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Na^+ channel β subunits: overachievers of the ion channel family

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Voltage-gated Na^+ channels (VGSCs) in mammals contain a pore-forming α subunit and one or more β subunits. There are five mammalian β subunits in total: β1, β1B, β2, β3, and β4, encoded by four genes: *SCN1B–SCN4B*. With the exception of the *SCN1B* splice variant, β1B, the β subunits are type I topology transmembrane proteins. In contrast, β1B lacks a transmembrane domain and is a secreted protein. A growing body of work shows that VGSC β subunits are multifunctional. While they do not form the ion channel pore, β subunits alter gating, voltage-dependence, and kinetics of VGSCα subunits and thus regulate cellular excitability *in vivo*. In addition to their roles in channel modulation, β subunits are members of the immunoglobulin superfamily of cell adhesion molecules and regulate cell adhesion and migration. β subunits are also substrates for sequential proteolytic cleavage by secretases. An example of the multifunctional nature of β subunits is β1, encoded by *SCN1B*, that plays a critical role in neuronal migration and pathfinding during brain development, and whose function is dependent on Na^+ current and γ-secretase activity. Functional deletion of *SCN1B* results in Dravet Syndrome, a severe and intractable pediatric epileptic encephalopathy. β subunits are emerging as key players in a wide variety of physiopathologies, including epilepsy, cardiac arrhythmia, multiple sclerosis, Huntington's disease, neuropsychiatric disorders, neuropathic and inflammatory pain, and cancer. β subunits mediate multiple signaling pathways on different timescales, regulating electrical excitability, adhesion, migration, pathfinding, and transcription. Importantly, some β subunit functions may operate independently of α subunits. Thus, β subunits perform critical roles during development and disease. As such, they may prove useful in disease diagnosis and therapy.

Keywords: adhesion, β subunit, development, excitability, voltage-gated Na^+ channel

INTRODUCTION

Mammalian voltage-gated Na^+ channels (VGSCs) exist as macromolecular complexes *in vivo*, comprising, at minimum, one pore-forming α subunit and one or more β subunits in a 1:1 stoichiometry for α:β (Catterall, 1992). Traditionally, VGSC β subunits have been termed "auxiliary." However, increasing evidence suggests that the β subunits are far from auxiliary, and, in fact, function as critical signaling molecules in their own right, perhaps even independently of α subunits. In this review, we will summarize the latest developments describing the growing, diverse, multifunctional roles of the β subunits, including their contribution to human disease.

MOLECULAR DIVERSITY AND FUNCTIONAL ARCHITECTURE

The topology of the canonical VGSC complex is shown in **Figure 1**. To date, five β subunits have been identified in mammals: β1, its alternative splice variant β1B (previously called β1A), β2, β3, and β4 (Isom et al., 1992, 1995; Kazen-Gillespie et al., 2000; Morgan et al., 2000; Qin et al., 2003; Yu et al., 2003). Each β subunit is encoded by one of four genes, *SCN1B–SCN4B*. With the exception of β1B, the β subunits share a similar type I membrane topology, including an extracellular N-terminal region immunoglobulin (Ig) loop, one transmembrane domain, and a small intracellular C-terminal domain (**Figure 2**). β2 and β4 are disulfide linked to VGSC α subunits, whereas β1 and β3 associate non-covalently (Isom et al., 1992, 1995; Morgan et al., 2000; Yu et al., 2003). The residues(s) responsible for the covalent interaction between β2/β4 and α have not yet been identified. Mutation studies have revealed that the A/A′ strand of the β1 Ig fold contains critical charged residues that interact with, and modulate the activity of, the α subunit whereas the intracellular domain is not involved (Mccormick et al., 1998). Less is known about the β subunit interaction sites on α subunits; however, an epilepsy-causing mutation, D1866Y, in the C-terminal cytoplasmic domain of $Na_v1.1$ disrupts modulation of Na^+ current by β1 (Spampanato et al., 2004). β1B shares the same N-terminal Ig domain as β1, but by virtue of retention of intron 3, has a different C-terminal region that lacks a transmembrane domain but contains a stop codon and polyadenylation site (Kazen-Gillespie et al., 2000; Qin et al., 2003). As a result, β1B is unique among the β subunits in that it is a soluble protein (Patino et al., 2011). For reasons that are not understood, the amino acid sequence of the β1B C-terminal domain is species-specific (Patino et al., 2011). Further species-specific alternative splicing events have been discovered within *SCN1B*, including splice variants of

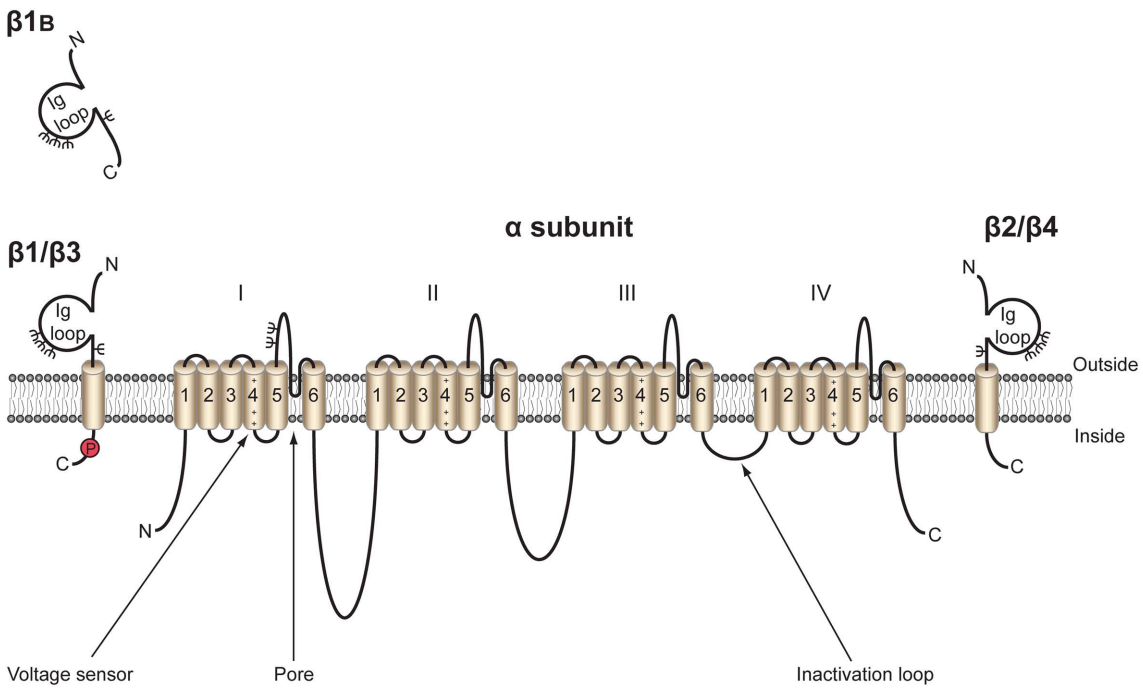

FIGURE 1 | Topology of the voltage-gated Na+ channel α and β subunits. VGSCs contain a pore-forming α subunit consisting of four homologous domains of six transmembrane segments (1–6). Segment 4 contains the voltage sensor (Catterall, 2000). VGSCs also contain one or more β subunits. β1, β2, β3, and β4 contain an extracellular immunoglobulin (Ig) loop, transmembrane domain, and an intracellular C-terminal domain (Isom et al., 1994). β1B also contains an Ig loop, but has a different C-terminus lacking a transmembrane domain, and is thus a soluble, secreted protein (Patino et al., 2011). β1 contains a tyrosine phosphorylation site in its C-terminus (Malhotra et al., 2004) ψ, glycosylation sites. β1 and β3 are non-covalently linked to α, whereas β2 and β4 are covalently linked through disulfide bonds. Figure was produced using Science Slides 2006 software.

the zebrafish *SCN1B* ortholog *scn1ba*, *scn1ba_tv1*, and *scn1ba_tv2* (Fein et al., 2007) with altered protein structure, and β1.2 in rat with an altered 3′ untranslated region (Dib-Hajj and Waxman, 1995). The tissue-specific expression profiles of each of the β subunits are subtly different, but clearly overlapping (**Table 1**). As with the α subunits, β subunits are highly expressed in excitable cells, including central and peripheral neurons, skeletal and cardiac muscle cells (Isom et al., 1992, 1995; Morgan et al., 2000; Yu et al., 2003; Maier et al., 2004; Lopez-Santiago et al., 2006, 2011; Brackenbury et al., 2010). Importantly, however, increasing evidence points to the expression of β subunits in a broad range of traditionally non-excitable cells, including stem cells, glia, vascular endothelial cells, and carcinoma cells (O'Malley and Isom, manuscript in preparation; Diss et al., 2008; Chioni et al., 2009; Andrikopoulos et al., 2011).

REGULATION OF EXCITABILITY BY INTERACTION WITH α SUBUNITS

Beginning with the initial report of β1 cloning in 1992 (Isom et al., 1992), numerous studies have demonstrated that all five β subunits alter gating and kinetics of α subunits expressed in heterologous cells (Catterall, 2000; Kazen-Gillespie et al., 2000; Qin et al., 2003; Yu et al., 2003). For example, in both *Xenopus* oocytes and mammalian cell lines, β1 and β2 increase the peak Na^+ current carried by $Na_v1.2$, accelerate inactivation, and shift the voltage-dependence of activation and inactivation to more negative potentials (Isom et al., 1992, 1994, 1995). However, inconsistencies between different reports documenting the magnitude and types of current modulation suggest that the cell background, including expression of endogenous β subunits and/or other interacting proteins, is a critical factor to consider when interpreting the data (Moran et al., 2000, 2003; Meadows and Isom, 2005). Furthermore, some of the more obvious effects of β1 and β2 on Na^+ current gating and kinetics in heterologous cells, especially *Xenopus* oocytes, do not appear to be reflected *in vivo*. In fact, results from null mouse models suggest that the effects of β1 and β2 on Na^+ currents *in vivo* are subtle and cell type-specific (Chen et al., 2002, 2004; Aman et al., 2009; Patino et al., 2009; Brackenbury et al., 2010).

Voltage-gated Na^+ channel β subunits have major effects on cellular excitability *in vivo*, suggesting that their subtle effects on Na^+ currents are functionally significant. For example, in *Scn1b* null mice, the fastest components of the compound action potential are slowed in the optic nerve (Chen et al., 2004). The heart rate is also slowed and action potentials in ventricular myocytes are slower to repolarize resulting in QT prolongation (Lopez-Santiago et al., 2007). *Scn1b* null mice are ataxic and, display frequent spontaneous bilateral myoclonic seizures from postnatal day (P)8–10 (Chen et al., 2004). Action potentials in *Scn1b* null CA3 neurons fire with a significantly higher peak voltage and significantly greater amplitude compared with wildtype neurons (Patino et al., 2009). In addition, the action potential firing

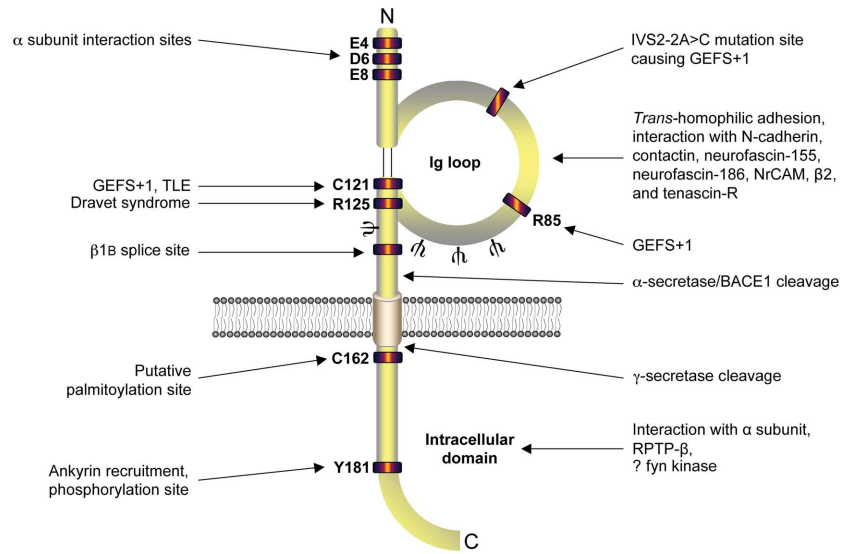

FIGURE 2 | Functional architecture of β1/β1B. β1 contains residues responsible for interaction with α subunit in its intracellular and extracellular domains (Mccormick et al., 1998; Spampanato et al., 2004). Mutation sites responsible for causing genetic epilepsy with febrile seizures plus (GEFS + 1), temporal lobe epilepsy (TLE), and Dravet syndrome are located in the extracellular immunoglobulin loop (Meadows et al., 2002; Wallace et al., 2002; Audenaert et al., 2003; Scheffer et al., 2007; Patino et al., 2009). Alternative splicing site for β1B (Kazen-Gillespie et al., 2000; Qin et al., 2003; Patino et al., 2011), putative palmitoylation site (Mcewen et al., 2004), ankyrin interaction site (Malhotra et al., 2002), tyrosine phosphorylation site (Malhotra et al., 2004), *N*-glycosylation sites (ψ; Mccormick et al., 1998), α/β/γ-secretase cleavage sites (Wong et al., 2005), receptor protein tyrosine phosphatase β (RPTPβ) interaction (Ratcliffe et al., 2000), and putative fyn kinase interaction (Malhotra et al., 2002, 2004; Brackenbury et al., 2008) are also marked. Figure was produced using Science Slides 2006 software.

rate is reduced in *Scn1b* null cerebellar granule neurons (Brackenbury et al., 2010). Reduced action potential firing in inhibitory interneurons (e.g., GABAergic granule neurons) may lead to overall hyperexcitability within the neuronal network, and result in hyperexcitability-related disorders, e.g., seizures (Oakley et al., 2011). In contrast, *Scn1b* null nociceptive dorsal root ganglion neurons are hyperexcitable (Lopez-Santiago et al., 2011). In the latter example, the hyperexcitability is proposed to be due to modulation of both Na^+ and K^+ currents by β1 or β1B (Lopez-Santiago et al., 2011). In support of this notion, β1 interacts with, and modulates, the gating of the inward rectifier $K_v4.3$ in heterologous cells (Deschenes et al., 2002, 2008).

β2 also regulates VGSC α subunits in neurons, and thereby electrical excitability. However, its role is proposed to be somewhat different to β1, and its effects on channel kinetics and voltage-dependence appear even subtler *in vivo*. Unlike *Scn1b* null mice, *Scn2b* null mice appear normal in neurological tests, although they display increased seizure susceptibility, and an elevated action potential threshold in the optic nerve (Chen et al., 2002, 2004). β2 associates with α subunits as the final step in neuronal VGSC biosynthesis, thereby permitting insertion of the complex into the plasma membrane, and increasing Na^+ current (Schmidt and Catterall, 1986; Isom et al., 1995). Thus, β2 plays an important role in stabilizing channel expression at the cell surface, and thereby maintaining normal action potential threshold. In agreement with this, there is ∼50% decreased expression of α subunits and Na^+ currents at the plasma membrane of *Scn2b* null hippocampal neurons (Chen et al., 2002). However, *Scn2b* deletion has no effect on Na^+ currents recorded from neurons isolated from the dentate gyrus, suggesting that, similar to β1, its effects are cell type-specific (Uebachs et al., 2010). In *Scn2b* null small-fast dorsal root ganglion neurons, tetrodotoxin-sensitive Na^+ current is reduced by ∼50% and kinetics of activation and inactivation are slowed. Consistent with this, the protein level of $Na_v1.7$ is reduced, whereas tetrodotoxin-resistant Na^+ current is unchanged (Lopez-Santiago et al., 2006). β2 may therefore specifically regulate tetrodotoxin-sensitive channels *in vivo*.

Similar to *Scn2b* null mice, *Scn3b* null mice behave normally and have full lifespans (Chen et al., 2002; Hakim et al., 2008). *Scn1b* may compensate for *Scn3b* deletion in brain, providing for an apparently normal neurological phenotype (Hakim et al., 2008, 2010a). *Scn3b* null hearts display ventricular arrhythmogenic properties, including shorter effective refractory periods, induced tachycardia, and shorter action potential durations, corresponding to reduced Na^+ current and hyperpolarized inactivation (Hakim et al., 2008). Atrial conduction abnormalities have also been reported in *Scn3b* null mice (Hakim et al., 2010a). These defects can be, in part, mitigated with the Class I antiarrhythmic agents flecainide and quinidine (Hakim et al., 2010b), supporting the conclusion that β3 modulates α subunit function in the heart.

The β4 intracellular domain may regulate α subunits in cerebellar Purkinje neurons by acting as an open-channel blocker of VGSCs that carry resurgent Na^+ current (Grieco et al., 2005). Silencing *Scn4b* in cerebellar granule neurons reduces resurgent and persistent Na^+ currents, hyperpolarizes voltage-dependence of inactivation of transient current, and reduces repetitive action

Table 1 | The β subunit family: tissue locations, interacting proteins, and disease association.

β subunit	Gene	Tissue locations	Interacting proteins	Disease	Reference
β1	*SCN1B*	CNS, heart, PNS, skeletal muscle	Ankyrin$_B$, ankyrin$_G$, β1, β2, contactin, K_v4.3, NF155, NF186, *N*-cadherin, NrCAM, tenascin-R, RPTPβ	Epilepsy, cardiac arrhythmia, cancer	Isom et al. (1992), Wallace et al. (1998), Xiao et al. (1999), Malhotra et al. (2000), Ratcliffe et al. (2000), Kaplan et al. (2001), Deschenes and Tomaselli (2002), Malhotra et al. (2002), Meadows et al. (2002), Wallace et al. (2002), Aronica et al. (2003), Audenaert et al. (2003), Chen et al. (2004), Davis et al. (2004), Malhotra et al. (2004), Mcewen and Isom (2004), Fein et al. (2007), Lopez-Santiago et al. (2007), Scheffer et al. (2007), Diss et al. (2008), Fein et al. (2008), Chioni et al. (2009), Watanabe et al. (2009), Brackenbury et al. (2010)
β1B	*SCN1B*	Adrenal gland, CNS, heart, PNS, skeletal muscle	β1	Epilepsy	Kazen-Gillespie et al. (2000), Qin et al. (2003), Patino et al. (2011)
β2	*SCN2B*	CNS, heart, PNS	Ankyrin$_G$ β1, β2, tenascin-C, tenascin-R	Altered pain response, cardiac arrhythmia, MS, seizure susceptibility	Isom et al. (1995), Srinivasan et al. (1998), Xiao et al. (1999), Malhotra et al. (2000), Chen et al. (2002), Yu et al. (2003), Mcewen et al. (2004), Pertin et al. (2005), Lopez-Santiago et al. (2006), O'Malley et al. (2009), Watanabe et al. (2009)
β3	*SCN3B*	Adrenal gland, CNS, heart, kidney, PNS	NF186	Epilepsy, cardiac arrhythmia, traumatic nerve injury	Morgan et al. (2000), Ratcliffe et al. (2001), Shah et al. (2001), Adachi et al. (2004), Casula et al. (2004), Chioni et al. (2009), Hu et al. (2009), Van Gassen et al. (2009), Valdivia et al. (2010), Wang et al. (2010), Olesen et al. (2011)
β4	*SCN4B*	CNS, heart, PNS, skeletal muscle	β1	Huntington's disease, long-QT syndrome	Yu et al. (2003), Davis et al. (2004), Oyama et al. (2006), Medeiros-Domingo et al. (2007), Aman et al. (2009)

CNS, central nervous system; MS, multiple sclerosis; NF155, neurofascin-155; NF186, neurofascin-186; PNS, peripheral nervous system; RPTPβ, receptor protein tyrosine phosphatase β.

potential firing (Bant and Raman, 2010). Resurgent Na^+ current is proposed to facilitate repetitive firing in cerebellar neurons (Khaliq et al., 2003; Bant and Raman, 2010). β4 thus appears to play a key role in regulating excitability. Finally, β4 plays an antagonistic role with β1 in regulating hippocampal neuron excitability: β4 slows inactivation and is proposed to promote excitability, whereas β1 promotes inactivation and is proposed to act as a brake on excitability (Aman et al., 2009). In summary, each of the β subunits regulates excitability through interaction with, and modulation of, α subunits in a cell type-specific and channel subtype-specific manner.

NON-CONDUCTING FUNCTIONS

β subunits are multifunctional (**Figure 2**). In addition to their "conducting" role in modulating Na^+ current kinetics and voltage-dependence, they are members of the Ig superfamily of cell adhesion molecules (CAMs) and participate in a number of "non-conducting" cell adhesion related activities (Isom et al., 1995; Yu et al., 2003). β1 and β2 both participate in *trans*-homophilic adhesion resulting in cellular aggregation and recruitment of ankyrin to points of cell–cell contact in *Drosophila* S2 cells (Malhotra et al., 2000). By contrast, β3, in spite of its high homology to β1, does not mediate homophilic adhesion (Mcewen et al., 2009) but participates in heterophilic adhesion (see below). Phosphorylation of tyrosine residue (Y)181 in the intracellular domain of β1 abrogates the recruitment of ankyrin$_B$ and ankyrin$_G$ in transfected Chinese hamster lung cells (Malhotra et al., 2002). In cardiac myocytes, phosphorylation of Y181 determines localization of β1 to intercalated disks with connexin-43, *N*-cadherin, and Na_v1.5, while non-phosphorylated β1 localizes in the t-tubules with ankyrin$_B$ (Malhotra et al., 2004). Thus, the phosphorylation state of Y181 may be important for regulating the subcellular distribution of β1. Interestingly, the intracellular domain of β1 interacts with receptor protein tyrosine phosphatase-β in rat brain neurons (Ratcliffe et al., 2000), potentially providing a mechanism for regulating Y181 phosphorylation. In addition, indirect evidence suggests that β1-mediated *trans*-homophilic adhesion results in fyn kinase activation in mouse cerebellar granule neurons (Brackenbury et al., 2008), which in turn could further fine tune phosphorylation of Y181.

The β subunits interact heterophilically with several other CAMs and extracellular matrix proteins. β1 interacts with VGSC β2, contactin, neurofascin-155, neurofascin-186, NrCAM, *N*-cadherin (Kazarinova-Noyes et al., 2001; Ratcliffe et al., 2001; Malhotra et al., 2004; Mcewen and Isom, 2004). Interaction between β1 and contactin, neurofascin-186, or VGSC β2 increases Na^+

current in heterologous systems (Kazarinova-Noyes et al., 2001; Mcewen et al., 2004), suggesting that β subunit-dependent adhesion may regulate α subunit function and excitability. Both β1 and β2 interact with the extracellular matrix protein tenascin-R (Xiao et al., 1999). β2 also interacts with tenascin-C (Srinivasan et al., 1998), but does not interact with contactin (Mcewen et al., 2004). Less is known about the heterophilic interactions of the other β subunits. β3, which does not interact with either β1 or contactin, does interact with neurofascin-186 (Ratcliffe et al., 2001; Mcewen et al., 2009). Although similar studies have not been performed for β1B, it has been proposed that its heterophilic binding partners are likely similar to those of β1, given that both molecules share an identical Ig domain (Patino and Isom, 2010).

β1, β2, β3, and β4 subunits are substrates for sequential proteolytic cleavage by enzymes from the secretase family. These β subunits contain cleavage sites for the β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) on the extracellular domain, adjacent to the transmembrane region (Wong et al., 2005; Gersbacher et al., 2010). β2 also contains a cleavage site for the α-secretase enzyme ADAM10 in the extracellular juxtamembrane region (Kim et al., 2005). Cleavage by BACE1 or α-secretase results in shedding of the extracellular Ig domain, leaving transmembrane C-terminal fragments (Kim et al., 2005; Wong et al., 2005). The C-terminal fragments are subsequently processed by γ-secretase at a site in the intracellular juxtamembrane region, thus releasing soluble intracellular domains into the cytoplasm (Kim et al., 2005; Wong et al., 2005). Although these four β subunits are cleaved by BACE1 *in vitro*, processing *in vivo* has so far been demonstrated only for β2 and β4 (Wong et al., 2005). Importantly, the signaling events responsible for initiating β subunit processing by the secretases, as well as potential developmental timing of these cleavage events *in vivo*, have not been investigated.

The functional effects of processing these β subunits appear critical to their *in vivo* function. For example, both the extracellular domain of β1, and its soluble splice variant, β1B, promote neurite outgrowth (Davis et al., 2004; Patino et al., 2011). Similarly, cleavage of the extracellular domain of β4 by BACE1 also increases neurite outgrowth (Miyazaki et al., 2007). Inhibition of γ-secretase activity reduces β2-dependent cell adhesion and migration, suggesting that the intracellular domain is important for promoting these functions (Kim et al., 2005). The β2 intracellular domain translocates to the nucleus of transfected SH-SY5Y cells and increases expression of *SCN1A*, suggesting that it may function, directly or indirectly, as a transcriptional regulator of VGSC α subunit expression (Kim et al., 2007). Further, the mRNA and protein levels of *Scn1a*/$Na_v1.1$ are reduced in the brains of *Bace1* null mice (Kim et al., 2011). Altered expression of α subunit mRNA and protein in the peripheral and central nervous systems of *Scn1b* and *Scn2b* null mice, as well as the hearts of *Scn1b* and *Scn3b* null mice (Chen et al., 2004; Lopez-Santiago et al., 2006, 2007, 2011; Hakim et al., 2008; Brackenbury et al., 2010), suggests that regulation of α subunit expression by β subunits may be widespread. Finally, cleavage of β4 by BACE1 in cerebellar Purkinje cells slows the decay of resurgent Na^+ current, thus promoting action potential firing (Huth et al., 2011), suggesting that secretase-mediated β subunit processing may modulate α subunit activity and thus neuronal excitability. However, another study from the same group indicated that BACE1 modulates α subunit gating in transfected human embryonic kidney cells and murine neuroblastoma cells, independent of its proteolytic effect on β2 and β4 (Huth et al., 2009). Thus the effects of BACE1 on α and β subunit function appear complex. Further work is required to understand the regulatory events involved in this putative signaling cascade and to establish whether BACE1 does indeed directly interact with α subunits in addition to β subunits.

ROLE OF β SUBUNITS IN DEVELOPMENT

β1 promotes neurite outgrowth in cerebellar granule neurons through *trans*-homophilic cell–cell adhesion (Davis et al., 2004). This mechanism operates through lipid rafts and requires fyn kinase, contactin, and Na^+ current (Brackenbury et al., 2008, 2010). β1-mediated neurite outgrowth also requires γ-secretase activity, suggesting that proteolytic processing of the intracellular domain may be important (**Figures 3A,B**). In contrast, neither β2 nor β4 promote neurite outgrowth in cerebellar granule neurons (Davis et al., 2004). However, β4 enhances neurite extension in neuroblastoma cells, and increases dendritic spine density in hippocampal neurons (Oyama et al., 2006; Miyazaki et al., 2007). In addition, β1 and β2 regulate the migration of fibroblasts away from the extracellular matrix protein tenascin-R (Xiao et al., 1999).

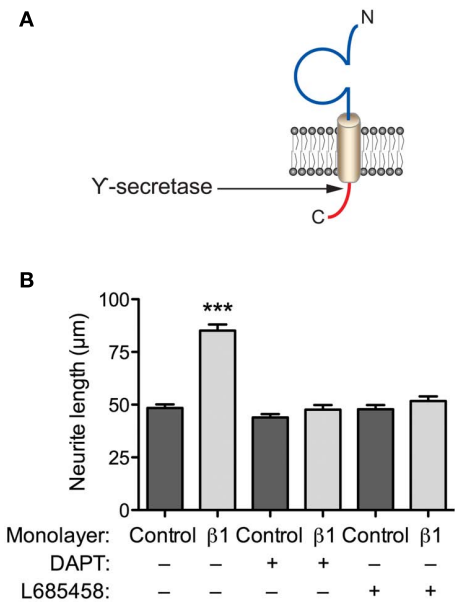

FIGURE 3 | β1-mediated neurite outgrowth requires γ-secretase activity. (A) Location of γ-secretase cleavage site on the intracellular domain of β1 (Wong et al., 2005). **(B)** Cerebellar granule neurons from postnatal day (P)14 wildtype mice were plated on top of monolayers of control or β1-expressing Chinese hamster lung cells, as described previously (Davis et al., 2004). Cultures were incubated with the either one of the γ-secretase inhibitors, L685458, or DAPT (both 1 μM); or control (DMSO) for 48 h (Kim et al., 2005). Cells were then fixed, processed for GAP43 immunocytochemistry, and neurite lengths measured, as described (Davis et al., 2004). Both L685458 and DAPT inhibited the increase in neurite length caused by β1 expressed in the monolayer. Data are mean + SEM ($n = 300$). Significance: $***P < 0.001$, ANOVA with Tukey's *post hoc* test.

Finally, β1 inhibits the migration of metastatic breast cancer cells (Chioni et al., 2009). Thus, β subunit-mediated neurite outgrowth and migration may be subtype and/or cell-specific.

The regulation of neurite outgrowth and migration by β subunits has consequences for development and organogenesis. In particular, β1 plays a critical role in neuronal pathfinding in postnatal-developing fiber tracts, coinciding with the onset of its expression from birth (Sutkowski and Catterall, 1990; Sashihara et al., 1995; Brackenbury et al., 2008). *Scn1b* null mice display a severe phenotype that includes growth retardation, ataxia, spontaneous seizures from P8–10, and death by P21 (Chen et al., 2004). In P14–16 *Scn1b* null mice, the pathfinding and migration of corticospinal axons is disrupted, leading to significant defasciculation of fibers at the pyramidal decussation (Brackenbury et al., 2008). The cerebellar parallel fibers are also defasciculated in P14 *Scn1b* null mice. The migration of granule neurons through the cerebellar molecular layer is disrupted, resulting in their accumulation in the external germinal layer, which is consequently thicker in *Scn1b* null mice than in wildtype littermates (Brackenbury et al., 2008). These cerebellar defects may contribute to the ataxic phenotype (Chen et al., 2004). Consistent with results in mice, abnormal pathfinding has also been reported in the olfactory nerve of zebrafish *scn1bb* morphants (Fein et al., 2008).

An important next step will be to determine whether defects in *Scn1b*-mediated cell–cell adhesion and migration in brain occur prior to the onset of convulsive seizures (Chen et al., 2004). It is possible that abnormal neuronal migration and pathfinding in the absence of β1/β1B-mediated cell adhesive interactions may lead to aberrant connections, resulting in neuronal hyperexcitability and epileptogenesis. Further work is required to establish the causational relationship between β1/β1B expression, cell adhesion, migration, and seizures during postnatal development of the nervous system.

ALTERATIONS IN VGSC PHARMACOLOGY BY β SUBUNITS

Studies have indicated that β subunits can alter the effect of pharmacological compounds on Na^+ currents carried by α subunits. For example, using heterologous systems, co-expression of β1 or β3 with $Na_v1.3$ in *Xenopus* oocytes attenuates the inhibitory effect of the antiarrhythmic agent and local anesthetic lidocaine on current amplitude and inactivation (Lenkowski et al., 2003). Further, the β1 C121W epilepsy mutation reduces tonic and use-dependent channel block in response to the antiepileptic drug phenytoin (Lucas et al., 2005). This altered sensitivity to phenytoin is proposed to be as a result of the altered gating caused by the mutation (Meadows et al., 2002), rather than a direct effect on the drug receptor. A recent *in vivo* study showed that the use-dependent reduction of transient Na^+ current caused by the anticonvulsant drug carbamazepine in wildtype hippocampal neurons was not observed in *Scn1b* or *Scn2b* null hippocampal neurons (Uebachs et al., 2010). However, carbamazepine caused a small hyperpolarizing shift in the voltage-dependence of activation of both transient and persistent Na^+ current. The hyperpolarizing shift in persistent Na^+ current was significantly increased in *Scn1b* null neurons at low carbamazepine concentrations, resulting in a complete loss in efficacy of the drug to reduce repetitive action potential firing (Uebachs et al., 2010). Thus, β1/β1B alters the pharmacological response of persistent Na^+ current to carbamazepine. Finally, in *Scn3b* null hearts, the VGSC-blocking antiarrhythmic agents flecainide and quinidine both modify ventricular effective refractory periods, resulting in anti-arrhythmogenic effects, in contrast to their effects in wildtype or *Scn5a* mutant hearts (Hakim et al., 2010b). Taken together, these findings have important clinical implications, suggesting that function-altering mutations and/or altered expression or localization of β subunits in patients may affect their sensitivity to VGSC-targeting drugs and thus therapeutic efficacy. Further work is required to establish whether or not β subunits alter pharmacological responses to additional VGSC-targeting therapeutics.

FUNCTIONAL RECIPROCITY BETWEEN α AND β SUBUNITS

Extensive evidence indicates that β subunits modulate channel gating of α subunits (see Regulation of Excitability by Interaction with α Subunits). Similarly, β1-mediated neurite outgrowth is inhibited by the VGSC-blocking toxin tetrodotoxin (Brackenbury et al., 2010). Thus, there is a potential for interplay between β1-mediated modulation of Na^+ current carried by α subunits and β1-mediated cell–cell adhesion/migration. β1 is required for normal high-frequency action potential firing in cerebellar granule neurons (**Figure 4A**). β1-mediated neurite outgrowth is abrogated in *Scn8a* null cerebellar granule neurons, suggesting that the mechanism requires Na^+ current carried by $Na_v1.6$ (**Figure 4B**). $Na_v1.6$ is vital for high-frequency repetitive firing in cerebellar neurons (Raman and Bean, 1997). Resurgent Na^+ current, carried by $Na_v1.6$, and which facilitates repetitive action potential firing (Raman and Bean, 1997; Khaliq et al., 2003; Bant and Raman, 2010), is reduced in *Scn1b* null cerebellar granule neurons (Brackenbury et al., 2010). The *Scn1b* null mutation disrupts the expression of $Na_v1.6$ at the axon initial segment (AIS) of cerebellar granule neurons (**Figure 4C**).

Taken together, these data suggest that there is a functional reciprocity between β1 and $Na_v1.6$ in cerebellar neurons, such that, on the one hand, β1 is required for normal localization of $Na_v1.6$ at the AIS, thus permitting resurgent Na^+ current, and repetitive action potential firing. On the other hand, $Na_v1.6$ is required for β1-mediated neurite outgrowth. Electrical activity generated at the AIS is proposed to provide a depolarizing signal to open $Na_v1.6$ channels at the growth cone, further promoting β1-mediated neurite outgrowth (**Figure 4D**; Brackenbury et al., 2008). This reciprocal relationship between β1 and $Na_v1.6$ is critical for postnatal cerebellar development (Chen et al., 2004; Van Wart and Matthews, 2006). Impaired localization of β1 at the AIS may thus lead to altered excitability. In agreement with this, in knock-in mice heterozygous for the β1C121W mutation, which disrupts β1-dependent adhesion and alters channel gating, mutant β1 protein is excluded from the AIS of pyramidal neurons, potentially contributing to febrile seizures (Meadows et al., 2002; Wimmer et al., 2010).

Future work will no doubt establish whether or not further complementary roles exist between VGSC α and β subunits, interacting in a coordinated fashion to regulate processes including excitability and neurite extension. It is already clear however, that β subunits function in macromolecular complexes with α subunits

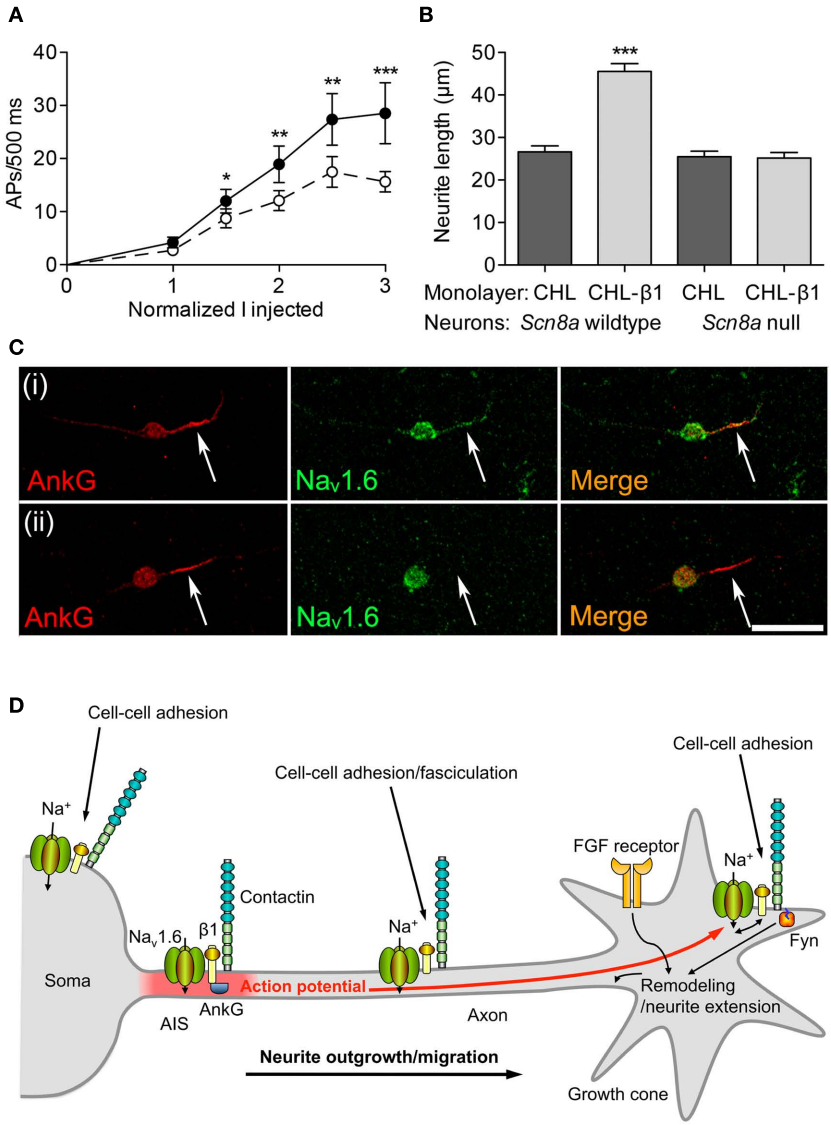

FIGURE 4 | Functional reciprocity between β1 and $Na_v1.6$. (A) Electrical excitability is impaired in *Scn1b* null cerebellar granule neurons. Action potential firing rate recorded from cerebellar granule neurons in brain slices from 12-day-old mice plotted as a function of injected current, normalized to action potential threshold for wildtype (filled circles) and *Scn1b* null (open circles). Data are mean ± SEM ($n \geq 15$). Significance: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; *t*-test. **(B)** β1-mediated neurite outgrowth is inhibited by the *Scn8a* null mutation. Neurite lengths of wildtype and *Scn8a* null cerebellar granule neurons grown on control Chinese hamster lung or β1-expressing monolayers ($n = 300$). Data are mean + SEM. Significance: $^{***}P < 0.001$, ANOVA with Tukey's *post hoc* test. **(C)** $Na_v1.6$ expression is reduced at the axon initial segment of *Scn1b* null cerebellar granule neurons. Wildtype and *Scn1b* null cerebellar granule neurons cultured *in vitro* for 14 days labeled with anti-ankyrin$_G$ (red) and $Na_v1.6$ antibodies (green). Scale bar, 20 μm. Arrows point to axon initial segment expressing ankyrin$_G$. **(D)** A model for Na^+ current involvement in β1-mediated neurite outgrowth. Complexes containing $Na_v1.6$, β1, and contactin are present throughout the neuronal membrane in the soma, neurite and growth cone. Localized Na^+ influx is necessary for β1-mediated neurite extension and migration. VGSC complexes along the neurite participate in cell–cell adhesion and fasciculation. β1 is also required for $Na_v1.6$ expression at the axon initial segment, and subsequent high-frequency action potential firing through modulation of resurgent Na^+current. Electrical activity may further promote β1-mediated neurite outgrowth at or near the growth cone. Thus, the developmental functions of β1 and $Na_v1.6$ are complementary, such that (1) Na^+ influx carried by $Na_v1.6$ is required for β1-mediated neurite outgrowth, and (2) β1 is required for normal expression/activity of $Na_v1.6$ at the axon initial segment. Fyn kinase and ankyrin$_G$ are likely also present in all complexes, but are only shown once in each panel for clarity. The FGF-mediated, β1-independent neurite outgrowth pathway is also shown. Figure reproduced with permission (Brackenbury et al., 2010).

to participate in signaling on multiple timescales to regulate excitability, adhesion, neurite outgrowth, and migration. A critical focus of future work will be to determine whether β subunits that are expressed independently of the ion-conducting pore also play roles in excitability *in vivo*, perhaps through regulation of axon guidance or fasciculation.

DYSREGULATION IN DISEASE

Voltage-gated Na^+ channel β subunits are implicated in a number of neurological diseases (**Table 1**) [reviewed extensively in Patino and Isom (2010)]. Of particular note is the growing list of mutations in *SCN1B* that are associated with genetic epilepsy with febrile seizures plus (GEFS) + 1 (OMIM 604233), a spectrum of disorders that includes mild to severe forms of epilepsy (Wallace et al., 1998, 2002; Audenaert et al., 2003; Burgess, 2005; Yamakawa, 2005; Scheffer et al., 2007; Patino et al., 2009, 2011). No GEFS + 1-causing mutations in the other β subunit genes have yet been identified. However, *Scn2b* null mice display increased seizure susceptibility (Chen et al., 2002). In addition, *SCN3B* is reduced in the hippocampus of patients with temporal lobe epilepsy, suggesting that altered β3 expression may contribute to or result from epilepsy (Van Gassen et al., 2009). The mutations in *SCN1B* may bias neurons toward hyperexcitability and epileptogenesis by one or both of two distinct mechanisms: (1) impaired regulation of α subunit-dependent excitability (Meadows et al., 2002; Chen et al., 2004; Spampanato et al., 2004; Patino et al., 2009; Wimmer et al., 2010); and/or (2) impaired cell–cell adhesive interactions (Meadows et al., 2002; Brackenbury et al., 2008; Fein et al., 2008; Patino et al., 2011). There may also be a causal relationship between VGSC α-β1 interactions, cell–cell adhesion, migration, and epilepsy. Further work is required to establish whether or not disrupted β1-dependent cell adhesion is indeed a prerequisite for seizure activity.

Changes in β2 expression have been implicated in altered pain sensation. *Scn2b* null mice are more sensitive to noxious thermal stimuli than wildtype mice (Lopez-Santiago et al., 2006). In addition, the spared nerve injury model of neuropathic pain results in increased β2 expression in rat sensory neurons, and mechanical allodynia-like behavior (Pertin et al., 2005). This behavior is absent in *Scn2b* null mice, suggesting that β2 expression may play an important role in neuropathic pain sensation (Pertin et al., 2005).

The β subunits play roles in neurodegenerative disease. The *Scn2b* null mutation is neuroprotective in the experimental allergic encephalomyelitis mouse model of multiple sclerosis (O'Malley et al., 2009). In addition, levels of *Scn4b* are reduced in mouse models of Huntington's disease prior to onset of motor symptoms, and a similar reduction has also been reported in patients (Oyama et al., 2006).

Indirect evidence suggests that β subunits may be involved in neuropsychiatric disorders. For example, $ankyrin_G$ and $Na_v1.6$, which both interact with β subunits, are linked genetically to bipolar disorder (Gargus, 2006; Wang et al., 2008). Migration defects observed in the cerebellum of *Scn1b* null mice may result in abnormal connections with the prefrontal cortex and posterior parietal cortex, thus providing a possible mechanism for β subunit involvement in mood disorders (Brackenbury et al., 2008; Strick et al., 2009). Similarly, migration defects in other brain areas in patients with *SCN1B* mutations may contribute to mental disorders. Pathological cellular migration regulated by β subunits extends beyond neurological diseases: β1 regulates cellular adhesion and migration in metastatic breast cancer cell lines (Chioni et al., 2009). β subunit transcripts are also expressed in prostate cancer cells and lung cancer cells (Roger et al., 2007; Diss et al., 2008), suggesting that their involvement in cancer may be widespread.

Finally, mutations in β subunits are associated with cardiac abnormalities (Wilde and Brugada, 2011). Mutations in *SCN1B* have been reported in patients with idiopathic ventricular fibrillation (Brugada syndrome; Watanabe et al., 2008; Hu et al., 2009; Valdivia et al., 2010). Mutations in *SCN1B*, *SCN2B*, and *SCN3B* are also associated with atrial fibrillation (Watanabe et al., 2009; Wang et al., 2010; Olesen et al., 2011). Mutations in *SCN3B* and *SCN4B* are associated with sudden infant death syndrome (Tan et al., 2010). These mutations are proposed to interfere with the ability of β subunits to modulate $Na_v1.5$ currents *in vivo* (Watanabe et al., 2008, 2009; Hu et al., 2009; Tan et al., 2010). A mutation in *SCN4B* results in long-QT syndrome (Medeiros-Domingo et al., 2007). Expression of a mutation linked to conduction disease and Brugada syndrome, $Scn5a^{1798insD/+}$, in a mouse strain (129P2) with markedly reduced *Scn4b* expression resulted in more severe cardiac conduction slowing than a strain with normal *Scn4b* levels (FVB/N), suggesting that *Scn4b* may be a genetic modifier of cardiac conduction (Remme et al., 2009). In summary, abnormal expression and/or function of β subunits appears to play an important role in a number of diseases, ranging from nervous system disorders, to cardiac abnormalities, and cancer.

CONCLUSION/OUTLOOK

Increasing new evidence supports the hypothesis that the VGSC β subunits are multifunctional. In addition, a growing list of mutations and *in vivo* studies indicate that the β subunits play important roles in a number of diseases due to abnormal function in both excitable and non-excitable cells. There is no doubt that the classical "conducting" role of β subunits as modulators of Na^+current is of paramount importance in regulating ion flux and excitability. However, there is a clear trend in the literature toward an increasingly important role for "non-conducting" functions, including cell adhesion, migration and pathfinding, and putative transcriptional regulation. As a result, the β subunits are integral components of VGSC macromolecular protein complexes, which can direct multiple signaling mechanisms on multiple timescales. Moreover, the cell adhesive, "non-conducting" properties of β subunits observed *in vitro* suggest that they may play critical functional roles independent of α subunits *in vivo*. The challenge now will be to clearly delineate the cell adhesive functions of β subunits from their roles in channel modulation during development and in pathophysiology. Clearer understanding of the interaction between the conducting and non-conducting functions of VGSC complexes will hopefully enable the full realization of their therapeutic potential.

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