to render $\beta 1$ incapable of modulating $\text{Na}_v 1.2$ currents when co-expressed in human embryonic kidney cells (Xu et al., 2007). Brugada syndrome- and cardiac block-associated nonsense mutations at W179 in $\beta 1B$ results in a truncated protein lacking the membrane spanning segment and intracellular C-terminus, which is incapable of modulating $\text{Na}_v 1.5$ currents in transfected Chinese hamster ovary cells (Watanabe et al., 2008). Another Brugada syndrome- and cardiac block-related missense mutation, E87Q, is also incapable of modulating $\text{Na}_v 1.5$ currents, and exerts a dominant negative effect on native $\beta 1$ (Watanabe et al., 2008). Clearly, mutations in $\beta 1$ and $\beta 1B$ have diverse effects in these various expression systems. Further studies are necessary to confirm/extend these findings in neurons and cardiac cells *in vivo*.

In the optic nerve of *Scn1b* null mice, the fastest components of the compound action potential are slowed, and the number of mature nodes of Ranvier are reduced, although $\text{Na}_v 1.6$ is localized normally to the nodal gap (Chen et al., 2004). In addition, *Scn1b* null mice appear ataxic and, from approximately postnatal day 10, display frequent spontaneous bilateral myoclonic seizures, suggesting that $\beta 1$ plays a key role in regulation of neuronal excitability during early postnatal development, possibly *via* modulation of α subunits (Chen et al., 2004). A similar ataxic, epileptic phenotype has been observed for *Scn1a* heterozygous and null mice, in which a reduction in Na^+ currents in hippocampal GABAergic inhibitory interneurons is postulated to underlie the hyperexcitability (Yu et al., 2006; Ragsdale, 2008). Given that reduced $\text{Na}_v 1.1$ protein was also observed in the hippocampus of *Scn1b* null mice (Chen et al., 2004), it is possible that $\beta 1$ may modulate neuronal excitability *in vivo* *via* regulation of *Scn1a*/ $\text{Na}_v 1.1$ expression.

Electrocardiograms recorded from *Scn1b* null mice display slowed heart rate (specifically prolonged QT and RR intervals), and action potentials recorded from *Scn1b* null acutely dissociated ventricular myocytes are slower to repolarize (Lopez-Santiago et al., 2007). In addition, acutely dissociated ventricular myocytes from *Scn1b* null mice have increased $\text{Na}_v 1.5$ protein expression, and increased peak and transient VGSC currents (Lopez-Santiago et al., 2007). Together, these findings suggest that $\beta 1$ may modulate cardiac excitability through regulation of $\text{Na}_v 1.5$ expression/activity. The transient outward current, carried by the inward rectifier voltage-gated K^+ channel $\text{K}_v 4.3$, and which underlies cardiac action potential repolarization (Ishihara et al., 2002), is modulated by VGSC $\beta 1$ when co-expressed in human embryonic kidney 293 cells (Deschenes and Tomaselli, 2002), raising the intriguing possibility that $\beta 1$ may also modulate cardiac excitability *via* K^+ channel(s).

Scn2b null mice display increased susceptibility to pilocarpine-induced seizures, but in contrast to *Scn1b* null mice, appear normal in other neurological tests (Chen et al., 2002; Chen et al., 2004). Association with $\beta 2$ and concomitant insertion into the plasma membrane are the final steps in VGSC biosynthesis in primary neurons *in vivo* (Schmidt and Catterall, 1986). Consistent with this, *Scn2b* null hippocampal neurons display decreased

tetrodotoxin-sensitive VGSC α subunit expression at the cell surface, and an elevation in threshold for action potential generation in the optic nerve (Chen et al., 2002). In the experimental allergic encephalomyelitis model of Multiple Sclerosis, survivability is enhanced in *Scn2b* null mice as a result of decreased $\text{Na}_v1.2$ upregulation in response to demyelination (O'Malley and Isom, 2006). Together, these findings suggest that in central nervous system neurons *in vivo*, it is likely that the major role of $\beta 2$ is in steady-state stabilization of VGSC complexes at the plasma membrane, rather than direct modulation of α subunit gating and/or kinetics. A slightly different situation exists in the peripheral nervous system. In small-fast dorsal root ganglion neurons isolated from *Scn2b* null mice, tetrodotoxin-sensitive Na^+ current is reduced, and kinetics of activation and inactivation are slowed, without a change in tetrodotoxin-resistant current, altering the pain response (Lopez-Santiago et al., 2006). The reduction in tetrodotoxin-sensitive current is supported by a reduction in tetrodotoxin-sensitive VGSC mRNA and protein (specifically *Scn9a*/ $\text{Na}_v1.7$), suggesting that $\beta 2$ may modulate α subunit mRNA and protein expression in DRG neurons (Lopez-Santiago et al., 2006). In addition, $\beta 2$ protein is upregulated in rat sensory neurons following spared nerve injury, resulting in mechanical allodynia-like behavior (Pertin et al., 2005). This spared nerve injury -induced behavior is absent in *Scn2b* null mice, suggesting that $\beta 2$ upregulation following spared nerve injury is functionally significant, likely through interaction with tetrodotoxin-sensitive VGSC α subunits (Pertin et al., 2005). In summary, $\beta 2$ may regulate tetrodotoxin-sensitive α subunit functional activity *in vivo* at multiple levels, including mRNA expression, protein stabilization/trafficking, and direct channel modulation, depending on the class of neuron and α subunit gene(s) being expressed.

3. Adhesion

It is well established that many voltage-gated ion channels are involved in signaling mechanisms, aside of ion conduction, often *via* their auxiliary subunits (Kaczmarek, 2006). As immunoglobulin superfamily cell adhesion molecules, $\beta 1$ and $\beta 2$ participate in *trans*-homophilic adhesion, resulting in cellular aggregation and ankyrin recruitment when expressed in *Drosophila* S2 cells, both in the presence and absence of VGSC α subunits (Figure 2) (Malhotra et al., 2000). $\beta 2$ interacts with the extracellular matrix proteins tenascin-C and tenascin-R (Srinivasan et al., 1998). Both $\beta 1$ and $\beta 2$ interact with fibronectin-like repeats and epidermal growth factor-like repeats in the amino-terminal domain of tenascin-R (Figure 2) (Xiao et al., 1999). In addition, epidermal growth factor-like peptide increases the Na^+ current amplitude recorded in *Xenopus* oocytes expressing $\text{Na}_v1.2$ and $\beta 2$, suggesting that tenascins may regulate VGSC cell surface localization and/or functional activity *via* adhesive interactions with the β subunits (Xiao et al., 1999).

$\beta 1$ interacts heterophilically with N-cadherin, and several immunoglobulin superfamily cell adhesion molecules, including contactin, neurofascin-155, neurofascin-186, NrCAM, and VGSC $\beta 2$, suggesting that $\beta 1$ may serve as a critical link between VGSCs and cell adhesion molecules at nodes of Ranvier (Figure 2) (Kazarinova-Noyes et al., 2001; Malhotra et al., 2004; McEwen and Isom, 2004; McEwen et al., 2004). $\beta 1$ - $\beta 1$ homophilic adhesion recruits ankyrin, thus providing a link between VGSCs and the cytoskeleton, which may be important in VGSC-regulated cellular adhesion and/or migration (Malhotra et al., 2002). Tyrosine residue Y181 in the intracellular domain of $\beta 1$ is required for the recruitment of ankyrin, and this interaction is phosphorylation state-dependent: phosphorylation of Y181 abrogates association of $\beta 1$ and ankyrin in heart membranes (Malhotra et al., 2002; Malhotra et al., 2004). Furthermore, phosphorylated $\beta 1$ colocalizes to the intercalated disks with connexin-43, N-cadherin, and $\text{Na}_v1.5$, while non-phosphorylated $\beta 1$ colocalizes with ankyrin in the t-tubules, suggesting that phosphorylation of Y181 may regulate the

subcellular distribution of $\beta 1$ and its interaction with other CAMs (Malhotra et al., 2004) in heart.

Interaction of $\beta 1$ with contactin, neurofascin-186, or $\beta 2$ adjusts the steady-state trafficking balance of VGSC α subunits in favor of externalization to the plasma membrane in transfected Chinese hamster lung cells, causing an increase in Na^+ current density (Kazarinova-Noyes et al., 2001; McEwen et al., 2004). These observations raise interesting questions regarding the regulation of cellular excitability *in vivo* by the β subunits. For example, is regulation of VGSC α subunit expression/activity in neurons dependent on cell-adhesive interactions *via* the β subunits? Might the multiple binding partners of $\beta 1$ also play a role in regulation of excitability? In considering these questions, it is important to note that all the epilepsy mutations reported to date, and the Brugada syndrome/heart block mutation E87Q, are within the $\beta 1$ immunoglobulin loop, suggesting that adhesion may be important clinically (Table 2). Indeed, the C121W mutation in $\beta 1$ disrupts *trans*-homophilic adhesion and cellular aggregation in transfected *Drosophila* S2 cells (Meadows et al., 2002). Further work is required to integrate the adhesive with the conductive roles of β subunits and assess whether abnormalities in this functional balance may be involved in VGSC channelopathies.

4. Neuronal migration and pathfinding

Neurite outgrowth in cerebellar granule neurons is enhanced when $\beta 1$ is expressed in a fibroblast cell monolayer substrate, *via trans*-homophilic cell adhesive interactions, and is blocked in cerebellar granule neurons from *Scn1b* null mice (Davis et al., 2004). In contrast, neither $\beta 2$ nor $\beta 4$ expression in fibroblast monolayers increased neurite extension in cerebellar granule neurons, suggesting that $\beta 1$ specifically is required (Davis et al., 2004). $\beta 4$ expression promotes neurite extension of mouse Neuro2a neuroblastoma cells, and increases dendritic spine density in hippocampal neurons, although it is not clear whether this is due to *trans*-homophilic adhesion (Oyama et al., 2006; Miyazaki et al., 2007). In addition, *SCN4B* mRNA is downregulated in brain in Huntington's disease and its loss may contribute to neuritic degeneration (Oyama et al., 2006). Therefore, β subunit-mediated neurite extension may be cell and/or gene-specific.

Recent evidence suggests a role for $\beta 1$ in regulation of neuronal migration and pathfinding in the developing CNS *in vivo*. In *Scn1b* null mice, the migration path of corticospinal axons, from the ventral pyramid region of the hindbrain across the midline to the dorsal column of the spinal cord, is disrupted, resulting in significant axonal defasciculation, both at the pyramidal decussation, and in the dorsal column (Brackenbury et al., 2008a). Other immunoglobulin superfamily cell adhesion molecules, including L1-CAM and NCAM, are also required for normal migration in the corticospinal tract. However, the functional role of $\beta 1$ appears to be unique. In both *L1cam* and *Ncam1* null mice, many axons fail to cross the midline at the pyramidal decussation, suggesting that these cell adhesion molecules serve as guidance cues for migrating corticospinal axons (Cohen et al., 1997; Rolf et al., 2002). In contrast, $\beta 1$ appears to modulate fasciculation rather than growth cone guidance *per se* in this tract (Brackenbury et al., 2008a). In addition, in the cerebellum of *Scn1b* null mice, there is defasciculation of parallel fibers and the migration of granule neurons through the molecular layer of the cerebellum is disrupted, resulting in accumulation of granule neurons in the external germinal layer (Brackenbury et al., 2008a).

$\beta 1$ -mediated neuronal migration is regulated by a signaling mechanism operating through lipid rafts (Figure 3) (Brackenbury et al., 2008a). Immunoglobulin superfamily cell adhesion molecules, including β subunits, localize to lipid rafts (Olive et al., 1995; Kasahara et al., 2002; Niethammer et al., 2002; Schafer et al., 2004; Wong et al., 2005; Brackenbury et al., 2008a). In addition, $\beta 1$ contains a putative palmitoylation site juxtaposed to the inner

membrane leaflet, which may be responsible for its recruitment to lipid rafts (McEwen et al., 2004). β 1-mediated neurite outgrowth is proposed to involve signaling through the lipid raft-associated non-receptor tyrosine kinase fyn, similar to NCAM (Beggs et al., 1994; Kolkova et al., 2000; Brackenbury et al., 2008a). However, non-lipid raft associated signaling *via* the fibroblast growth factor receptor tyrosine kinase, which can also regulate NCAM-mediated neurite outgrowth, has no effect on β 1-mediated neurite outgrowth even though a subpopulation of β 1 subunits localizes to non-raft regions in brain (Niethammer et al., 2002; Sanchez-Heras et al., 2006; Maness and Schachner, 2007; Brackenbury et al., 2008a). The level of β 1 protein is significantly reduced, and β 1-mediated neurite outgrowth is inhibited, in mice lacking the glycoposphatidylinositol-anchored cell adhesion molecule contactin (*Cntn* null), suggesting that contactin may be required to enhance β 1 expression and/or activity (Brackenbury et al., 2008a). Interestingly, *Cntn* null mice have a similar phenotype to *Scn1b* null mice, including ataxia, defective CGN migration, and early postnatal lethality (Berglund et al., 1999), raising the possibility that the *Cntn* null may also be a phenotypic *Scn1b* hypomorph. Further work is required to establish the relationship between contactin and β 1 expression/activity.

5. Proteolytic cleavage

VGSC β subunits are potential secretase substrates *in vivo* and are postulated to undergo sequential proteolytic cleavage in a similar fashion to the amyloid precursor protein (Figure 4). All four VGSC β subunits contain cleavage sites for the β -site amyloid precursor protein-cleaving enzyme (BACE1; also called β -secretase), on the extracellular face, juxtaposed to the transmembrane region (Wong et al., 2005). Cleavage at this site results in shedding of the soluble extracellular immunoglobulin domain, and generation of remaining membrane-bound β subunit C-terminal fragments (Wong et al., 2005). In addition, β 2 is cleaved by the α -secretase enzyme ADAM10, at a site adjacent to the transmembrane region, resulting in immunoglobulin domain shedding and generation of β 2-C-terminal fragments (Kim et al., 2005). Following ectodomain shedding by BACE1 and/or α -secretase, the β subunits undergo cleavage by presenilin-1 and/or presenilin-2, in the γ -secretase complex, thus releasing soluble β subunit intracellular domains (Kim et al., 2005; Wong et al., 2005).

The functional consequences of β subunit cleavage by the secretases appear complex, and may be subunit- and cell type-dependent. The soluble extracellular domain of β 1 increases neurite length of cerebellar granule neurons grown *in vitro* (Davis et al., 2004), suggesting that the shed ectodomain may be required for β subunit-mediated neurite outgrowth. Co-expression of BACE1 with β 4 in Neuro2a cells enhances β 4-mediated neurite extension, and can be partially recapitulated by expressing the β 4 C-terminal fragment alone (Miyazaki et al., 2007), suggesting that in the case of β 4, the C-terminal fragment is important for regulation of neurite outgrowth. Pharmacological inhibition of γ -secretase activity in Chinese hamster ovary cells expressing β 2 prevents cellular aggregation by β 2 *trans* homophilic adhesion, and reduces the cells' transverse migration in a wound-heal assay (Kim et al., 2005). In addition, γ -secretase inhibitor treatment reduces migration of SH-SY5Y neuroblastoma cells in a modified Boyden chamber assay (Kim et al., 2005). In contrast, overexpression of β 2 C-terminal fragments blocks transverse migration of Chinese hamster ovary cells, suggesting that the cleavage of β 2 C-terminal fragments and the consequent release of the shorter β 2 intracellular domain are required for β 2-mediated adhesion/migration (Kim et al., 2005).

The β 2 intracellular domain translocates to the nucleus of transfected SH-SY5Y cells (Kim et al., 2007). In addition, the β 2 intracellular domain increases the mRNA and protein levels of *SCN1A/Na_v1.1*, suggesting that this fragment may regulate transcription in a manner similar to that reported for some other secretase-cleaved proteins, including the amyloid

precursor protein (Table 3) (Kim et al., 2007; Selkoe and Wolfe, 2007). In *Scn2b* null DRG neurons, the mRNA levels of *Scn3a*, *Scn8a*, *Scn9a*, *Scn3b* and *Scn4b* are reduced, as are Na_v1.1 and Na_v1.7 protein levels (Lopez-Santiago et al., 2006), suggesting that β2 may regulate expression of multiple VGSC genes in a cell type-specific manner. Evidence suggests that β1 may also regulate VGSC α subunit mRNA and protein expression. Cardiomyocytes from *Scn1b* null mice have increased *Scn5a* mRNA and Na_v1.5 protein levels (Lopez-Santiago et al., 2007). In addition, in *Scn1b* null mice, there is a downregulation of Na_v1.1 protein and upregulation of Na_v1.3 protein in a subset of *Scn1b* null pyramidal neurons in the CA2/CA3 region of the hippocampus, which may contribute to the seizure-susceptible phenotype of these mice (Chen et al., 2004). Further work is required to establish directly whether β subunit intracellular domains do indeed function as transcriptional regulators of α subunit expression.

6. Novel role in cancer cells

VGSCs are widely expressed in cancer cells both *in vitro* and *in vivo*, and the functional activity of the pore has been shown to potentiate various cellular behaviors associated with the metastatic cascade, including migration and invasion (Diss et al., 2004; Brackenbury et al., 2008b). Recently, it has been shown that *SCN1B*, *SCN2B*, and *SCN4B* mRNAs are expressed in human prostate cancer and breast cancer cell lines (Chioni et al., 2006; Diss et al., 2007). In breast cancer, *SCN1B* mRNA is most abundant of the four, and *SCN1B* mRNA and β1 protein levels are significantly higher in weakly metastatic MCF-7 cells than strongly metastatic MDA-MB-231 cells (Chioni et al., 2006). Downregulation of *SCN1B*/β1 using siRNA in MCF-7 cells reduces adhesion and increases migration, suggesting that β1 may control migration of breast cancer cells via cell adhesive interactions (Chioni et al., 2006; Brackenbury et al., 2008b). Similar to results in *Scn1b* null cardiomyocytes, downregulation of *SCN1B*/β1 in MCF-7 cells results in an increase in the mRNA level of the neonatal splice variant of *SCN5A* and neonatal Na_v1.5 protein, suggesting that β1 may regulate neonatal *SCN5A* expression (Chioni et al., 2006). Together, these findings suggest that VGSC α and β subunits play complex roles in metastatic cancer cells. Further work is required to establish the respective roles of these subunits in metastatic cancer progression *in vivo*, and to assess their suitability as diagnostic and/or therapeutic targets.

7. Conclusion

An increasing body of evidence has identified multiple functional roles for the VGSC β subunits. In addition to their 'classical' role as modulators of the ionic activity of the VGSC ion conducting pore, they play important roles in cell adhesion, migration and pathfinding, and putative transcriptional regulation, possibly independent of the α subunit. We propose that discussion of the β subunits as 'auxiliary' is increasingly inappropriate, in light of the emerging evidence. Rather, the β subunits should be considered as integral components of VGSC macromolecular protein complexes, which can interact with, and direct multiple intracellular and intercellular signaling cascades on multiple timescales. This would fit in well with the idea that voltage-gated ion channels carry out significant non-conducting functions (Isom, 2001; Meadows and Isom, 2005; Kaczmarek, 2006; Brackenbury et al., 2008b).

VGSC macromolecular signaling complexes likely vary in composition depending on the cell type and/or subcellular domain. Based on current evidence, VGSC complexes in central neurons include an α subunit, one or more β subunits, cytoskeletal proteins, e.g. ankyrins, secretases, e.g. BACE1 and γ-secretase, kinases e.g. fyn, and other cell adhesion molecules, e.g. contactin (Figure 5). In addition, further signaling components may also be involved. For example, the intracellular fibroblast growth factor homologous factor 14 interacts with α

subunits and controls VGSC targeting to the axon initial segment, thus regulating neuronal excitability, although whether it also interacts with β subunits is not known (Lou et al., 2005; Goldfarb et al., 2007; Laezza et al., 2007).

8. Expert opinion

VGSC β subunits regulate key cellular processes, including electrical excitability, adhesion, transcription, migration, pathfinding, and invasion (Malhotra et al., 2000; Chen et al., 2002; Chen et al., 2004; Chioni et al., 2006; Kim et al., 2007; Brackenbury et al., 2008a). This has important clinical implications, in terms of our understanding of aspects of central nervous system development/plasticity, and diverse pathophysiological conditions, including epilepsy, cardiac conduction disease, Multiple Sclerosis, Huntington's disease, pain, and cancer (Wallace et al., 1998; Meadows et al., 2002; Wallace et al., 2002; Audenaert et al., 2003; Chioni et al., 2006; Lopez-Santiago et al., 2006; O'Malley and Isom, 2006; Oyama et al., 2006; Lopez-Santiago et al., 2007; Scheffer et al., 2007; Brackenbury et al., 2008a; Watanabe et al., 2008). However, the majority of studies to date have employed basic research. The challenge for future studies is now to translate both the current *in vitro* and *in vivo* data to the clinic.

β subunits may serve as useful diagnostic tools. Genetic screening for mutation(s), e.g. C121W in $\beta 1$ (Wallace et al., 1998; Meadows et al., 2002), could provide useful information to clinicians as regards epilepsy type and appropriate treatment. Given that several mutations in $\beta 1$ have now been associated with at least two types of epilepsy (Wallace et al., 1998; Meadows et al., 2002; Wallace et al., 2002; Audenaert et al., 2003; Scheffer et al., 2007), it is likely that further epilepsy-related mutations may exist in the β subunits. Further work should be directed towards elucidating this possibility. In addition, mutations in $\beta 1$ and $\beta 1B$ may also be significant in prediction of cardiac arrhythmia and/or sudden cardiac death (Watanabe et al., 2008). Loss and/or genetic absence of *SCN4B* may provide a prognostic marker for Huntington's disease (Oyama et al., 2006). In addition, *SCN1B*/ $\beta 1$ may serve as a marker for cancer progression (Chioni et al., 2006). For example, loss of $\beta 1$ expression in breast cancer biopsies might indicate transition to a metastatic phenotype. The interesting possibility that VGSC subunits, including $\beta 1$, may be involved in metastatic cell behavior is in agreement with the hypothesis that cancer progression has some of the hallmarks of neuronal development (Liotta and Clair, 2000; Onganer et al., 2005; Brackenbury et al., 2008b). Further work is required to evaluate the potential of $\beta 1$ as a prognostic marker in cancer.

β subunits may be useful therapeutic targets. Given that β subunits are key components of VGSC macromolecular signaling complexes (Isom, 2001; Meadows and Isom, 2005; Brackenbury et al., 2008b), it is possible that targeting β subunit expression/activity to fine-tune VGSC signaling may be efficacious in the treatment of VGSC channelopathies. For example, targeting the phosphorylation state of $\beta 1$ using a drug directed at Y181 may be useful in treatment of cardiac arrhythmia and/or neuronal excitability (Malhotra et al., 2002; Chen et al., 2004; Malhotra et al., 2004). A soluble form of the extracellular $\beta 1$ immunoglobulin loop is capable of rescuing neurite outgrowth of cerebellar granule neurons *in vitro* (Davis et al., 2004). Therefore, it is possible that in their soluble form, β subunit immunoglobulin domains could be effective therapeutic tools. For example, application of a soluble $\beta 1$ may be efficacious in preventing migration of metastatic cancer cells. Clearly, much further work is needed to ascertain whether/how the β subunits could be used in/as therapeutic treatments.

Electrical activity driven by VGSCs is required for various processes in which the β subunits are implicated, including neuronal migration and pathfinding, myelination, and

cancer metastasis (Dubin et al., 1986; Kalil et al., 1986; Casagrande and Condo, 1988; Grimes et al., 1995; Demerens et al., 1996; Fraser et al., 2003; Davis et al., 2004; Fraser et al., 2005; Brackenbury and Djamgoz, 2006; Chioni et al., 2006; O'Malley and Isom, 2006; Brackenbury et al., 2008a; Palmer et al., 2008). This raises the intriguing possibility that drugs targeting the pore-forming α subunit could influence β subunit-mediated functions, e.g. adhesion and/or migration. Further work should clarify if/how activity of VGSC α subunits can modulate signaling through the β subunits.

In conclusion, there is increasing evidence implicating VGSC β subunits in a plethora of disease conditions. It is likely that the β subunits will prove useful in diagnosis and therapy. However, the nature of the evidence to date has been for the most part pre-clinical. Future advances will certainly address this issue and will provide further insight into the important and diverse roles played by these proteins.

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References

- Adachi K, Toyota M, Sasaki Y, Yamashita T, Ishida S, Ohe-Toyota M, Maruyama R, Hinoda Y, Saito T, Imai K, Kudo R, Tokino T. Identification of SCN3B as a novel p53-inducible proapoptotic gene. *Oncogene*. 2004; 23:7791–7798. [PubMed: 15334053]
- Adelman JP. Proteins that interact with the pore-forming subunits of voltage-gated ion channels. *Curr Opin Neurobiol*. 1995; 5:286–295. [PubMed: 7580150]
- Audenaert D, Claes L, Ceulemans B, Lofgren A, Van Broeckhoven C, De Jonghe P. A deletion in *SCN1B* is associated with febrile seizures and early-onset absence epilepsy. *Neurology*. 2003; 61:854–856. [PubMed: 14504340]
- Barres BA, Chun LL, Corey DP. Ion channels in vertebrate glia. *Annu Rev Neurosci*. 1990; 13:441–474. [PubMed: 2158266]
- Beggs HE, Soriano P, Maness PF. NCAM-dependent neurite outgrowth is inhibited in neurons from Fyn-minus mice. *J Cell Biol*. 1994; 127:825–833. [PubMed: 7962063]
- Berglund EO, Murai KK, Fredette B, Sekerkova G, Marturano B, Weber L, Mugnaini E, Ranscht B. Ataxia and abnormal cerebellar microorganization in mice with ablated contactin gene expression. *Neuron*. 1999; 24:739–750. [PubMed: 10595523]
- Brackenbury WJ, Davis TH, Chen C, Slat EA, Detrow MJ, Dickendesher TL, Ranscht B, Isom LL. Voltage-gated Na⁺ channel β 1 subunit-mediated neurite outgrowth requires fyn kinase and contributes to central nervous system development in vivo. *J Neurosci*. 2008a; 28:3246–3256. [PubMed: 18354028]
- Brackenbury WJ, Djamgoz MB. Activity-dependent regulation of voltage-gated Na⁺ channel expression in Mat-LyLu rat prostate cancer cell line. *J Physiol*. 2006; 573(Pt 2):343–356. [PubMed: 16543264]
- Brackenbury WJ, Djamgoz MB, Isom LL. An emerging role for voltage-gated Na⁺ channels in cellular migration: Regulation of central nervous system development and potentiation of invasive cancers. *The Neuroscientist*. 2008b In press.
- Casagrande VA, Condo GJ. The effect of altered neuronal activity on the development of layers in the lateral geniculate nucleus. *J Neurosci*. 1988; 8:395–416. [PubMed: 3339423]
- Catterall WA. Cellular and molecular biology of voltage-gated sodium channels. *Physiological Reviews*. 1992a; 72:S15–S48. [PubMed: 1332090]
- Catterall WA. Cellular and molecular biology of voltage-gated sodium channels. *Physiol Rev*. 1992b; 72:S15–48. [PubMed: 1332090]
- Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron*. 2000; 26:13–25. [PubMed: 10798388]

- Chen C, Bharucha V, Chen Y, Westenbroek RE, Brown A, Malhotra JD, Jones D, Avery C, Gillespie PJ 3rd, Kazen-Gillespie KA, Kazarinova-Noyes K, Shrager P, Saunders TL, Macdonald RL, Ransom BR, Scheuer T, Catterall WA, Isom LL. Reduced sodium channel density, altered voltage dependence of inactivation, and increased susceptibility to seizures in mice lacking sodium channel beta 2-subunits. *Proc Natl Acad Sci U S A*. 2002; 99:17072–17077. [PubMed: 12481039]
- Chen C, Westenbroek RE, Xu X, Edwards CA, Sorenson DR, Chen Y, McEwen DP, O'malley HA, Bharucha V, Meadows LS, Knudsen GA, Vilaythong A, Noebels JL, Saunders TL, Scheuer T, Shrager P, Catterall WA, Isom LL. Mice lacking sodium channel β 1 subunits display defects in neuronal excitability, sodium channel expression, and nodal architecture. *J Neurosci*. 2004; 24:4030–4042. [PubMed: 15102918]
- Chioni AM, Isom LL, Djamgoz MB. Adhesion of human breast cancer cell lines: role of voltage-gated sodium channel β 1 subunit. *Proc Physiol Soc*. 2006; 3:PC187.
- Clare JJ, Tate SN, Nobbs M, Romanos MA. Voltage-gated sodium channels as therapeutic targets. *Drug Discov Today*. 2000; 5:506–520. [PubMed: 11084387]
- Cohen NR, Taylor JS, Scott LB, Guillery RW, Soriano P, Furley AJ. Errors in corticospinal axon guidance in mice lacking the neural cell adhesion molecule L1. *Curr Biol*. 1997; 8:26–33. [PubMed: 9427628]
- Cummins TR, Sheets PL, Waxman SG. The roles of sodium channels in nociception: Implications for mechanisms of pain. *Pain*. 2007; 131:243–257. [PubMed: 17766042]
- Davis TH, Chen C, Isom LL. Sodium channel β 1 subunits promote neurite outgrowth in cerebellar granule neurons. *J Biol Chem*. 2004; 279:51424–51432. [PubMed: 15452131]
- Demerens C, Stankoff B, Logak M, Anglade P, Allinquant B, Couraud F, Zalc B, Lubetzki C. Induction of myelination in the central nervous system by electrical activity. *Proc Natl Acad Sci U S A*. 1996; 93:9887–9892. [PubMed: 8790426]
- Deschenes I, Tomaselli GF. Modulation of Kv4.3 current by accessory subunits. *FEBS Lett*. 2002; 528:183–188. [PubMed: 12297301]
- Dib-Hajj SD, Waxman SG. Genes encoding the beta 1 subunit of voltage-dependent Na⁺ channel in rat, mouse and human contain conserved introns. *FEBS Lett*. 1995; 377:485–488. [PubMed: 8549781]
- Dichgans M, Freilinger T, Eckstein G, Babini E, Lorenz-Depiereux B, Biskup S, Ferrari MD, Herzog J, Van Den Maagdenberg AM, Pusch M, Strom TM. Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. *Lancet*. 2005; 366:371–377. [PubMed: 16054936]
- Diss JK, Fraser SP, Djamgoz MB. Voltage-gated Na⁺ channels: multiplicity of expression, plasticity, functional implications and pathophysiological aspects. *Eur Biophys J*. 2004; 33:180–193. [PubMed: 14963621]
- Diss JK, Fraser SP, Walker MM, Patel A, Latchman DS, Djamgoz MB. beta-Subunits of voltage-gated sodium channels in human prostate cancer: quantitative in vitro and in vivo analyses of mRNA expression. *Prostate Cancer Prostatic Dis*. 2007; 10:1038/sj.pcan.4501012
- Dubin MW, Stark LA, Archer SM. A role for action-potential activity in the development of neuronal connections in the kitten retinogeniculate pathway. *J Neurosci*. 1986; 6:1021–1036. [PubMed: 3701407]
- Fein AJ, Meadows LS, Chen C, Slat EA, Isom LL. Cloning and expression of a zebrafish SCN1B ortholog and identification of a species-specific splice variant. *BMC Genomics*. 2007; 8:226. [PubMed: 17623064]
- Fontaine B, Khurana TS, Hoffman EP, Bruns GA, Haines JL, Trofatter JA, Hanson MP, Rich J, McFarlane H, Yasek DM, Et Al. Hyperkalemic periodic paralysis and the adult muscle sodium channel alpha-subunit gene. *Science*. 1990; 250:1000–1002. [PubMed: 2173143]
- Fraser SP, Diss JK, Chioni AM, Mycielska M, Pan H, Yamaci RF, Pani F, Siwy Z, Krasowska M, Grzywna Z, Brackenbury WJ, Theodorou D, Koyuturk M, Kaya H, Battaloglu E, Tamburo De Bella M, Slade MJ, Tolhurst R, Palmieri C, Jiang J, Latchman DS, Coombes RC, Djamgoz MB. Voltage-gated sodium channel expression and potentiation of human breast cancer metastasis. *Clin Cancer Res*. 2005; 11:5381–5389. [PubMed: 16061851]

- Fraser SP, Salvador V, Manning EA, Mizal J, Altun S, Raza M, Berridge RJ, Djamgoz MB. Contribution of functional voltage-gated Na⁺ channel expression to cell behaviors involved in the metastatic cascade in rat prostate cancer: I. lateral motility. *J Cell Physiol*. 2003; 195:479–487. [PubMed: 12704658]
- Gautron S, Dos Santos G, Pinto-Henrique D, Koulakoff A, Gros F, Berwald-Netter Y. The glial voltage-gated sodium channel: cell- and tissue-specific mRNA expression. *Proc Natl Acad Sci U S A*. 1992; 89:7272–7276. [PubMed: 1379737]
- George AL Jr. Inherited disorders of voltage-gated sodium channels. *J Clin Invest*. 2005; 115:1990–1999. [PubMed: 16075039]
- Goldfarb M, Schoorlemmer J, Williams A, Diwakar S, Wang Q, Huang X, Giza J, Tchetchik D, Kelley K, Vega A, Matthews G, Rossi P, Ornitz DM, D'angelo E. Fibroblast growth factor homologous factors control neuronal excitability through modulation of voltage-gated sodium channels. *Neuron*. 2007; 55:449–463. [PubMed: 17678857]
- Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, Kallen RG, Mandel G, Meisler MH, Netter YB, Noda M, Tamkun MM, Waxman SG, Wood JN, Catterall WA. Nomenclature of voltage-gated sodium channels. *Neuron*. 2000; 28:365–368. [PubMed: 11144347]
- Gosling M, Harley SL, Turner RJ, Carey N, Powell JT. Human saphenous vein endothelial cells express a tetrodotoxin-resistant, voltage-gated sodium current. *J Biol Chem*. 1998; 273:21084–21090. [PubMed: 9694861]
- Grimes JA, Fraser SP, Stephens GJ, Downing JE, Laniado ME, Foster CS, Abel PD, Djamgoz MB. Differential expression of voltage-activated Na⁺ currents in two prostatic tumour cell lines: contribution to invasiveness in vitro. *FEBS Lett*. 1995; 369:290–294. [PubMed: 7649275]
- Hille, B. Ionic channels of excitable membranes. Sunderland (Massachusetts): Sinauer Associates Inc; 1992.
- Hodgkin AL, Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol*. 1952; 117:500–544. [PubMed: 12991237]
- Ishihara K, Yan DH, Yamamoto S, Ehara T. Inward rectifier K(+) current under physiological cytoplasmic conditions in guinea-pig cardiac ventricular cells. *J Physiol*. 2002; 540:831–841. [PubMed: 11986372]
- Isom LL. Sodium channel β subunits: anything but auxiliary. *The Neuroscientist*. 2001; 7:42–54. [PubMed: 11486343]
- Isom LL, Catterall WA. Na⁺ channel subunits and Ig domains. *Nature*. 1996; 383:307–308. [PubMed: 8848042]
- Isom LL, De Jongh KS, Catterall WA. Auxiliary subunits of voltage-gated ion channels. *Neuron*. 1994; 12:1183–1194. [PubMed: 7516685]
- Isom LL, De Jongh KS, Patton DE, Reber BF, Offord J, Charbonneau H, Walsh K, Goldin AL, Catterall WA. Primary structure and functional expression of the b1 subunit of the rat brain sodium channel. *Science*. 1992; 256:839–842. [PubMed: 1375395]
- Isom LL, Ragsdale DS, De Jongh KS, Westenbroek RE, Reber BF, Scheuer T, Catterall WA. Structure and function of the b2 subunit of brain sodium channels, a transmembrane glycoprotein with a CAM motif. *Cell*. 1995; 83:433–442. [PubMed: 8521473]
- Kaczmarek LK. Non-conducting functions of voltage-gated ion channels. *Nat Rev Neurosci*. 2006; 7:761–771. [PubMed: 16988652]
- Kalil RE, Dubin MW, Scott G, Stark LA. Elimination of action potentials blocks the structural development of retinogeniculate synapses. *Nature*. 1986; 323:156–158. [PubMed: 3018590]
- Karadottir R, Hamilton NB, Bakiri Y, Attwell D. Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. *Nat Neurosci*. 2008; 11:450–456. [PubMed: 18311136]
- Kasahara K, Watanabe K, Kozutsumi Y, Oohira A, Yamamoto T, Sanai Y. Association of GPI-anchored protein TAG-1 with src-family kinase Lyn in lipid rafts of cerebellar granule cells. *Neurochem Res*. 2002; 27:823–829. [PubMed: 12374219]
- Kazarinova-Noyes K, Malhotra JD, McEwen DP, Mattei LN, Berglund EO, Ranscht B, Levinson SR, Schachner M, Shrager P, Isom LL, Xiao Z-C. Contactin associates with Na⁺ channels and increases their functional expression. *J Neurosci*. 2001; 21:7517–7525. [PubMed: 11567041]

- Kazen-Gillespie KA, Ragsdale DS, D'andrea MR, Mattei LN, Rogers KE, Isom LL. Cloning, localization, and functional expression of sodium channel β 1A subunits. *J Biol Chem.* 2000; 275:1079–1088. [PubMed: 10625649]
- Kim DY, Carey BW, Wang H, Ingano LA, Binshtok AM, Wertz MH, Pettingell WH, He P, Lee VM, Woolf CJ, Kovacs DM. BACE1 regulates voltage-gated sodium channels and neuronal activity. *Nat Cell Biol.* 2007; 9:755–764. [PubMed: 17576410]
- Kim DY, Mackenzie Ingano LA, Carey BW, Pettingell WP, Kovacs DM. Presenilin/gamma - secretase-mediated cleavage of the voltage-gated sodium channel beta 2 subunit regulates cell adhesion and migration. *J Biol Chem.* 2005; 280:23251–23261. [PubMed: 15833746]
- Kolkova K, Novitskaya V, Pedersen N, Berezin V, Bock E. Neural cell adhesion molecule-stimulated neurite outgrowth depends on activation of protein kinase C and the Ras-mitogen-activated protein kinase pathway. *J Neurosci.* 2000; 20:2238–2246. [PubMed: 10704499]
- Laezza F, Gerber BR, Lou JY, Kozel MA, Hartman H, Craig AM, Ornitz DM, Nerbonne JM. The FGF14(F145S) mutation disrupts the interaction of FGF14 with voltage-gated Na⁺ channels and impairs neuronal excitability. *J Neurosci.* 2007; 27:12033–12044. [PubMed: 17978045]
- Liotta LA, Clair T. Cancer. Checkpoint for invasion. *Nature.* 2000; 405:287–288. [PubMed: 10830943]
- Lopez-Santiago LF, Meadows LS, Ernst SJ, Chen C, Malhotra JD, McEwen DP, Speelman A, Noebels JL, Maier SK, Lopatin AN, Isom LL. Sodium channel *Scn1b* null mice exhibit prolonged QT and RR intervals. *J Mol Cell Cardiol.* 2007; 43:636–647. [PubMed: 17884088]
- Lopez-Santiago LF, Pertin M, Morisod X, Chen C, Hong S, Wiley J, Decosterd I, Isom LL. Sodium channel beta2 subunits regulate tetrodotoxin-sensitive sodium channels in small dorsal root ganglion neurons and modulate the response to pain. *J Neurosci.* 2006; 26:7984–7994. [PubMed: 16870743]
- Lou JY, Laezza F, Gerber BR, Xiao M, Yamada KA, Hartmann H, Craig AM, Nerbonne JM, Ornitz DM. Fibroblast growth factor 14 is an intracellular modulator of voltage-gated sodium channels. *J Physiol.* 2005; 569:179–193. [PubMed: 16166153]
- Malhotra JD, Kazen-Gillespie K, Hortsch M, Isom LL. Sodium channel β subunits mediate homophilic cell adhesion and recruit ankyrin to points of cell-cell contact. *J Biol Chem.* 2000; 275:11383–11388. [PubMed: 10753953]
- Malhotra JD, Koopmann MC, Kazen-Gillespie KA, Fettman N, Hortsch M, Isom LL. Structural requirements for interaction of sodium channel β 1 subunits with ankyrin. *J Biol Chem.* 2002; 277:26681–26688. [PubMed: 11997395]
- Malhotra JD, Thyagarajan V, Chen C, Isom LL. Tyrosine-phosphorylated and nonphosphorylated sodium channel beta1 subunits are differentially localized in cardiac myocytes. *J Biol Chem.* 2004; 279:40748–40754. [PubMed: 15272007]
- Maness PF, Schachner M. Neural recognition molecules of the immunoglobulin superfamily: signaling transducers of axon guidance and neuronal migration. *Nat Neurosci.* 2007; 10:19–26. [PubMed: 17189949]
- McEwen DP, Isom LL. Heterophilic interactions of sodium channel beta1 subunits with axonal and glial cell adhesion molecules. *J Biol Chem.* 2004; 279:52744–52752. [PubMed: 15466474]
- McEwen DP, Meadows LS, Chen C, Thyagarajan V, Isom LL. Sodium channel β 1 subunit-mediated modulation of Nav1.2 currents and cell surface density is dependent on interactions with contactin and ankyrin. *J Biol Chem.* 2004; 279:16044–16049. [PubMed: 14761957]
- Meadows LS, Isom LL. Sodium channels as macromolecular complexes: implications for inherited arrhythmia syndromes. *Cardiovasc Res.* 2005; 67:448–458. [PubMed: 15919069]
- Meadows LS, Malhotra J, Loukas A, Thyagarajan V, Kazen-Gillespie KA, Koopman MC, Kriegler S, Isom LL, Ragsdale DS. Functional and biochemical analysis of a sodium channel β 1 subunit mutation responsible for Generalized Epilepsy with Febrile Seizures Plus Type 1. *J Neurosci.* 2002; 22:10699–10709. [PubMed: 12486163]
- Medeiros-Domingo A, Kaku T, Tester DJ, Iturralde-Torres P, Itty A, Ye B, Valdivia C, Ueda K, Canizales-Quinteros S, Tusie-Luna MT, Makielski JC, Ackerman MJ. SCN4B-encoded sodium channel beta4 subunit in congenital long-QT syndrome. *Circulation.* 2007; 116:134–142. [PubMed: 17592081]

- Miyazaki H, Oyama F, Wong HK, Kaneko K, Sakurai T, Tamaoka A, Nukina N. BACE1 modulates filopodia-like protrusions induced by sodium channel beta4 subunit. *Biochem Biophys Res Commun.* 2007; 361:43–48. [PubMed: 17644063]
- Morgan K, Stevens EB, Shah B, Cox PJ, Dixon AK, Lee K, Pinnock RD, Hughes J, Richardson PJ, Mizuguchi K, Jackson AP. $\beta 3$: An additional auxiliary subunit of the voltage-sensitive sodium channel that modulates channel gating with distinct kinetics. *Proc Natl Acad Sci USA.* 2000; 97:2308–2313. [PubMed: 10688874]
- Niethammer P, Delling M, Sytnyk V, Dityatev A, Fukami K, Schachner M. Cosignaling of NCAM via lipid rafts and the FGF receptor is required for neuritogenesis. *J Cell Biol.* 2002; 157:521–532. [PubMed: 11980923]
- O'malley HA, Isom LL. The role of sodium channel $\beta 2$ subunits in neuroprotection. Society for Neuroscience (Annual Meeting Abstracts). 2006 727.722/D761.
- Olive S, Dubois C, Schachner M, Rougon G. The F3 neuronal glycosylphosphatidylinositol-linked molecule is localized to glycolipid-enriched membrane subdomains and interacts with L1 and fyn kinase in cerebellum. *J Neurochem.* 1995; 65:2307–2317. [PubMed: 7595520]
- Onganer PU, Seckl MJ, Djamgoz MB. Neuronal characteristics of small-cell lung cancer. *Br J Cancer.* 2005; 93:1197–1201. [PubMed: 16265346]
- Oyama F, Miyazaki H, Sakamoto N, Becquet C, Machida Y, Kaneko K, Uchikawa C, Suzuki T, Kurosawa M, Ikeda T, Tamaoka A, Sakurai T, Nukina N. Sodium channel beta4 subunit: down-regulation and possible involvement in neuritic degeneration in Huntington's disease transgenic mice. *J Neurochem.* 2006; 98:518–529. [PubMed: 16805843]
- Palmer CP, Mycielska ME, Burcu H, Osman K, Collins T, Beckerman R, Perrett R, Johnson H, Aydar E, Djamgoz MB. Single cell adhesion measuring apparatus (SCAMA): application to cancer cell lines of different metastatic potential and voltage-gated Na^+ channel expression. *Eur Biophys J.* 2008; 37:359–368. [PubMed: 17879092]
- Pertin M, Ji RR, Berta T, Powell AJ, Karchewski L, Tate SN, Isom LL, Woolf CJ, Gilliard N, Spahn DR, Decosterd I. Upregulation of the voltage-gated sodium channel $\beta 2$ subunit in neuropathic pain models: characterization of expression in injured and non-injured primary sensory neurons. *J Neurosci.* 2005; 25:10970–10980. [PubMed: 16306410]
- Qin N, D'andrea MR, Lubin ML, Shafae N, Codd EE, Correa AM. Molecular cloning and functional expression of the human sodium channel beta1B subunit, a novel splicing variant of the beta1 subunit. *Eur J Biochem.* 2003; 270:4762–4770. [PubMed: 14622265]
- Ragsdale DS. How do mutant Nav1.1 sodium channels cause epilepsy? *Brain Res Rev.* 2008
- Ratcliffe CF, Qu Y, McCormick KA, Tibbs VC, Dixon JE, Scheuer T, Catterall WA. A sodium channel signaling complex: modulation by associated receptor protein tyrosine phosphatase b. *Nature Neurosci.* 2000; 3:437–444. [PubMed: 10769382]
- Rolf B, Bastmeyer M, Schachner M, Bartsch U. Pathfinding errors of corticospinal axons in neural cell adhesion molecule-deficient mice. *J Neurosci.* 2002; 22:8357–8362. [PubMed: 12351709]
- Sanchez-Heras E, Howell FV, Williams G, Doherty P. The fibroblast growth factor receptor acid box is essential for interactions with N-cadherin and all of the major isoforms of neural cell adhesion molecule. *J Biol Chem.* 2006; 281:35208–35216. [PubMed: 17005551]
- Schafer DP, Bansal R, Hedstrom KL, Pfeiffer SE, Rasband MN. Does paranode formation and maintenance require partitioning of neurofascin 155 into lipid rafts? *J Neurosci.* 2004; 24:3176–3185. [PubMed: 15056697]
- Scheffer IE, Harkin LA, Grinton BE, Dibbens LM, Turner SJ, Zielinski MA, Xu R, Jackson G, Adams J, Connellan M, Petrou S, Wellard RM, Briellmann RS, Wallace RH, Mulley JC, Berkovic SF. Temporal lobe epilepsy and GEFS+ phenotypes associated with SCN1B mutations. *Brain.* 2007; 130:100–109. [PubMed: 17020904]
- Schmidt JW, Catterall WA. Biosynthesis and processing of the alpha subunit of the voltage-sensitive sodium channel in rat brain neurons. *Cell.* 1986; 46:437–444. [PubMed: 2425982]
- Selkoe DJ, Wolfe MS. Presenilin: running with scissors in the membrane. *Cell.* 2007; 131:215–221. [PubMed: 17956719]

- Srinivasan J, Schachner M, Catterall WA. Interaction of voltage-gated sodium channels with the extracellular matrix molecules tenascin-C and tenascin-R. *Proc Natl Acad Sci U S A*. 1998; 95:15753–15757. [PubMed: 9861042]
- Wallace RH, Scheffer IE, Parasivam G, Barnett S, Wallace GB, Sutherland GR, Berkovic SF, Mulley JC. Generalized epilepsy with febrile seizures plus: mutation of the sodium channel subunit SCN1B. *Neurology*. 2002; 58:1426–1429. [PubMed: 12011299]
- Wallace RH, Wang DW, Singh R, Scheffer IE, George AL Jr, Phillips HA, Saar K, Reis A, Johnson EW, Sutherland GR, Berkovic SF, Mulley JC. Febrile seizures and generalized epilepsy associated with a mutation in the Na⁺-channel beta1 subunit gene SCN1B. *Nat Genet*. 1998; 19:366–370. [PubMed: 9697698]
- Watanabe H, Koopmann TT, Le Scouarnec S, Yang T, Ingram CR, Schott JJ, Demolombe S, Probst V, Anselme F, Escande D, Wiesfeld AC, Pfeufer A, Kaab S, Wichmann HE, Hasdemir C, Aizawa Y, Wilde AA, Roden DM, Bezzina CR. Sodium channel beta1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. *J Clin Invest*. 2008
- Waxman SG. Axonal conduction and injury in multiple sclerosis: the role of sodium channels. *Nat Rev Neurosci*. 2006; 7:932–941. [PubMed: 17115075]
- Waxman SG. Channel, neuronal and clinical function in sodium channelopathies: from genotype to phenotype. *Nat Neurosci*. 2007; 10:405–409. [PubMed: 17387329]
- Wong HK, Sakurai T, Oyama F, Kaneko K, Wada K, Miyazaki H, Kurosawa M, De Strooper B, Saftig P, Nukina N. beta subunits of voltage-gated sodium channels are novel substrates of BACE1 and gamma-secretase. *J Biol Chem*. 2005; 280:23009–23017. [PubMed: 15824102]
- Xiao ZC, Ragsdale DS, Malhotra JD, Mattei LN, Braun PE, Schachner M, Isom LL. Tenascin-R is a functional modulator of sodium channel beta subunits. *J Biol Chem*. 1999; 274:26511–26517. [PubMed: 10473612]
- Xu R, Thomas EA, Gazina EV, Richards KL, Quick M, Wallace RH, Harkin LA, Heron SE, Berkovic SF, Scheffer IE, Mulley JC, Petrou S. Generalized epilepsy with febrile seizures plus-associated sodium channel beta1 subunit mutations severely reduce beta subunit-mediated modulation of sodium channel function. *Neuroscience*. 2007; 148:164–174. [PubMed: 17629415]
- Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, Mcknight GS, Scheuer T, Catterall WA. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci*. 2006; 9:1142–1149. [PubMed: 16921370]
- Yu FH, Westenbroek RE, Silos-Santiago I, McCormick KA, Lawson D, Ge P, Ferriera H, Lilly J, Distefano PS, Catterall WA, Scheuer T, Curtis R. Sodium channel beta4, a new disulfide-linked auxiliary subunit with similarity to beta2. *J Neurosci*. 2003; 23:7577–7585. [PubMed: 12930796]
- Zhao P, Barr TP, Hou Q, Dib-Hajj SD, Black JA, Albrecht PJ, Petersen K, Eisenberg E, Wymer JP, Rice FL, Waxman SG. Voltage-gated sodium channel expression in rat and human epidermal keratinocytes: Evidence for a role in pain. *Pain*. 2008

Figure 1. Basic functional architecture of voltage-gated Na⁺ channels (VGSCs)

VGSCs contain one pore-forming α subunit in association with two β subunits (Catterall, 1992a). The α subunits consist of four homologous domains, each containing six transmembrane segments (1–6). Segment 4 contains positively charged residues making the voltage sensor (Catterall, 2000). The β subunits contain one extracellular immunoglobulin (Ig) loop, one transmembrane segment, and an intracellular C-terminal domain (Isom et al., 1994). The tyrosine phosphorylation site in $\beta 1$ is shown (Malhotra et al., 2004). ψ , glycosylation sites. $\beta 2$ and $\beta 4$ are covalently linked to the α subunit through disulfide bonds, although the residue(s) responsible for this interaction have not yet been identified.

Figure 2. Adhesion partners of $\beta 1$

$\beta 1$ interacts homophilically and heterophilically with tenascin-R, NrCAM, neurofascin (NF)155, NF186, contactin, N-cadherin and VGSC $\beta 2$ (Xiao et al., 1999; Malhotra et al., 2000; Malhotra et al., 2002; McEwen and Isom, 2004; McEwen et al., 2004). Interactions with cell adhesion molecules can be *trans* or *cis*, although are shown here in *trans*. The intracellular domain of $\beta 1$ recruits ankyrin_G or ankyrin_B (Ank) in response to homophilic adhesion and this interaction is dependent on tyrosine phosphorylation of Y181 (Malhotra et al., 2002; Malhotra et al., 2004). EGF-L, epidermal growth factor-like repeat; FN III, fibronectin III-like repeat; Ig, immunoglobulin domain; GPI, glycosylphosphatidylinositol anchor; Cadh, cadherin repeat.

Figure 3. Model of $\beta 1$ -mediated neurite extension at the growth cone and axonal fasciculation
Trans-adhesive interactions between $\beta 1$ on an opposing glial cell and a lipid raft $\beta 1$ -contactin complex at the growth cone of a migrating cerebellar granule neuron initiate a signaling cascade *via* fyn kinase, resulting in neurite outgrowth and migration (Brackenbury et al., 2008a). In addition, $\beta 1$ - $\beta 1$ *trans*-adhesion between neurons is proposed to result in axonal fasciculation (Brackenbury et al., 2008a). The possible functional association of $\beta 1$ with α subunits in these processes is unclear.

Figure 4. Proteolytic processing of β subunits

Sequential cleavage of β subunits by (1) α -secretase and/or β -site amyloid precursor protein-cleaving enzyme (BACE1), followed by (2) γ -secretase yields soluble extracellular N-terminal domains and small intracellular C-terminal domains (Kim et al., 2005; Wong et al., 2005). The soluble immunoglobulin domains are responsible for regulation of adhesion and migration (Davis et al., 2004; Kim et al., 2005). β subunit intracellular domains regulate neurite outgrowth and are putative regulators of VGSC gene transcription (Kim et al., 2007; Miyazaki et al., 2007).

Figure 5. The VGSC macromolecular signaling complex

Pore-forming VGSC α subunits associate with two β subunits (in this case, $\beta 1$ and $\beta 2$) (Catterall, 2000). Through the immunoglobulin domains of the β subunits, the complex can associate heterophilically with cell adhesion molecules, e.g. contactin (McEwen and Isom, 2004; McEwen et al., 2004; Brackenbury et al., 2008a). Secretases, e.g. the β -site amyloid precursor protein-cleaving enzyme (BACE1) and γ -secretase may exist in the complex in order to permit regulated proteolytic cleavage of the VGSC β subunits to release intracellular domains (ICDs) (Kim et al., 2005; Wong et al., 2005; Kim et al., 2007). $\beta 1$ interacts with receptor protein tyrosine phosphatase β (RPTP β) (Ratcliffe et al., 2000), and depending on the phosphorylation state of Y181 (P), can recruit the cytoskeleton *via* ankyrin (Ank) (Malhotra et al., 2000; Malhotra et al., 2002). $\beta 1$ likely interacts with fyn kinase to permit neurite outgrowth/migration (Brackenbury et al., 2008a).

Table 1The VGSC β subunits.

Protein	Gene symbol	Tissue locations	Disease	Reference
$\beta 1$	<i>SCN1B</i>	CNS, heart, PNS, skeletal muscle	Epilepsy, cardiac arrhythmia, cancer	(Isom et al., 1992; Wallace et al., 1998; Meadows et al., 2002; Wallace et al., 2002; Audenaert et al., 2003; Chen et al., 2004; Chioni et al., 2006; Diss et al., 2007; Lopez-Santiago et al., 2007; Scheffer et al., 2007)
$\beta 1A/\beta 1B$	<i>SCN1B</i>	Adrenal gland, CNS, heart, PNS, skeletal muscle	Epilepsy?	(Kazen-Gillespie et al., 2000; Qin et al., 2003)
$\beta 2$	<i>SCN2B</i>	CNS, heart, PNS	Altered pain response, seizure susceptibility, MS	(Isom et al., 1995; Chen et al., 2002; Lopez-Santiago et al., 2006; O'Malley and Isom, 2006)
$\beta 3$	<i>SCN3B</i>	Adrenal gland, CNS, kidney, PNS	Cancer	(Morgan et al., 2000; Adachi et al., 2004; Chioni et al., 2006)
$\beta 4$	<i>SCN4B</i>	CNS, heart, PNS, skeletal muscle	Huntington's disease	(Yu et al., 2003; Oyama et al., 2006)

Abbreviations: CNS, central nervous system; MS, Multiple Sclerosis; PNS, peripheral nervous system.

Table 2Human mutations in $\beta 1$.

Mutation	Functional consequence	Disease	Reference
IVS2-2A>C	Possible misfolding of Ig domain	GEFS, GEFS+	(Audenaert et al., 2003)
R85C/H	-	GEFS, GEFS+	(Scheffer et al., 2007)
C121W	Slowed inactivation, increased availability at hyperpolarized potentials, reduced rundown, disrupted homophilic adhesion	GEFS, GEFS+, TLE	(Wallace et al., 1998; Meadows et al., 2002; Wallace et al., 2002; Scheffer et al., 2007)
W179 (in $\beta 1B$)	Truncated protein, loss of function	Cardiac block, Brugada syndrome	(Watanabe et al., 2008)
E87Q	Loss of function, dominant negative	Cardiac block, Brugada syndrome	(Watanabe et al., 2008)

Abbreviations: GEFS, generalized epilepsy with febrile seizures; TLE, temporal lobe epilepsy.

Table 3Possible gene targets of β subunits.

β subunit	Cell/tissue	Gene target	Effect	Reference
$\beta 1$	Cardiomyocyte	<i>Scn5a</i>	Increased in <i>Scn1b</i> null	(Lopez-Santiago et al., 2007)
$\beta 1$	CA2/CA3 hippocampal neurons	<i>Scn1a</i> , <i>Scn3a</i>	Decreased $\text{Na}_v1.1$, increased $\text{Na}_v1.3$ in <i>Scn1b</i> null	(Chen et al., 2004)
$\beta 1$	MCF-7	<i>Neonatal SCN5A</i>	Increased following downregulation of <i>SCN1B</i> / $\beta 1$	(Chioni et al., 2006)
$\beta 2$	SH-SY5Y	<i>SCN1A</i>	Increased in presence of $\beta 2$ intracellular domain	(Kim et al., 2007)
$\beta 2$	Dorsal root ganglia	<i>Scn3a</i> , <i>Scn8a</i> , <i>Scn9a</i> , <i>Scn3b</i> , <i>Scn4b</i>	Decreased in <i>Scn2b</i> null	(Lopez-Santiago et al., 2006)