

Reduced Sodium Channel Na_v1.1 Levels in BACE1-null Mice^{*[5]}

Received for publication, April 15, 2010, and in revised form, December 22, 2010 Published, JBC Papers in Press, December 29, 2010, DOI 10.1074/jbc.M110.134692

Doo Yeon Kim^{†1}, Manuel T. Gersbacher^{†1}, Perrine Inquimbert[§], and Dora M. Kovacs^{‡2}

From the [†]Neurobiology of Disease Laboratory, Genetics and Aging Research Unit, Massachusetts General Institute for Neurodegenerative Disease, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts 02129 and the [§]Department of Neurology, F. M. Kirby Neurobiology Center, Children's Hospital, Boston, Massachusetts 02115

The Alzheimer BACE1 enzyme cleaves numerous substrates, with largely unknown physiological consequences. We have previously identified the contribution of elevated BACE1 activity to voltage-gated sodium channel Na_v1.1 density and neuronal function. Here, we analyzed physiological changes in sodium channel metabolism in BACE1-null mice. Mechanistically, we first confirmed that endogenous BACE1 requires its substrate, the β-subunit Na_vβ₂, to regulate levels of the pore-forming α-subunit Na_v1.1 in cultured primary neurons. Next, we analyzed sodium channel α-subunit levels in brains of BACE1-null mice at 1 and 3 months of age. At both ages, we found that Na_v1.1 protein levels were significantly decreased in BACE1-null *versus* wild-type mouse brains, remaining unchanged in BACE1-heterozygous mouse brains. Interestingly, levels of Na_v1.2 and Na_v1.6 α-subunits also decreased in 1-month-old BACE1-null mice. In the hippocampus of BACE1-null mice, we found a robust 57% decrease of Na_v1.1 levels. Next, we performed surface biotinylation studies in acutely dissociated hippocampal slices from BACE1-null mice. Hippocampal surface Na_v1.1 levels were significantly decreased, but Na_v1.2 surface levels were increased in BACE1-null mice perhaps as a compensatory mechanism for reduced surface Na_v1.1. We also found that Na_vβ₂ processing and Na_v1.1 mRNA levels were significantly decreased in brains of BACE1-null mice. This suggests a mechanism consistent with BACE1 activity regulating mRNA levels of the α-subunit Na_v1.1 via cleavage of cell-surface Na_vβ₂. Together, our data show that endogenous BACE1 activity regulates total and surface levels of voltage-gated sodium channels in mouse brains. Both decreased Na_v1.1 and elevated surface Na_v1.2 may result in a seizure phenotype. Our data caution that therapeutic BACE1 activity inhibition in Alzheimer disease patients may affect Na_v1 metabolism and alter neuronal membrane excitability in Alzheimer disease patients.

Alzheimer disease (AD)³ is a devastating neurodegenerative disorder characterized by progressive memory loss. In the late

* This work was supported, in whole or in part, by National Institutes of Health grants from NIA (to D. M. K. and D. Y. K.).

[5] The on-line version of this article (available at <http://www.jbc.org>) contains supplemental Figs. 1–4.

¹ Both authors equally contributed to this work.

² To whom correspondence should be addressed. E-mail: dora_kovacs@hms.harvard.edu.

³ The abbreviations used are: AD, Alzheimer disease; APP, amyloid β-precursor protein; Aβ, amyloid β; Na_v1, voltage-gated sodium channel; Na_vβ₂, voltage-gated sodium channel β₂ subunit; Na_vβ₂-CTF, Na_vβ₂ C-terminal fragment; β₂-ICD, Na_vβ₂ intracellular domain; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; ANOVA, analysis of variance.

stages of the disease, neuropsychiatric symptoms include depression, aggressiveness, agitation, and generalized anxiety (1). Pathological hallmarks of AD patient brains consist of extracellular amyloid deposits (senile plaques), neurofibrillary tangles, and loss of neurons and synapses in the hippocampus and the cerebral cortex (2, 3). The main component of amyloid deposits is a short amyloid β (Aβ) peptide most commonly of 40–42 amino acids, which derives from the amyloid precursor protein (APP). To generate Aβ, APP is first cleaved by β-site APP-cleaving enzyme 1 (BACE1) and subsequently by γ-secretase. Because of their central role in Aβ generation, it is not surprising that the two secretases are among the prime drug targets for AD treatment (4, 5).

BACE1 is a transmembrane aspartic protease that is ubiquitously expressed with the highest levels in the brain (6, 7). BACE1 levels and activity are increased in AD patient brains (8–10). The reason why BACE1 is elevated in AD brains is unknown, although it has been shown that depletion of the adaptor protein GGA3 and phosphorylation of the translation initiation factor eIF2α increase BACE1 levels (11, 12). Cellular and oxidative stresses as well as energy starvation have an effect on the enzyme function (11, 13, 14). Currently, only a few proteins are known as physiological substrates of BACE1 (15–19). To understand the physiological function of BACE1, it is essential to characterize BACE1-mediated cleavage of its neuronal substrates.

Voltage-gated sodium channels (Na_v1s) consist of one pore-forming α-subunit, associated with one or two β-accessory subunits (20). Currently, 10 α- and 4 β-subunits have been reported (21). The β-subunits are type I single-transmembrane proteins that regulate the localization, trafficking, and inactivation of the α-subunits by direct interaction (20, 22–24). Of the four β-subunits, the β₂-subunit (Na_vβ₂) appears to have a major role in the regulation of the total and cell surface density of sodium channels in neurons (25–27). Na_vβ₂-null mice show altered response to pain and a reduced threshold for seizures because of defects in electrical excitability in neurons (26, 27).

BACE1-null mice have revealed abnormalities in behavior, cognitive, and emotional functions (28–31). An imbalance in sodium channel function may contribute to some of these phenotypes. Indeed, the auxiliary β₂-subunit of voltage-gated sodium channels undergoes sequential processing by BACE1 and γ-secretase similar to APP (18, 19). We found that BACE1-mediated cleavage of Na_vβ₂ alters neuronal activity (32). Moreover, elevated levels of the pore-forming α-subunit Na_v1.1 are observed in AD brains with elevated BACE1 expression and also in mice overexpressing human BACE1 (32). The β₂-intra-

cellular domain ($\beta 2$ -ICD), derived from *BACE1* and γ -secretase-mediated cleavage of $\beta 2$, regulates $\text{Na}_v1.1$ expression. However, $\text{Na}_v1.1$ trafficking to the cell membrane is impaired resulting in reduced $\text{Na}_v1.1$ surface levels, which leads to reduced sodium current densities (32).

Here, we asked whether endogenous *BACE1* activity regulates Na_v1 metabolism in neuronal cells under physiological conditions. First, we found that $\text{Na}_v\beta 2$ is essential for *BACE1* activity-dependent regulation of $\text{Na}_v1.1$ mRNA and protein levels in cultured embryonic neurons. Next, we also found that endogenous *BACE1* activity regulates total and surface levels of $\text{Na}_v1.1$ especially in adult hippocampus. Interestingly, $\text{Na}_v1.2$ appears to compensate for decreased surface levels of $\text{Na}_v1.1$.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—*N*-[*N*-3,5-Difluorophenacetyl]-*L*-alanyl]-*S*-phenylglycine *t*-butyl ester and *BACE* inhibitor IV were purchased from Calbiochem. *BACE1* monoclonal antibody (3D5) was a kind gift from Dr. Robert Vassar (Northwestern University), and anti- $\text{Na}_v\beta 2$ directed against the C terminus of endogenous $\text{Na}_v\beta 2$ was from Dr. Nobuyuki Nukina (RIKEN Brain Science Institute). The following antibodies were purchased for this study: anti-GAPDH (Chemicon); anti- $\text{Na}_v1.1$ antibody (University of California, Davis/National Institutes of Health NeuroMab Facility); anti- $\text{Na}_v1.2$, anti- $\text{Na}_v1.3$, and anti- $\text{Na}_v1.6$ (Alomone Labs); anti- Na_v1s (Sigma); anti-N-cadherin (BD Transduction Laboratories); and anti-synaptophysin (StressGen).

Primary Neuronal Cultures—Mouse primary hippocampal/cortical neuronal cultures were prepared and maintained in Neurobasal media (Invitrogen) supplemented with B27 and 0.5 mM *L*-glutamine as described previously (18). The neuronal cultures were maintained at 37 °C in a humidified 5% CO_2 atmosphere for 14 days. For rat primary neuronal culture, cryopreserved rat hippocampal/cortical regions were purchased from BrainBits (Springfield, IL), dissociated, and plated on poly-D-lysine/laminin-coated plates. The neuronal cultures were maintained for 14–17 days in Neurobasal media (Invitrogen) supplemented with B27 and 0.5 mM *L*-glutamine.

Breeding—All animal procedures were approved by the Subcommittee on Research Animal Care on the Use and Care of Animals, Massachusetts General Hospital. *BACE1*-null and wild-type control mice (C57BL/6J background) were purchased from The Jackson Laboratory (Bar Harbor, ME). *BACE1*-heterozygous knock-out mice were generated by crossing *BACE1*-null and wild-type control mice. Genotypes were determined by genomic PCR using the Allele-in-One mouse tail direct PCR system (Allele Biotechnology, San Diego) using the following primers: 5'-AGGCAGCTTTGTGGAG-ATGGTG-3', 5'-CGGGAAATGGAAAGGCTACTCC-3', and 5'-TGGATGTGGAATGTGTGCGAG-3'. $\text{Na}_v\beta 2$ -null and control mice were kindly provided by Dr. Lori L. Isom (University of Michigan). Generation and genotyping of $\text{Na}_v\beta 2$ -null mice were described previously (26).

Preparation of Mouse Brain Lysates—One- to 3-month-old mice were sacrificed, and brains were immediately removed and stored at -80 °C until use. To prepare total brain lysates, the frozen brains were powdered under liquid nitrogen with the

use of mortar and pestle. The aliquots were stored at -80 °C for individual experiments. For total brain extraction, 50–100 μg of brain powder was extracted in 5 volumes of SDS extraction buffer (10 mM Tris-HCl (pH 6.8), 2% SDS, 1 mM EDTA, 150 mM NaCl, a protease inhibitor mixture (Roche Applied Science), 2 mM 1,10-phenanthroline, 2 μM MG132, and 1 mM PMSF). After incubation on ice for 10 min, samples were sonicated at 4 °C for 5 min, passed through 0.22- μm pore-sized filter in spin-x centrifuge tubes (Costar, Corning, NY), and centrifuged for 45 min at 14,000 rpm. To prepare crude brain membrane fractions, the brain powder was resuspended in TBS extraction buffer (10 mM Tris-HCl (pH 6.8), 1 mM EDTA, 150 mM NaCl, a protease inhibitor mixture (Roche Applied Science), 2 mM PNT, 2 μM MG132, and 1 mM PMSF) and passed through 22-gauge needle three times. After brief centrifugation (500 $\times g$, 5 min), the supernatant was then centrifuged again at 10,000 $\times g$ for 1 h. The membrane pellet was then resuspended in TBS extraction buffer, and the protein level was determined by BCA assay. For SDS-PAGE analysis, the crude membrane fractions were directly mixed with 4 \times lithium dodecyl sulfate sample buffer (Invitrogen) supplemented with 8% (v/v) β -mercaptoethanol and heated at 95 °C for 5 min. To prepare hippocampal brain lysates, brains were microdissected under microscope immediately after the brains were removed. Hippocampi were then directly lysed in SDS extraction buffer (10 mM Tris-HCl (pH 6.8), 1.5% SDS, 1 mM EDTA, 150 mM NaCl, a protease inhibitor mixture (Roche Applied Science), 2 mM PNT, 2 μM MG132, and 1 mM PMSF). RNeasy mini kit (Qiagen) was used to extract total RNA from the brains. Approximately 30 mg of brain powder was dissolved in 600 μl of buffer RLT containing 1% β -mercaptoethanol by vortexing. Lysates were applied to QIAshredder (Qiagen). The instructions for RNeasy mini kit (Qiagen) were followed.

Confocal Immunofluorescence Staining of Hippocampal Sections—*BACE1*-null, *BACE1* heterozygote knock-out, and wild-type control mice were perfused with 12 ml of PBS and 1% paraformaldehyde, 1% sucrose solution, respectively. Brains were rapidly removed, further fixed in 1% paraformaldehyde, 1% sucrose solution for 3 h at 4 °C, transferred to 30% sucrose solution, and incubated for 48 h at 4 °C. 40- μm sections were cut, washed three times with PBS, and incubated with blocking solution containing 0.1% Triton X-100 and 5% purified goat IgG (Jackson ImmunoResearch) for 12 h. The sections were then incubated with primary antibody solution containing anti- $\text{Na}_v1.1$ antibody (monoclonal, 1:20 dilution, University of California, Davis/National Institutes of Health NeuroMab Facility), 0.1% Triton X-100, and 5% goat IgG for 24 h 4 °C. After washing three times, sections were incubated with secondary antibody solution with anti-mouse Alexa Fluor488 (Invitrogen) for 3 h at room temperature. Immunostained sections were mounted and analyzed by Olympus IX70 fluorescence microscope equipped with a confocal Disk Spinning Unit (Olympus). Images were captured and analyzed by IPLab software.

Western Blot Analysis—20–100 μg of protein were resolved on 4–12% gradient BisTris gels, 12% BisTris gels, or 16% Tricine gels (Invitrogen), depending on the individual experiment as described. The blots were visualized by enhanced chemiluminescence (ECL). The images were captured by using BioMax

Reduced $Na_v1.1$ Levels in *BACE1*-null Mice

film (Eastman Kodak Co.) or VersaDoc imaging system (Bio-Rad) and quantitated by using QuantityOne software (Bio-Rad).

Quantitative RT-PCR—Levels of sodium channel genes were analyzed by quantitative RT-PCR performed in a Light Cycler PCR system (Bio-Rad). 1 μ g of total RNA was used for the synthesis of cDNA using oligo(dT) primers following the protocol of the SuperScript first-strand synthesis system for RT-PCR (Invitrogen). Relative quantification of mRNA expression of *Scn1a* ($Na_v1.1$) compared with endogenous expression of GAPDH was analyzed by TaqMan real time PCR. Primers were purchased from Applied Biosystems (catalogue numbers Mn01329052-m1, Mn00488110-m1, and Mn01270368-m1). Expression analysis was performed with 40 two-step cycles for 30 s at 95 °C and 60 s 60 °C as described in the supplier's protocol (Applied Biosystems) using a Bio-Rad iCycler. Sodium channel mRNA was normalized to GAPDH mRNA by the comparative *CT* method.

Acute Hippocampal Slice Preparation—Hippocampal neurons from *BACE1*-transgenic and control mice (1–2 month) were acutely isolated using standard procedures (26, 33). Mice were decapitated under deep isoflurane anesthesia, and brains were rapidly removed and iced. Slices (400 μ m) were cut and transferred to a low calcium buffer containing 15 mM HEPES (pH 7.4), 140 mM sodium isethionate, 2 mM KCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, and 23 mM glucose. Slices were incubated for more than 1 h in $NaHCO_3$ -buffered Earle's balanced salt solution (Sigma) bubbled with 95% O_2 and 5% CO_2 at room temperature.

Surface Biotinylation—Surface biotinylation of acutely prepared hippocampal slices was performed according to a method described by Thomas-Crusells *et al.* (34) with slight modifications. The slices were prepared as described earlier and incubated with 100 μ M Sulfo-NHS-Biotin (Pierce) in Earle's balanced salt solution bubbled with 95% O_2 and 5% on ice for 80 min under dark conditions. The reaction was stopped by washing three times with cold Earle's balanced salt solution containing 100 μ M L-lysine. Hippocampal regions were rapidly separated from the individual slices under a dissecting microscope. The isolated hippocampi were extracted with a lysis buffer containing 10 mM Tris-HCl (pH 6.8), 1 mM EDTA, 150 mM NaCl, 0.2% SDS, 0.5% sodium deoxycholate, 1% Triton X-100, and protease inhibitors, followed by a spin at 16,000 \times g. Biotinylated proteins were captured by Neutravidin beads (Pierce) at 4 °C overnight.

Statistical Analyses—All statistical analyses were performed using a two-tailed Student's *t* test or one-way ANOVA followed by a post hoc Tukey's test. Error bars represented in graphs denote the means \pm S.E.

RESULTS

Na_v β_2 Mediates the Effect of BACE1 on $Na_v1.1$ Levels in Primary Neurons—Previously, we found that *BACE1* and γ -secretase sequentially cleave $Na_v\beta_2$ to release the intracellular domain of $Na_v\beta_2$ (β_2 -ICD). In neuroblastoma cells, β_2 -ICD is localized to the nucleus and regulates mRNA and protein levels of $Na_v1.1$ (32). To test whether endogenous *BACE1* activity regulates $Na_v1.1$ through the processing of $Na_v\beta_2$ *in vivo*, we

treated cultured rat cortical/hippocampal neurons with *BACE1* inhibitors and analyzed $Na_v1.1$ levels. We found that β_2 -ICD treatment decreased $Na_v1.1$ levels up to 50% in a dose-dependent manner (Fig. 1A). Interestingly, we also found that $Na_v1.2$ levels were dose-dependently increased upon β_2 -ICD treatment, suggesting potential compensatory changes (supplemental Fig. 1, A and B). Previously, we have shown that recombinant β_2 -ICD expression significantly elevates *Scn1a* ($Na_v1.1$) mRNA levels in rat neuroblastoma cells (32). Similarly to $Na_v1.1$, $Na_v1.2$ may also be regulated at the level of β_2 -ICD. Therefore, we tested the effect of recombinant β_2 -ICD on *Scn2a* ($Na_v1.2$) mRNA levels. Unlike *Scn1a* ($Na_v1.1$), *Scn2a* ($Na_v1.2$) mRNA levels did not change in a statistically meaningful way under the same conditions (supplemental Fig. 1C). These results suggest that a potential compensatory change of $Na_v1.2$ may not be directly regulated by β_2 -ICD.

To explore whether $Na_v\beta_2$ is required for *BACE1* activity-dependent $Na_v1.1$ decrease, we took advantage of primary neuronal cultures from $Na_v\beta_2$ -null mice (26). Cultured hippocampal/cortical neurons (14 days *in vitro*) from wild-type and $Na_v\beta_2$ -null mice were treated with *BACE1* inhibitors, followed by analysis of $Na_v1.1$ mRNA and protein levels. As expected, in wild-type neurons we found that two different *BACE1* inhibitors, β_2 -ICD or inhibitor IV, decrease $Na_v1.1$ protein levels by 42–70% (Fig. 1, B and C). *Scn1a* ($Na_v1.1$) mRNA levels were also decreased by β_2 -ICD or inhibitor IV (supplemental Fig. 1D). These data show that *BACE1* activity is required for maintaining $Na_v1.1$ levels in cultured rat and mouse neurons. In contrast, in $Na_v\beta_2$ -null neurons $Na_v1.1$ levels were unaffected by the same *BACE1* inhibitor treatments (Fig. 1, D and E). These data show that $Na_v\beta_2$ is required for *BACE1*-dependent regulation of $Na_v1.1$ levels in cultured embryonic neurons.

Decreased Na_v1 Levels in *BACE1*-null Mice—Next, we investigated whether endogenous *BACE1* activity is required for maintaining normal Na_v1 α -subunit levels in adult brains. Brain samples were prepared from 1- and 3-month-old *BACE1*-null (*BACE1*-KO), *BACE1*-heterozygous knock-out (*BACE1*-HE), and aged-matched wild-type control mice (WT). One month of age was chosen because it is the minimum age for analyzing adult Na_v1 s and is also age-compatible with acute preparation of hippocampal slices without inducing significant neuronal death. All animals were healthy; however, *BACE1*-null mice were generally smaller in size than wild-type and *BACE1*-HE animals as reported previously (28). Furthermore, increased mortality was observed among *BACE1*-null mice.

We compared total brain Na_v1 α -subunits levels in 1-month-old *BACE1*-null and *BACE1*-heterozygous knock-out with age-matched wild-type mice. To avoid degradation of protease-prone Na_v1 α -subunits, brains were rapidly removed, powdered under liquid nitrogen, aliquoted, and stored at -80 °C until extraction. Because Na_v1 α -subunits are hydrophobic integral membrane proteins, extraction conditions with strong ionic detergents were required to solubilize α -subunits from insoluble membrane fractions. We compared several extraction conditions, and we found that extraction with 2% SDS showed the most consistent results, tightly comparable with a method based on direct boiling with SDS-PAGE sample

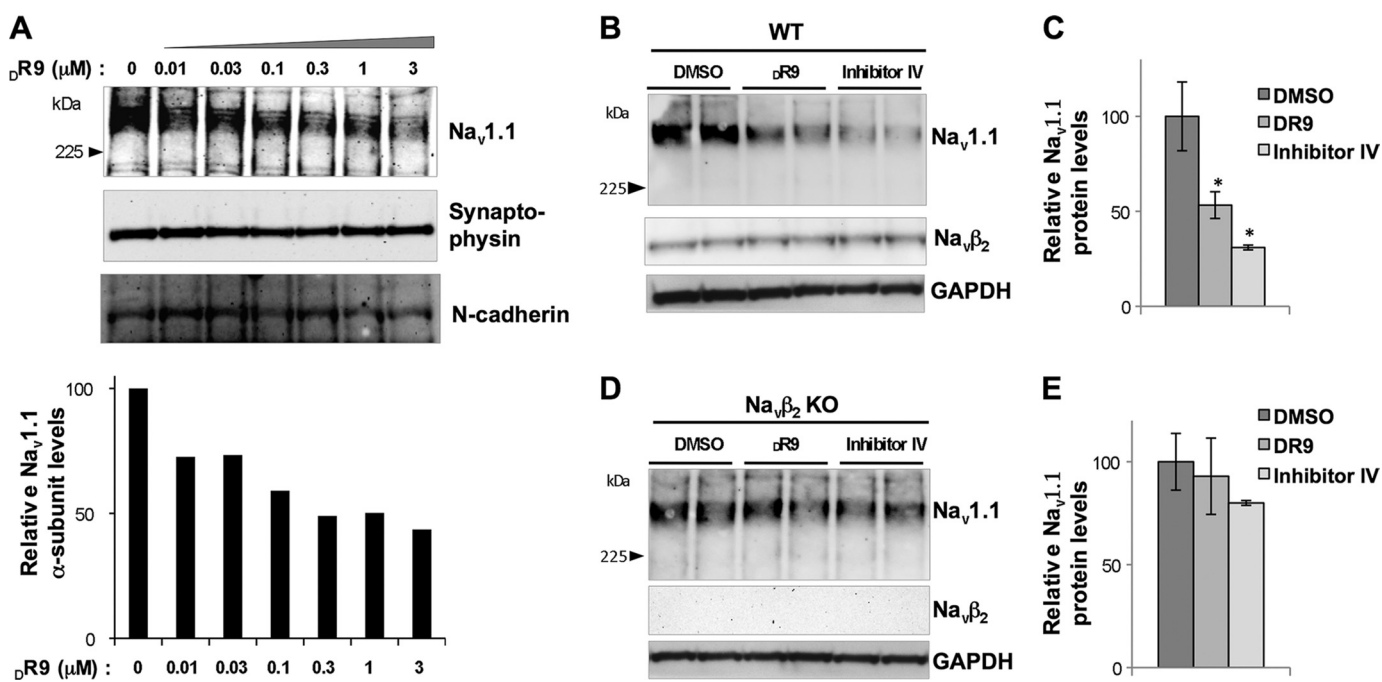


FIGURE 1. $Na_v\beta_2$ is required for BACE1 activity-dependent regulation of $Na_v1.1$ levels. Primary neuronal culture (14 days *in vitro*) from $Na_v\beta_2$ -null mice ($Na_v\beta_2$ KO), wild-type control mice (WT), and wild-type rat were treated with control vehicle (DMSO) or BACE1 inhibitors (α DR9 and/or inhibitor IV) for 48–72 h. $Na_v1.1$ protein levels were analyzed by Western blot. *A*, upper panel, $Na_v1.1$ Western blot analysis of rat primary neurons (14 days *in vitro*) treated with various concentrations of α DR9 and control vehicle DMSO for 72 h; lower panel, quantitative analysis of $Na_v1.1$ protein levels. *B*, representative Western blot showing changes in $Na_v1.1$ levels upon BACE1 inhibitor treatment in cultured WT neurons. *C*, quantitative analysis of $Na_v1.1$ protein levels upon BACE1 inhibitor treatment in cultured WT neurons. *D*, representative Western blot showing $Na_v1.1$ levels of cultured $Na_v\beta_2$ KO neurons treated with DMSO, α DR9, or inhibitor IV. *E*, quantitative analysis of $Na_v1.1$ protein levels of cultured $Na_v\beta_2$ KO neurons treated with DMSO, α DR9, or inhibitor IV. One-way ANOVA followed by a post hoc Tukey's test was used for statistical analysis (*, $p < 0.05$; $n = 3$ per each condition).

loading buffer (Fig. 2; for detailed buffer formulation and conditions, please see “Experimental Procedures”).

Western blot analysis showed more than 50% decrease of $Na_v1.1$ levels in brain extracts from BACE1-null as compared with age-matched wild-type control mice, although no significant difference was detected between BACE1-heterozygous knock-out and wild-type controls (Fig. 2). These data suggest that more than 50% decrease in BACE1 expression is required for decreasing $Na_v1.1$ levels in adult mouse brains. Interestingly, we also observed significant decreases in $Na_v1.2$ and $Na_v1.6$ levels, two additional major CNS Na_v1 α -subunits (Fig. 2). Levels of $Na_v1.3$, an embryonic Na_v1 α -subunit, did not change (data not shown). The supplemental Fig. 2 shows a littermate analysis of BACE1-null and BACE1-heterozygous knock-out mice. As expected, we found more than 50% decrease of $Na_v1.1$ levels in BACE1-null mice as compared with BACE1-heterozygous knock-out littermate controls at the same age.

Finally, we investigated whether the changes in $Na_v1.1$ levels were also seen in older mice. For this purpose, Na_v1 levels were analyzed in whole brain extracts from 3-month-old BACE1-null and wild-type control mice (Fig. 3A). Similar to 1-month-old mice, we observed a significant $\sim 20\%$ decrease in $Na_v1.1$ and $Na_v1.2$ levels in BACE1-null mice but no significant changes in $Na_v1.6$ levels (Fig. 3B). Interestingly, decreases in Na_v1 α -subunit levels were less pronounced at 3 months *versus* 1 month of age, suggesting the possibility that the lack of BACE1 activity affects Na_v1 α -subunit levels more prominently at early ages (Fig. 3, A and B). High expression of BACE1 in early

postnatal stages may also explain the strong effects of BACE1 knock-out on Na_v1 α -subunit levels in young mouse brains (15). $Na_v1.3$ levels were hardly detectable, because this subunit is predominantly expressed in early developmental stages (data not shown). Together, these data indicate that endogenous BACE1 activity regulates Na_v1 α -subunit levels in adult neurons.

$Na_v1.1$ Protein Levels in the Hippocampus of BACE1-null Mice—We then investigated the effect of BACE1 ablation in the hippocampus, which is a highly affected brain region in patients with Alzheimer disease. Hippocampal regions were dissected from the brains of 3-month-old BACE1-null and wild-type control mice, extracted, and analyzed for Na_v1 subunits by Western blot analysis. We observed a robust 57% decrease of $Na_v1.1$ levels in BACE1-null as compared with wild-type control mice (Fig. 4, A and B). Interestingly, we could not detect any significant changes in $Na_v1.2$ or $Na_v1.6$ levels in these samples, suggesting that $Na_v1.1$ is the major Na_v1 α -subunit affected by BACE1 activity in the hippocampal region (Fig. 4, A and B).

We also investigated $Na_v1.1$ distribution in the hippocampal region of 3-month-old BACE1-null mice by immunofluorescence (Fig. 4C). Using a monoclonal $Na_v1.1$ antibody (Neuromab), we mainly detected $Na_v1.1$ in neuronal cell bodies of hippocampal neurons (35, 36). As shown in Fig. 4C, we found that $Na_v1.1$ levels were significantly decreased in the dentate gyrus, hilus, and CA3 regions (Fig. 4C, upper and middle panels). At higher magnification, we confirmed that somatic $Na_v1.1$ staining is specifically decreased in BACE1-null mice in the same regions (Fig. 4C, bottom panels). We also found similar but

Reduced $\text{Na}_v1.1$ Levels in BACE1 -null Mice

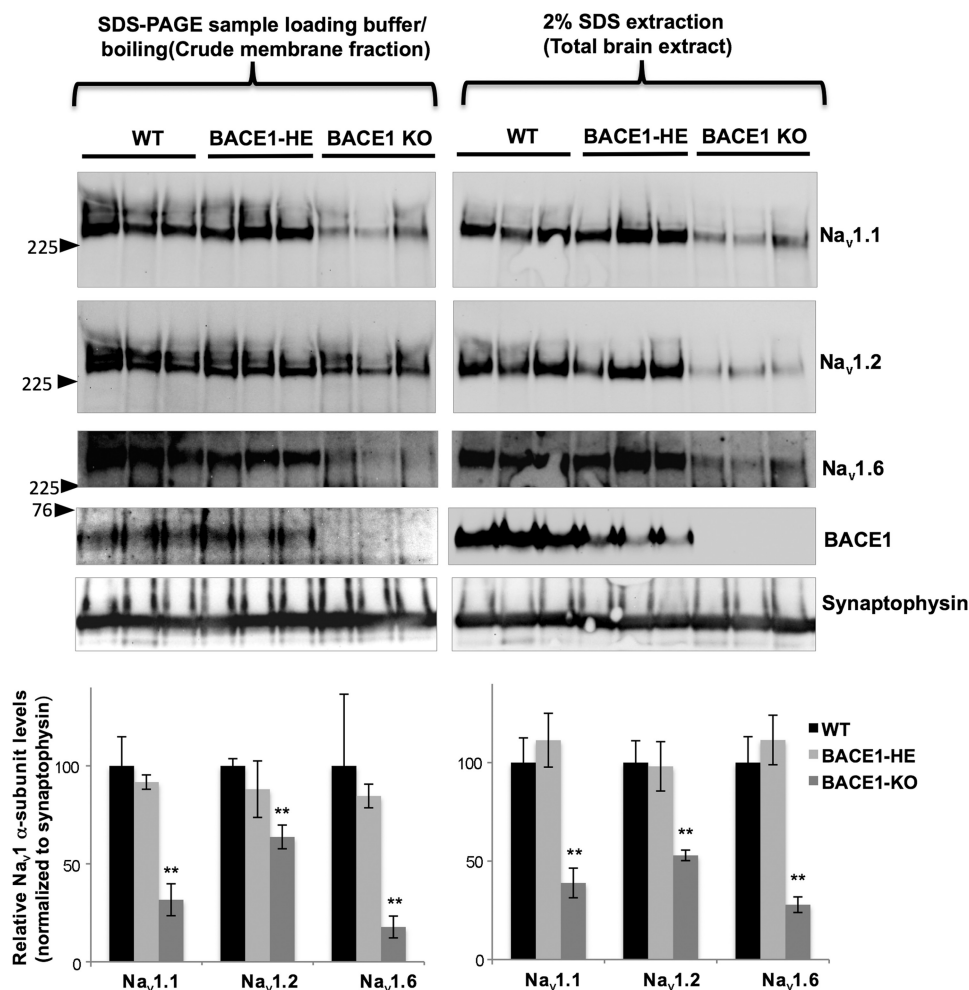


FIGURE 2. $\text{Na}_v1.1$ α -subunit levels are decreased in brains from BACE1 -null mice. Upper left panel, Western blot showing $\text{Na}_v1.1$, $\text{Na}_v1.2$, and $\text{Na}_v1.6$ α -subunit levels in brain membrane fractions prepared from 1-month-old wild-type (WT), BACE1 -heterozygous knock-out (BACE1-HE), and BACE1 -null mice (BACE1-KO). Lower left panel, graph showing normalized $\text{Na}_v1.1$ protein levels in WT, BACE1-HE , and BACE1-KO normalized against synaptophysin (ANOVA followed by a post hoc Tukey's test; *, $p < 0.05$; **, $p < 0.01$; $n = 3$ for WT, BACE1-HE , and BACE1-KO , respectively). Upper right panel, Western blot showing $\text{Na}_v1.1$, $\text{Na}_v1.2$ and $\text{Na}_v1.6$ α -subunit levels in total brain extract prepared from 1-month-old WT, BACE1-HE , and BACE1-KO ; 2% SDS was used to extract total brain homogenates. Lower right panel, a graph showing normalized $\text{Na}_v1.1$ protein levels in WT, BACE1-HE , and BACE1-KO normalized against synaptophysin (ANOVA followed by a post hoc Tukey's test; **, $p < 0.01$; $n = 3$ for WT, BACE1-HE , and BACE1-KO , respectively).

more dramatic decreases of $\text{Na}_v1.1$ in 1-month-old BACE1 -null mice as compared with age-matched controls (supplemental Fig. 3). These data show that ablation of BACE1 significantly decreases $\text{Na}_v1.1$ levels in adult hippocampal neurons.

Altered Surface Na_v1 Levels in BACE1 -null Brain Slices—In neurons, newly synthesized Na_v1 α -subunits accumulate inside the cell body, and only a small fraction of active channels reaches the cell surface (22). To explore whether endogenous BACE1 activity modulates surface expression of Na_v1 α -subunits, we performed surface biotinylation analysis using acute hippocampal slices prepared from BACE1-HE and BACE1 -null mice as described previously (32). The biotinylated surface proteins were captured, and total and surface Na_v1 α -subunit levels were analyzed by Western blot analysis (Fig. 5A). As expected, total levels of $\text{Na}_v1.1$ were largely decreased in BACE1 -null as compared with BACE1-HE (Fig. 5A, left panel). Similar to the total $\text{Na}_v1.1$ levels, surface levels of $\text{Na}_v1.1$ also largely and significantly decreased in BACE1 -null as compared with BACE1-HE (Fig. 5A, right panel). When quantitated, we found 32% decrease in BACE1 -null as compared with BACE1-HE

control mice (Fig. 5B). Surface expression of $\text{Na}_v1.2$ showed a significant increase, in contrast with $\text{Na}_v1.1$ (Fig. 5, A and C). This suggests a compensatory increase in $\text{Na}_v1.2$ sodium channel density in adult mice. We also compared $\text{Na}_v1.1$ surface levels between BACE1 -null with wild-type control mice and found a similar 35% decrease (Fig. 5, D and E). Our data show that endogenous BACE1 activity regulates surface expression of Na_v1 α -subunit levels in adult neurons and suggest a compensatory regulation between $\text{Na}_v1.1$ and $\text{Na}_v1.2$ surface levels.

In addition to Na_v1 α -subunit surface levels, we also investigated surface expression of full-length $\text{Na}_v\beta_2$ in acute brain slices from BACE1-HE and BACE1 -null mice. Interestingly, we found that surface levels of full-length $\text{Na}_v\beta_2$ increased by 1.92-fold in BACE1 -null as compared with BACE1-HE (± 0.06 , Fig. 6A). Similarly, the APP, another well known physiological substrate of BACE1 , also showed increased surface expression of its full-length mature form in BACE1 -null mice (Fig. 6A). These surface level changes suggest that ablation of BACE1 decreased the cleavage of these substrates at the cell surface because

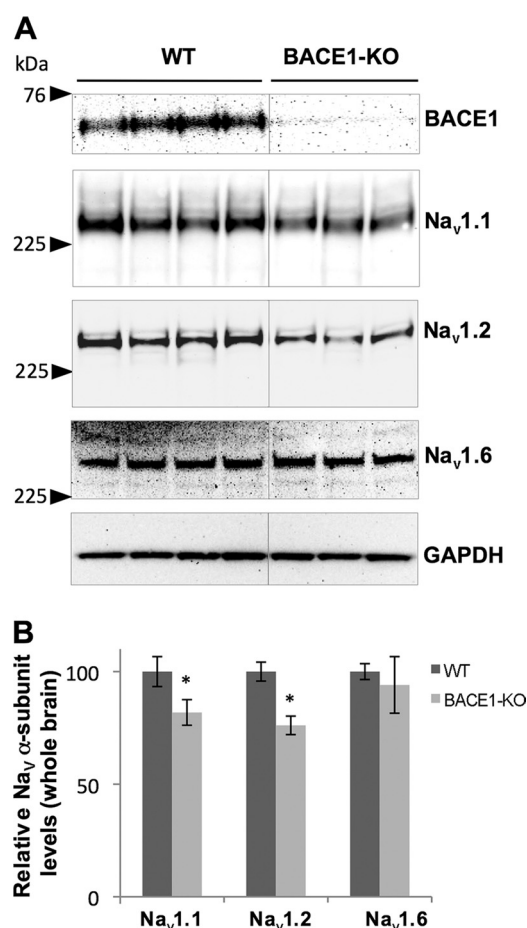


FIGURE 3. $Na_v1.1$ and $Na_v1.2$ are decreased in 3-month-old BACE1-null mice. *A*, Western blot showing $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$ α -subunit levels in total brain extracts from 3-month-old wild-type (WT) and BACE1-null mice (BACE1-KO). *B*, quantitative analysis showing normalized $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$ protein levels in WT and BACE1-KO (Student *t* test; *, $p < 0.05$; $n = 4$ for both WT and BACE1-KO).

BACE1 is known to cleave its substrates at the cell surface or in the endosomal recycling compartment (37).

Previously, we found that BACE1 and γ -secretase sequentially cleave $Na_v\beta_2$ to release β_2 -ICD (32). As shown in our previous study and [supplemental Fig. 1C](#), β_2 -ICD is localized to the nucleus and regulates mRNA and protein levels of $Na_v1.1$ in neuroblastoma cells. To test whether decreased $Na_v1.1$ proteins levels corresponded to reduced $Na_v\beta_2$ processing, we assessed levels of the BACE1 cleavage product $Na_v\beta_2$ C-terminal fragment ($Na_v\beta_2$ -CTF). Consistent with previous reports (18, 19), we found that ablation of BACE1 reduced generation of $Na_v\beta_2$ -CTF by 40% both in total brain extract from 1-month-old BACE1-KO and in hippocampal regions from 3-month-old BACE1-KO mice (Fig. 6B). We were not able to directly detect β_2 -ICD because the $Na_v\beta_2$ antibody was not sensitive enough to detect β_2 -ICD, which was present in very low levels. However, together these data strongly suggest that endogenous BACE1 activity modulates $Na_v\beta_2$ processing, and ablation of BACE1 decreases $Na_v\beta_2$ -CTF and possibly β_2 -ICD.

Next, we investigated whether decreased $Na_v\beta_2$ processing also results in reduced *Scn1a* ($Na_v1.1$) mRNA in brains of BACE1-null mice (32). In brain samples from 1-month-old WT, BACE1-HE, and BACE1-null, we measured *Scn1a*

($Na_v1.1$) mRNA levels by the TaqMan real time RT-PCR method. Similar to its protein levels, we found a significant decrease of *Scn1a* ($Na_v1.1$) mRNA in BACE1-null as compared with wild-type mice (Fig. 6C). Interestingly, we were not able to detect significant changes of *Scn2a* ($Na_v1.2$) or *Scn8a* ($Na_v1.6$) mRNA levels, suggesting that changes at the protein level may be responsible for decreases in $Na_v1.2$ or $Na_v1.6$ in BACE1-null mice ([supplemental Fig. 4](#)). Together, our mechanistic data strongly suggest that decreased $Na_v\beta_2$ processing is responsible for the decrease of $Na_v1.1$ protein levels by decreasing *scn1a* mRNA in BACE1-null mice.

DISCUSSION

Here, we show that expression levels of three major brain Na_v1 channels are strongly affected by BACE1 deletion during early postnatal brain development. Levels of the $Na_v1.1$ α -subunit showed the most dramatic decrease. These changes are predicted to have significant effects on the development of excitability. Ablation of BACE1 results in ~40–50% decrease of $Na_v1.1$ protein and mRNA levels in whole brains of 1-month-old mice and up to 56% in the hippocampus of 3-month-old mice (Figs. 2–4 and [supplemental Figs. 2–4](#)). We also found that the decrease in $Na_v1.1$ in BACE1-null mice was less pronounced at 3 months *versus* 1 month of age, suggesting the possibility that the lack of BACE1 activity affects Na_v1 α -subunit levels more prominently at early ages. $Na_v1.2$ α -subunit levels were also decreased in whole brain samples from 1- and 3-month-old mice but not in the hippocampus. In addition, we found that $Na_v1.6$ α -subunit levels were also decreased in brains of 1-month-old but not in 3-month-old mice. These data demonstrate that BACE1 regulates adult brain Na_v1 α -subunit levels in physiological conditions, possibly in an age-dependent and/or region-specific manner.

Previously, we reported that BACE1/ γ -secretase activities promote the release of the β_2 -ICD, which localizes to the nucleus and increases mRNA and protein levels of the pore-forming $Na_v1.1$ α -subunit in neuroblastoma cells (18, 32). Elevated BACE1 activity also increases $Na_v\beta_2$ cleavages and $Na_v1.1$ levels in brains of BACE1-transgenic mice and AD patients (32). Although BACE1 cleaves all four β -subunits *in vitro*, only $Na_v\beta_2$ and $Na_v\beta_4$ were reported as physiological substrates in mouse brains (19). Consistent with our previous findings, we found a significant decrease of $Na_v\beta_2$ C-terminal fragment levels in the hippocampus of BACE1-null mice (Fig. 6B). Therefore, endogenous BACE1 activity is essential for $Na_v\beta_2$ cleavage in the hippocampus *in vivo*. Fig. 1 also suggests a crucial role of $Na_v\beta_2$ in BACE1-dependent regulation of $Na_v1.1$ levels at least in the embryonic neuronal cells. Although BACE1 inhibitors largely decreased *Scn1a* ($Na_v1.1$) mRNA and protein levels in cultured cortical/hippocampal neurons, the same inhibitors did not change $Na_v1.1$ levels in $Na_v\beta_2$ -null neurons (Fig. 1 and [supplemental Fig. 1, A, B, and D](#)). Together, these data support the notion that $Na_v\beta_2$ and its processing are essential for regulating $Na_v1.1$ levels under physiological conditions. Interestingly, we were not able to detect significant changes of *Scn2a* ($Na_v1.2$) or *Scn8a* ($Na_v1.6$) mRNA levels, although $Na_v1.2$ and $Na_v1.6$ protein levels are significantly decreased in the brains from 1-month-old BACE1-null mice. This suggests an alterna-

Reduced $Na_v1.1$ Levels in $BACE1$ -null Mice

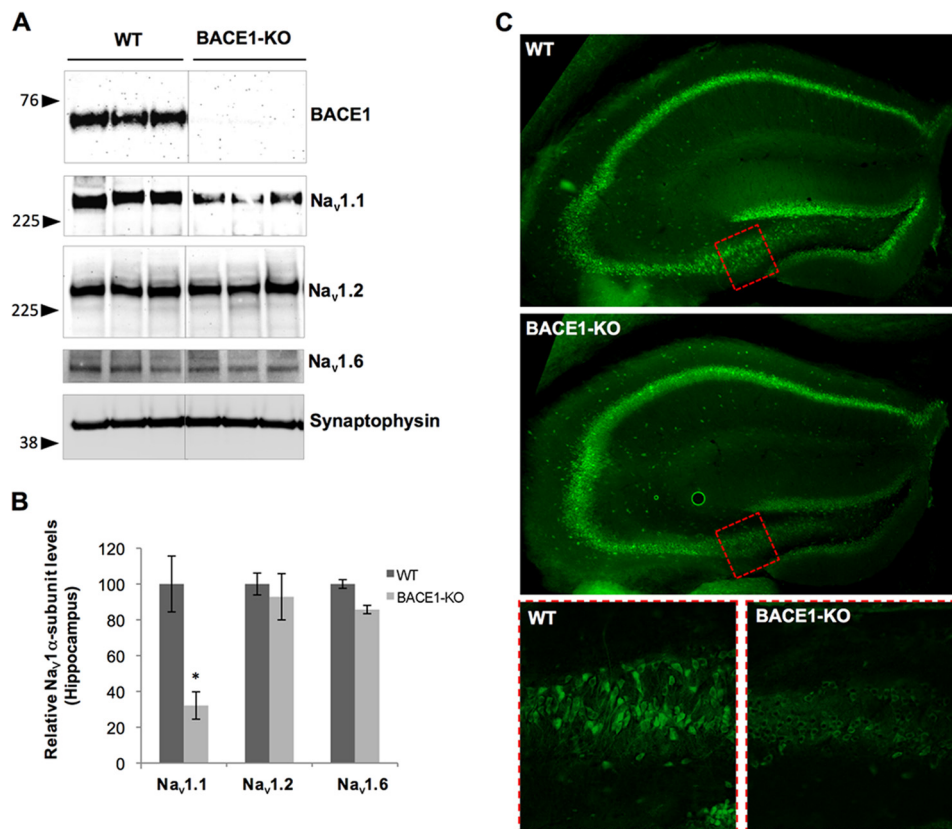


FIGURE 4. Hippocampal $Na_v1.1$ levels are decreased in $BACE1$ -null mice. *A*, Western blot analysis of $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$ from WT and $BACE1$ -KO mice. *B*, quantitative analysis of $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$ levels in hippocampal brain extracts from $BACE1$ -KO and WT mice (Student's *t* test; *, $p < 0.01$; $n = 3$ for WT and $n = 4$ for $BACE1$ -KO). *C*, confocal immunofluorescence analysis of $Na_v1.1$ (green) in hippocampal regions of 3-month-old WT or $BACE1$ -KO mice. *Top* and *middle* panels, low magnification ($\times 50$) images of hippocampal region stained with $Na_v1.1$ staining (green). The red dotted box indicates the area of high magnification shown in the *bottom* panels. *Bottom* panels, high magnification ($\times 200$) images with $Na_v1.1$ staining (*bottom left* panel (WT); *bottom right* panel, $BACE1$ -KO).

tive molecular pathway mediating the $Na_v1.2$ or $Na_v1.6$ decrease in $BACE1$ -null mice (Fig. 6C and supplemental Fig. 4). Altered processing of other neuronal $BACE1$ substrates may contribute to these Na_v1 α -subunit changes. Further studies will be required to fully characterize the underlying molecular mechanism of Na_v1 α -subunit changes.

As shown in Fig. 5, ablation of $BACE1$ results in $\sim 40\%$ decrease of surface $Na_v1.1$ levels in hippocampal neurons, which is comparable with the decreased levels of total $Na_v1.1$ in the same region. Therefore, surface expression of $Na_v1.1$ is regulated by the decrease of total $Na_v1.1$ levels in $BACE1$ -null mice in physiological conditions. On the contrary, we found that $Na_v1.2$ surface levels are significantly increased (Fig. 5, *A* and *D*). This suggests a compensatory mechanism between $Na_v1.1$ and $Na_v1.2$ surface levels. However, disconnected total *versus* cell surface distributions of $Na_v1.2$ in $BACE1$ -null mice are reminiscent of $Na_v1.1$ distribution in $BACE1$ -overexpressing systems. In neuroblastoma cells and adult hippocampal neurons from $BACE1$ -transgenic mice, overexpressed $BACE1$ paradoxically reduced cell surface expression of Na_v1 α -subunits, although total $Na_v1.1$ α -subunits levels increased (32). The increased $Na_v1.1$ was retained inside the cells and accumulated in HSP70-positive punctate structures, suggesting that $Na_v1.1$ trafficking to cell surface was inhibited by $BACE1$ overexpression (32). Together, these data indicate that expression of Na_v1 α -subunits on the cell surface is under tight regulation, and

changes in intracellular α -subunit levels may not always reflect surface expression of each channel. Decreased $BACE1$ -mediated cleavage of surface $Na_v\beta_2$ may differentially affect $Na_v1.1$ and $Na_v1.2$ surface trafficking. Further studies will be required to clarify how $BACE1$ -mediated cleavage of $Na_v\beta_2$ can differentially regulate the trafficking of each α -subunit.

Neuronal Na_v1 channels are known to be up-regulated or redistributed upon nerve injury or demyelination (38–41). In experimental allergic encephalomyelitis, an animal model of multiple sclerosis, levels and distribution of $Na_v1.2$ and $Na_v1.6$ are altered in demyelinated axons within acute multiple sclerosis plaques (39–41). $BACE1$ -null mice also show mild but significant hypomyelination in the CNS and peripheral nervous system, and these might contribute to the elevated $Na_v1.2$ surface levels shown in $BACE1$ -null mice (15, 31, 42). Interestingly, a recent study showed that $Na_v\beta_2$ also mediates demyelination-induced Na_v1 α -subunit regulation in the same multiple sclerosis model (43). Together with our data, this study suggests the interesting possibility that in $BACE1$ -null mice $Na_v\beta_2$ not only is an essential Na_v1 β -subunit in regulating changes in Na_v1 α -subunit levels via generation of its ICD but also indirectly via reduced myelination, which is a well characterized phenotype of $BACE1$ -null mice. Further studies are required to clarify whether $Na_v\beta_2$ also plays a role in regulating Na_v1 α -subunit levels under mild hypomyelination in addition to demyelinating conditions.

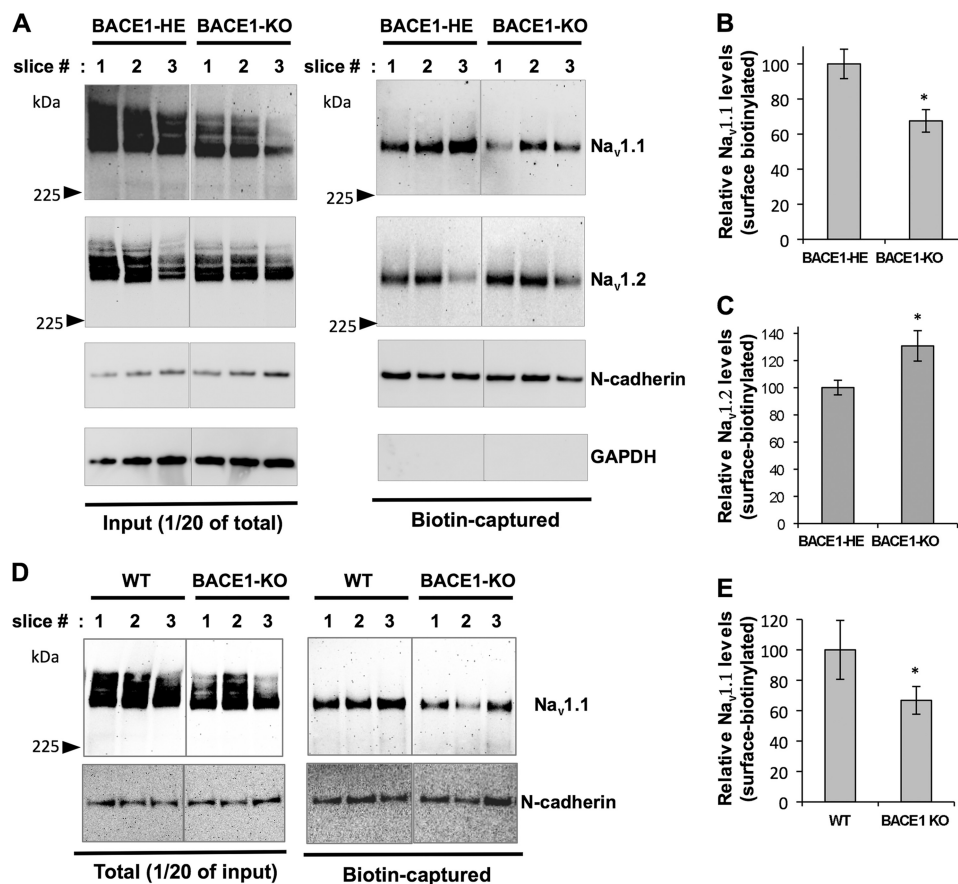


FIGURE 5. Surface Na_v1 α -subunit levels are altered in BACE1 -null hippocampal slices. Surface biotinylation experiments were performed on acutely prepared hippocampal slices from WT, BACE1-HE , and BACE1-KO mice. 2–3 matching slice pairs from similar locations of the brains were selected for the experiments. The slices with the same numbers are matching pairs. **A**, representative Western blot showing total and biotin-captured surface $\text{Na}_v1.1$ and $\text{Na}_v1.2$ levels in hippocampal slices (Slice #1–3) from BACE1-HE and BACE1-KO . **B**, representative Western blot showing total and biotin-captured surface $\text{Na}_v1.1$ levels in hippocampal slices (Slice #1–3) from wild-type control (WT) and BACE1-KO . **C**, graph showing quantitated surface $\text{Na}_v1.1$ levels in BACE1-HE and BACE1-KO (Student's *t* test; *, $p < 0.01$; $n = 7$ slices from three BACE1-HE and $n = 8$ from three BACE1-KO mice). Surface N-cadherin levels were used to normalize the surface $\text{Na}_v1.1$ and $\text{Na}_v1.2$ levels. **D**, graph showing quantitated surface $\text{Na}_v1.2$ levels in BACE1-HE and BACE1-KO (Student's *t* test; *, $p < 0.01$; $n = 7$ slices from three BACE1-HE and $n = 8$ from three BACE1-KO mice). **E**, graph showing quantitated surface $\text{Na}_v1.1$ levels in WT and BACE1-KO (Student's *t* test; *, $p < 0.01$; $n = 8$ slices from three WT and BACE1-KO mice, respectively).

Deletion of the $\text{Na}_v1.1$ gene does not significantly affect sodium current density in hippocampal pyramidal neurons because of high expression of $\text{Na}_v1.2$ and other α -subunits (35, 36, 44). However, $\text{Na}_v1.1$ plays a major role in regulating sodium currents in hippocampal GABAergic interneurons. Indeed, 50% decrease of $\text{Na}_v1.1$ dramatically reduced sodium current in GABAergic interneurons (36). Similarly, 40–50% decrease of total and surface $\text{Na}_v1.1$ levels in BACE1 -null mice may decrease sodium current in hippocampal GABAergic interneurons. This would result in electrical imbalances and the vulnerability to epileptic seizures as previously shown in $\text{Na}_v1.1$ -null mice (36). Increase of $\text{Na}_v1.2$ surface expression may also contribute to the electrical imbalances by selectively increasing sodium currents in hippocampal pyramidal neurons.

While we were revising this manuscript, two independent groups reported that BACE1 -null mice showed increased seizure susceptibility and spontaneous behavioral seizure phenotypes (45, 46). These results clearly support our prediction, based on altered $\text{Na}_v1.1$ and $\text{Na}_v1.2$ surface expression in BACE1 -null mice (Fig. 5). Hu *et al.* (45) showed that surface levels of $\text{Na}_v1.2$ are increased in cultured hippocampal neu-

rons from BACE1 -null mice, which is also consistent with our results on increased $\text{Na}_v1.2$ surface levels in adult hippocampus from BACE1 -null mice (Fig. 5). In addition to $\text{Na}_v1.2$ changes, we also found reduced surface levels of $\text{Na}_v1.1$, which allow us to propose a novel $\text{Na}_v1.1$ -based mechanism for the increased seizure susceptibility of BACE1 -null mice (Fig. 6D). Indeed, mutations of the $\text{Na}_v1.1$ α -subunit are frequently associated with inherited familial epileptic seizures in humans (44).

Early electrical deficits may also explain the enhanced lethality of BACE1/2 -null mice at very early ages and their body weight loss (28, 46). $\text{Na}_v1.1$ -null and -heterozygous mice show severe and fatal seizure phenotypes (36, 44). A selective loss of $\text{Na}_v1.1$ -mediated sodium currents in inhibitory neurons is proposed as a molecular mechanism underlying these severe phenotypes. Interestingly, all the major Na_v1 α -subunit levels are decreased in BACE1 -null mice (Fig. 2) and may not be selective to one neuronal population. Therefore, neuronal excitability in BACE1 -null mice may be generally reduced all over the brain rather than in specific circuits, which may not lead to a severe and fatal electrical imbalance in neural circuits. This may explain why BACE1 -

Reduced $Na_v1.1$ Levels in $BACE1$ -null Mice

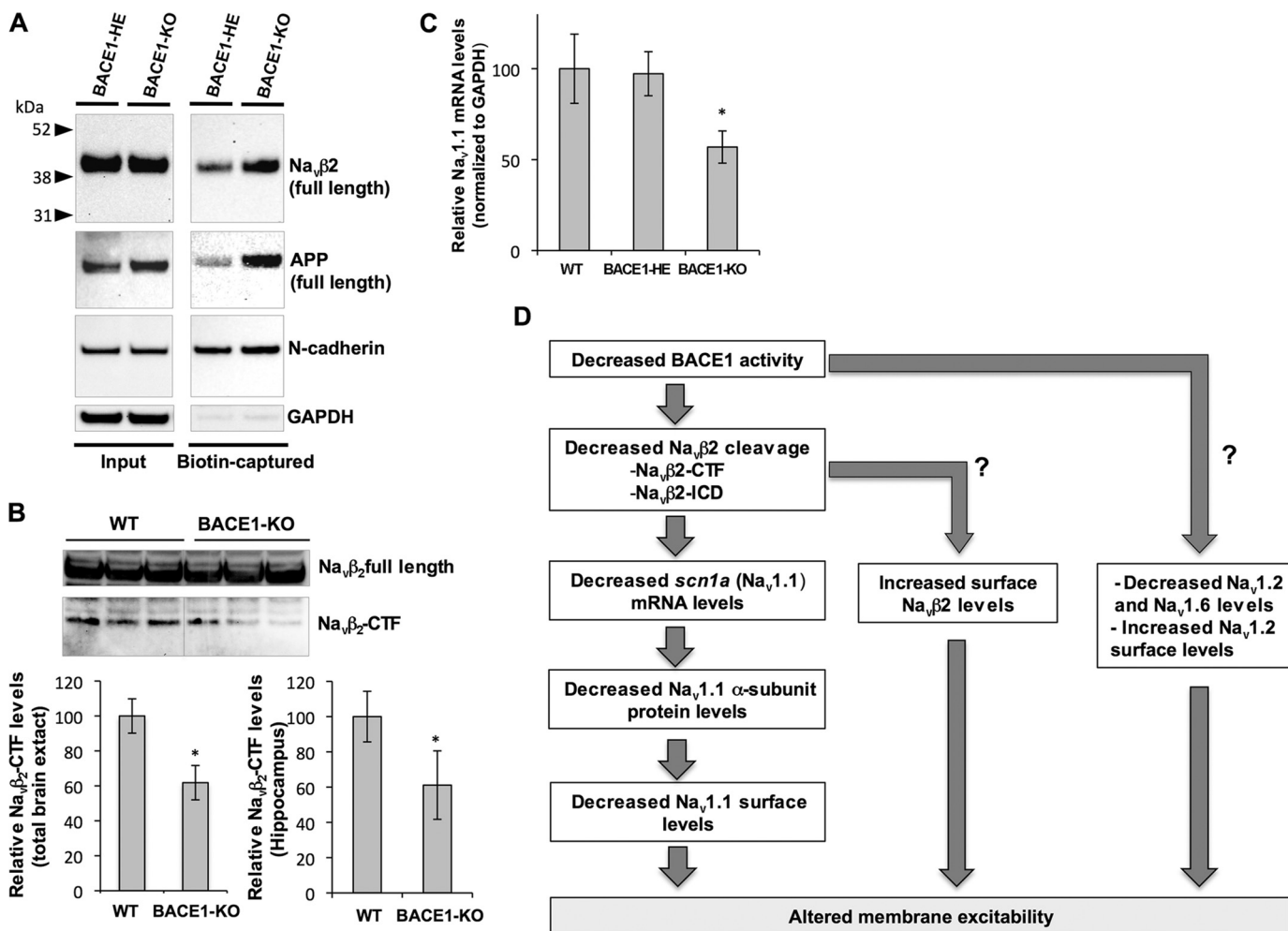


FIGURE 6. $Na_v\beta_2$ processing and $scn1a$ ($Na_v1.1$) mRNA levels are decreased in brains of $BACE1$ -null mice. **A**, surface $Na_v\beta_2$ and APP levels are largely decreased in brain slices from $BACE1$ -null ($BACE1-KO$) as compared with $BACE1$ -heterozygous knock-out ($BACE1-HE$). Slice biotinylation experiments were performed as described in Fig. 5. **B**, upper panel, representative Western blot showing full-length $Na_v\beta_2$ and C-terminal fragment ($Na_v\beta_2$ -CTF) levels in hippocampal brain extracts prepared from WT and $BACE1-KO$ mice. Lower panel, quantitative analysis of $Na_v\beta_2$ -CTF levels in total brain extract from 1-month-old WT and $BACE1-KO$ (left) and hippocampal extracts from 3-month-old WT and $BACE1-KO$ WT and $BACE1-KO$ mice (right, Student's *t* test; *, $p < 0.01$; $n = 3$ per each condition). **C**, TaqMan real time RT-PCR analysis of $scn1a$ ($Na_v1.1$) mRNA levels in 1-month-old WT, $BACE1-HE$, and $BACE1-KO$ (ANOVA followed by a post hoc Tukey's test; *, $p < 0.05$; $n = 5$ for WT, $n = 8$ for $BACE1-HE$, and $n = 6$ for $BACE1-KO$). GAPDH levels were used to normalize $Scn1a$ ($Na_v1.1$) mRNA levels. **D**, diagram summarizing altered sodium channel metabolism in $BACE1$ -null mice.

null mice do not show a fatal seizure phenotype as predicted by the drastic Na_v1 α -subunit decreases at the early age (Fig. 2).

Na_v1 α -subunits are large hydrophobic membrane proteins with multiple transmembrane domains (24). It is technically challenging to analyze total Na_v1 α -subunits because they are highly insoluble in mild detergent extraction conditions. To overcome this technical problem, we used several extraction conditions and compared the results (Fig. 2). We found that 1.5–2% of SDS extraction conditions showed the most consistent results, tightly comparable with a method based on direct boiling of membrane fractions in the presence of SDS-PAGE sample loading buffer. The direct boiling method has been commonly used for sodium channel analysis by other groups (27, 36). In these conditions, we found that $Na_v1.1$ and $Na_v1.2$ levels are consistently decreased especially in young adult $BACE1$ -null mice as compared with $BACE1-HE$ and wild-type controls (Fig. 2). These data are consistent with the results from Hu *et al.* and Hitt *et al.* (45, 46). However, Hitt *et al.* (46)

reported that $Na_v1.2$ and $Na_v1.6$ levels were not significantly changed in $BACE1$ -null mice (45). The discrepancy regarding $Na_v1.2$ may derive from differential extraction conditions (2% SDS versus 1% Triton X-100) or different ages of the mice. Indeed, we found that the decrease in $Na_v1.2$ is much less pronounced (~20%) in 3-month-old mice as compared with 1-month-old mice (~40–50%) (Figs. 2 and 3). Consistent with the data from Hitt *et al.* (46), we found that $Na_v1.2$ levels were not significantly changed in the hippocampal region of 3-month-old mice, although $Na_v1.1$ shows a dramatic decrease in the same samples (Fig. 4). However, Hitt *et al.* (46) did not analyze levels of $Na_v1.2$ at the cell surface. Because the vast majority of sodium channels is retained inside the cells, total $Na_v1.2$ levels may not reflect changes at the cell surface. We found that $Na_v1.1$ levels decrease at the cell surface, although $Na_v1.2$ levels increase in agreement with Hu *et al.* (45). Taken together, these data argue that the lack of $BACE1$ activity dynamically modulates changes in Na_v1 α -subunit levels depending on

age/brain region and that changes in cell surface Na_v1 α -subunit levels are likely to cause epileptic seizures in BACE1-null mice. Further studies will be required to fully address BACE1 effects on Na_v1 α -subunit levels *in vivo*.

BACE1 is one of the prime drug targets for therapeutic treatment of AD patents. Genetic ablation of BACE1 showed decreased A β levels and ameliorated cognitive function in animal models (47). Our findings that the ablation of BACE1 leads to decreased sodium channel levels suggest that complete block of BACE1 is likely to cause side effects through altered sodium current densities. However, our studies on Na_v1 levels in BACE1-heterozygous mice show that 50% decrease of BACE1 activity does not significantly decrease Na_v1.1 levels (Fig. 1), although partial reduction of BACE1 results in dramatic reduction in A β plaques, neuritic burden, and synaptic deficits in older mice (48). Therefore, it will be important to find a therapeutic window for inhibiting BACE1 activity to block A β burden, without significantly affecting Na_v1 metabolism.

Acknowledgments—We thank Dr. Lori L. Isom (University of Michigan) for providing us Na_v β ₂-null and control mice, Dr. Nobuyuki Nukina (RIKEN Brain Science Institute) for the gift of the C-terminal Na_v β ₂ antibody, Dr. Robert Vassar (Northwestern University) for the BACE1 monoclonal antibody 3D5, and Dr. Jordan Tang (Oklahoma Medical Research Foundation) for β DR9.

REFERENCES

- Zayas, E., and Grossberg, G. (1996) *J. Clin. Psychiatry* **57**, Suppl. 7, 46–54
- de Leon, M. J., Convit, A., DeSanti, S., Golomb, J., Tarshish, C., Rusinek, H., Bobinski, M., Ince, C., Miller, D. C., and Wisniewski, H. M. (1995) *Neuroimaging Clin. N. Am.* **5**, 1–17
- Hardy, J., and Selkoe, D. J. (2002) *Science* **297**, 353–356
- Vassar, R. (2001) *J. Mol. Neurosci.* **17**, 157–170
- Wolfe, M. S. (2008) *Curr. Alzheimer Res.* **5**, 158–164
- Sinha, S., Anderson, J. P., Barbour, R., Basji, G. S., Caccavello, R., Davis, D., Doan, M., Dovey, H. F., Frigon, N., Hong, J., Jacobson-Croak, K., Jewett, N., Keim, P., Knops, J., Lieberburg, I., Power, M., Tan, H., Tatsuno, G., Tung, J., Schenk, D., Seubert, P., Suomensari, S. M., Wang, S., Walker, D., Zhao, J., McConlogue, L., and John, V. (1999) *Nature* **402**, 537–540
- Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., Teplow, D. B., Ross, S., Amarante, P., Loeloff, R., Luo, Y., Fisher, S., Fuller, J., Edenson, S., Lile, J., Jarosinski, M. A., Biere, A. L., Curran, E., Burgess, T., Louis, J. C., Collins, F., Treanor, J., Rogers, G., and Citron, M. (1999) *Science* **286**, 735–741
- Fukumoto, H., Cheung, B. S., Hyman, B. T., and Irizarry, M. C. (2002) *Arch. Neurol.* **59**, 1381–1389
- Tyler, S. J., Dawbarn, D., Wilcock, G. K., and Allen, S. J. (2002) *Biochem. Biophys. Res. Commun.* **299**, 373–376
- Yang, L. B., Lindholm, K., Yan, R., Citron, M., Xia, W., Yang, X. L., Beach, T., Sue, L., Wong, P., Price, D., Li, R., and Shen, Y. (2003) *Nat. Med.* **9**, 3–4
- Tesco, G., Koh, Y. H., Kang, E. L., Cameron, A. N., Das, S., Sena-Esteves, M., Hiltunen, M., Yang, S. H., Zhong, Z., Shen, Y., Simpkins, J. W., and Tanzi, R. E. (2007) *Neuron* **54**, 721–737
- O'Connor, T., Sadleir, K. R., Maus, E., Velliquette, R. A., Zhao, J., Cole, S. L., Eimer, W. A., Hitt, B., Bembinster, L. A., Lammich, S., Lichtenthaler, S. F., Hébert, S. S., De Strooper, B., Haass, C., Bennett, D. A., and Vassar, R. (2008) *Neuron* **60**, 988–1009
- Zacchetti, D., Chieragatti, E., Bettgazzi, B., Mihailovich, M., Sousa, V. L., Grohovaz, F., and Meldolesi, J. (2007) *Neurodegener. Dis.* **4**, 117–126
- Velliquette, R. A., O'Connor, T., and Vassar, R. (2005) *J. Neurosci.* **25**, 10874–10883
- Willem, M., Garratt, A., Novak, B., Citron, M., Kaufmann, S., Rittger, A., Destrooper, B., Saftig, P., Birchmeier, C., and Haass, C. (2006) *Science* **314**, 664–666
- Kitazume, S., Tachida, Y., Oka, R., Kotani, N., Ogawa, K., Suzuki, M., Dohmae, N., Takio, K., Saido, T. C., and Hashimoto, Y. (2003) *J. Biol. Chem.* **278**, 14865–14871
- Lichtenthaler, S. F., Dominguez, D. I., Westmeyer, G. G., Reiss, K., Haass, C., Saftig, P., De Strooper, B., and Seed, B. (2003) *J. Biol. Chem.* **278**, 48713–48719
- Kim, D. Y., Ingano, L. A., Carey, B. W., Pettingell, W. H., and Kovacs, D. M. (2005) *J. Biol. Chem.* **280**, 23251–23261
- Wong, H. K., Sakurai, T., Oyama, F., Kaneko, K., Wada, K., Miyazaki, H., Kurosawa, M., De Strooper, B., Saftig, P., and Nukina, N. (2005) *J. Biol. Chem.* **280**, 23009–23017
- Isom, L. L. (2001) *Neuroscientist* **7**, 42–54
- Yu, F. H., and Catterall, W. A. (2003) *Genome Biol.* **4**, 207
- Schmidt, J. W., and Catterall, W. A. (1986) *Cell* **46**, 437–444
- Catterall, W. (2002) *Novartis Found. Symp.* **241**, 206–218; discussion 218–232
- Catterall, W. A. (2000) *Neuron* **26**, 13–25
- Isom, L. L., Ragsdale, D. S., De Jongh, K. S., Westenbroek, R. E., Reber, B. F., Scheuer, T., and Catterall, W. A. (1995) *Cell* **83**, 433–442
- Chen, C., Bharucha, V., Chen, Y., Westenbroek, R. E., Brown, A., Malhotra, J. D., Jones, D., Avery, C., Gillespie, P. J., 3rd., Kazen-Gillespie, K. A., Kazarinova-Noyes, K., Shrager, P., Saunders, T. L., Macdonald, R. L., Ransom, B. R., Scheuer, T., Catterall, W. A., and Isom, L. L. (2002) *Proc. Natl. Acad. Sci. U.S.A.* **99**, 17072–17077
- Lopez-Santiago, L. F., Pertin, M., Morisod, X., Chen, C., Hong, S., Wiley, J., Decesterd, L., and Isom, L. L. (2006) *J. Neurosci.* **26**, 7984–7994
- Dominguez, D., Tournoy, J., Hartmann, D., Huth, T., Cryns, K., Deforce, S., Serneels, L., Camacho, I. E., Marjaux, E., Craessaerts, K., Roebroek, A. J., Schwake, M., D'Hooge, R., Bach, P., Kalinke, U., Moechars, D., Alzheimer, C., Reiss, K., Saftig, P., and De Strooper, B. (2005) *J. Biol. Chem.* **280**, 30797–30806
- Laird, F. M., Cai, H., Savonenko, A. V., Farah, M. H., He, K., Melnikova, T., Wen, H., Chiang, H. C., Xu, G., Koliatsos, V. E., Borchelt, D. R., Price, D. L., Lee, H. K., and Wong, P. C. (2005) *J. Neurosci.* **25**, 11693–11709
- Savonenko, A. V., Melnikova, T., Laird, F. M., Stewart, K. A., Price, D. L., and Wong, P. C. (2008) *Proc. Natl. Acad. Sci. U.S.A.* **105**, 5585–5590
- Hu, X., Hicks, C. W., He, W., Wong, P., Macklin, W. B., Trapp, B. D., and Yan, R. (2006) *Nat. Neurosci.* **9**, 1520–1525
- Kim, D. Y., Carey, B. W., Wang, H., Ingano, L. A., Binshtok, A. M., Wertz, M. H., Pettingell, W. H., He, P., Lee, V. M., Woolf, C. J., and Kovacs, D. M. (2007) *Nat. Cell Biol.* **9**, 755–764
- Davis, T. H., Chen, C., and Isom, L. L. (2004) *J. Biol. Chem.* **279**, 51424–51432
- Thomas-Crusells, J., Vieira, A., Saarma, M., and Rivera, C. (2003) *J. Neurosci. Methods* **125**, 159–166
- Westenbroek, R. E., Merrick, D. K., and Catterall, W. A. (1989) *Neuron* **3**, 695–704
- Yu, F. H., Mantegazza, M., Westenbroek, R. E., Robbins, C. A., Kalume, F., Burton, K. A., Spain, W. J., McKnight, G. S., Scheuer, T., and Catterall, W. A. (2006) *Nat. Neurosci.* **9**, 1142–1149
- Vassar, R., Kovacs, D. M., Yan, R., and Wong, P. C. (2009) *J. Neurosci.* **29**, 12787–12794
- Westenbroek, R. E., Noebels, J. L., and Catterall, W. A. (1992) *J. Neurosci.* **12**, 2259–2267
- Craner, M. J., Newcombe, J., Black, J. A., Hartle, C., Cuzner, M. L., and Waxman, S. G. (2004) *Proc. Natl. Acad. Sci. U.S.A.* **101**, 8168–8173
- Waxman, S. G., Craner, M. J., and Black, J. A. (2004) *Trends Pharmacol. Sci.* **25**, 584–591
- Craner, M. J., Damarjian, T. G., Liu, S., Hains, B. C., Lo, A. C., Black, J. A., Newcombe, J., Cuzner, M. L., and Waxman, S. G. (2005) *Glia* **49**, 220–229
- Hu, X., He, W., Diaconu, C., Tang, X., Kidd, G. J., Macklin, W. B., Trapp, B. D., and Yan, R. (2008) *FASEB J.* **22**, 2970–2980
- O'Malley, H. A., Shreiner, A. B., Chen, G. H., Huffnagle, G. B., and Isom,

Reduced Na_v1.1 Levels in BACE1-null Mice

- L. L. (2009) *Mol. Cell. Neurosci.* **40**, 143–155
44. Catterall, W. A., Kalume, F., and Oakley, J. C. (2010) *J. Physiol.* **588**, 1849–1859
45. Hu, X., Zhou, X., He, W., Yang, J., Xiong, W., Wong, P., Wilson, C. G., and Yan, R. (2010) *J. Neurosci.* **30**, 8819–8829
46. Hitt, B. D., Jaramillo, T. C., Chetkovich, D. M., and Vassar, R. (2010) *Mol. Neurodegener.* **5**, 31
47. Ohno, M., Sametsky, E. A., Younkin, L. H., Oakley, H., Younkin, S. G., Citron, M., Vassar, R., and Disterhoft, J. F. (2004) *Neuron* **41**, 27–33
48. McConlogue, L., Buttini, M., Anderson, J. P., Brigham, E. F., Chen, K. S., Freedman, S. B., Games, D., Johnson-Wood, K., Lee, M., Zeller, M., Liu, W., Motter, R., and Sinha, S. (2007) *J. Biol. Chem.* **282**, 26326–26334