Na\(^+\) Channel Expression and Neuronal Function in the Na\(^+\)/H\(^+\) Exchanger 1 Null Mutant Mouse

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Xia, Ying, Peng Zhao, Jin Xue, Xiang Q. Gu, Xiaolu Sun, Hang Yao, and Gabriel G. Haddad. Na\(^+\) Channel expression and neuronal function in the Na\(^+\)/H\(^+\) exchanger 1 null mutant mouse. J Neurophysiol 89: 229–236, 2003. First published October 10, 2002; 10.1152/jn.00488.2002. Mice lacking Na\(^+\)/H\(^+\) exchanger 1 (NHE1) suffer from recurrent seizures and die early postnatally. Although the mechanisms for seizures are not well established, our previous electrophysiological work has shown that neuronal excitability and Na\(^+\) current density are increased in hippocampal CA1 neurons of these mutant mice. However, it is unknown whether this increased density is related to altered expression or functional regulation of Na\(^+\) channels. In this work, we asked three questions: is the increased excitability limited to CA1 neurons, is the increased Na\(^+\) current density related to an increased Na\(^+\) channel expression, and, if so, which Na\(^+\) channel subtype(s) is upregulated? Using neurophysiological, autoradiographic, and immunoblotting techniques, we showed that both CA1 and cortical neurons have an increase in membrane excitability and Na\(^+\) current density; Na\(^+\) channel subtype I is significantly increased in the hippocampus and Na\(^+\) channel subtype II is increased in the cortex. Our results demonstrate that mice lacking NHE1 upregulate their Na\(^+\) channel expression in the hippocampal and cortical regions selectively; this leads to an increase in Na\(^+\) current density and membrane excitability. We speculate that neuronal overexcitability due to Na\(^+\) channel upregulation in the hippocampus and cortex forms the basis of epileptic seizures in NHE1 mutant mice.

INTRODUCTION

Neuronal function depends in a major way on plasma membrane protein expression (Banasik et al. 2000; Cummins et al. 1994; Fanning and Anderson 1999; Haddad and Jiang 1993). An abnormal expression of membrane proteins may lead to disturbed neuronal function and neurological disorders (Banasik et al. 2000; Haddad and Jiang 1993; Steinlein et al. 2000; Xia et al. 2000). For example, mice lacking Na\(^+\)/H\(^+\) exchanger 1 (NHE1) show epileptic seizures, which appear by age 2–3 wk postnatally, leading to an early death in most cases (Bell et al. 1999; Cox et al. 1997). The basis for the seizures in the mice is not well understood.

Using an electrophysiological approach, we have recently observed that neuronal excitability and Na\(^+\) current density are increased in freshly dissociated hippocampal neurons from these mice (Gu et al. 2001). Because Na\(^+\) channels are critical for setting the firing threshold and neuronal excitability (Cummins et al. 1993; Catterall 1992; Urenjak and Obrenovitch 1996; Xia et al. 2000), it is highly likely that an increase in the macroscopic Na\(^+\) currents could play a central role in facilitating the induction of seizures in these mutant mice. It is not known, however, whether this Na\(^+\) current upregulation is related to an increase in Na\(^+\) channel density or to an alteration in Na\(^+\) channel conductance or open probability. Furthermore, it is not clear as to whether these changes in the Na\(^+\) current are limited to one region or more generalized in the CNS. We have therefore performed this work to determine whether Na\(^+\) channel expression is altered in multiple regions of the NHE1 null mutant brain. To provide correlative functional analysis, we also used electrophysiological techniques to study the properties of neurons in two different regions, i.e., the CA1 area and the neocortex.

METHODS

Animals

B6SJL, +/swe (slow-wave epilepsy) mice were obtained from Jackson Laboratories (Bar Harbor, ME) (Cox et al. 1997). These heterozygous mice were mated in our institution and the resulting homozygous NHE1 mutant (25%) F1 mice progeny were used between postnatal days 20 and 30. All animal procedures were performed in accordance with the guidelines of the Animal Care Committee of Yale University School of Medicine, which is accredited by the American Association of Laboratory Animal Care.

NHE1 mutant mice genotyping

Although homozygous mutant mice had a clear phenotype consisting of locomotor ataxia in the hindlimbs and slow, wide-based gait and coarse truncal instability starting at ~2–3 wk of age, we performed genotyping on all presumed mutant mice to confirm the phenotype with a PCR-based test. This has been described in previous publications (Yao et al. 1999).

Chemicals and reagents

\(^3\)H-saxitoxin (21 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). Unlabeled saxitoxin (STX) was purchased from

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Calbiochem (La Jolla, CA) and tetrodotoxin (TTX) was from Sigma Chemical (St. Louis, MO). All other chemicals and reagents were purchased from Sigma Chemicals and Aldrich (Milwaukee, WI).

**Tissue preparation**

The animals were decapitated after halothane inhalational anesthesia and their brains were rapidly removed. For patch-clamp recording, the CA1 and neocortex were dissected out for neuronal dissociation. For autoradiographic studies, brains from age-matched control mice were simultaneously removed and frozen in dry ice-cold 2-methylbutane and then stored at −80°C. Ten-micrometer coronal sections were cut in a Hacker-Bright cryostat at −20°C and mounted onto gelatin-coated microscope slides. For Western blots, the hippocampal and cortical tissues were dissected out and pooled for microsomal preparations.

**Cell preparation for patch recording**

The hippocampus and cortex were removed from the animals and sliced into 7–10 transverse sections of 400 μm thick. The slices were immediately transferred to a container with 10 ml of fresh, oxygenated, and slightly stirred HEPES buffer at room temperature. After 30 min of exposure to trypsin (0.08%) and 20 min of protease (0.05%) digestion, the slices were washed several times with HEPES buffer and left in oxygenated solution. The CA1 or cortical tissues were then dissected out and triturated in a small volume (0.25 ml) of HEPES buffer.

**Electrophysiological solutions**

The external HEPES solution bathing neurons for enzymatic treatment and electrophysiological recording contained (in mM) 130 NaCl, 3 KCl, 1 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 glucose (pH 7.4). The pipette solution for whole cell patch electrodes contained (in mM) 138 KCl, 0.2 CaCl₂, 1 MgCl₂, 10 HEPES (Na⁺ salt), and 10 EGTA, and an adjusted pH of 7.4 with Tris. Osmolarity of all solutions was adjusted to 290 mOsm.

**Recording criteria**

**MORPHOLOGICAL CRITERIA.** Cells were used if they had a smooth surface, had a three-dimensional contour, and were pyramidal in shape. Similar criteria have been used by us (Cummins et al. 1993; Gu et al. 2001) and others (Hamill et al. 1981) on freshly triturated neurons.

**ELECTROPHYSIOLOGICAL CRITERIA.** Neurons were considered for recording if the seal resistance was >5 GΩ, a holding current was <0.1 nA (command potential −100 mV), and series resistance was <10 MΩ. The series resistances were compensated at 90% level with the Axopatch 1C amplifier (Axon Instruments). Under these conditions, the error caused by uncompensated series resistances was <5 mV. To obtain adequate voltage clamp and minimize the space-clamp problem, only small neurons with short processes were used in these experiments. Junction potentials were nulled for each individual cell with the Axopatch 1C amplifier. All recordings were performed at room temperature (22–24°C).

**3H-STX binding and autoradiography**

The methods used are the same as in our previous work (Cummins et al. 1993; Xia and Haddad 1993; Xia et al. 2000). In brief, mouse brains were cut at three major levels, i.e., rostral, midbrain and brain stem levels. At each level, two to four consecutive sections were used for autoradiography. All slice-mounted slides were preincubated for 15 min in the binding buffer (in mM) 20 Tris-HCl (pH 7.4), 140 NaCl, 5.4 KCl, 2.8 CaCl₂, and 1.3 MgSO₄ to remove putative endogenous ligand. Brain tissues were then labeled at room temperature for 60 min in the same buffer with 4 nM [³H]-STX. Nonspecific binding was measured in separate slices from the same brain levels of the same mice by adding 5 μM of unlabeled TTX in the binding buffer. The incubated slides were rinsed in seven different jars containing the same buffer without any labeled or unlabelled STX at 4°C and finally in a jar containing cold distilled water. The total rinsing time was ~30 s. Well-dried slides were organized in cassettes and exposed to ³H-Ulfronfilm (Fluca) with tritium standards for 3 wk. All age-matched pairs of mutant and control mice were performed simultaneously from tissue preparation to film exposure. The films were then developed in Kodak D-19 developer. The resulting autoradiograms were processed by means of a computerized image system. After the film background was corrected, STX binding density was quantified by Scion Image analysis software.

**Immunoblotting**

The tissues of cortex and hippocampus from four animals were separately dissected out, weighed, pooled, and transferred to lysis buffer as one sample (4 × vol/wt; 200 mM mannitol, 80 mM HEPES, 41 mM KOH, 1 μM pepstatin A, 1 μM leupeptin, 230 mM phenylmethylsulfonyl fluoride, and 1 mM ethylenediamine tetrahydrochloride; pH 7.5) for the microsomal preparation according to a method described by Grassl and Aronson (1986). The tissues were then homogenized with a Teflon-glass homogenizer (Thomas Scientific, Swedesboro, NJ). The homogenate was centrifuged at 1,000g, 4°C for 10 min to remove cellular debris. The supernatant was re-centrifuged at 100,000g, 4°C in a Beckman SW40T rotor for 1 h. The resulting pellet was re-suspended in 200–500 μl of lysis buffer and protein concentrations were determined using a DC Protein Assay kit (Bio-Rad, Hercules, CA). Thirty micrograms of membrane protein of each region-purified rabbit polyclonal antibodies against Na⁺ channel type I (1:800 dilution), type II (1:600), and type III (1:120) (Sigma) were applied, respectively. Protein signals were detected using an ECL chemiluminescence system (Amersham). For normalization, all the membranes were stripped and re-probed with affinity-purified goat polyclonal antibody to actin at the dilution of 1:1000 (Santa Cruz Biotechnology, Santa Cruz, CA). Four to five pooled samples were studies for each group.

**Data analysis**

In the electrophysiological experiments, data from the same group were averaged and subjected to one-tailed Student t-test. For STX binding density, all measurements from bilateral areas were averaged as a single value for a given section image. At least two-section images per brain level were analyzed in either mutant or control mice. All values from different sections in a given animal were averaged as one value for a brain region. To compare alterations in STX binding density in various brain regions, changes in STX binding density in mutant mice were converted to percent change based on its control.
RESULTS

Increased neuronal excitability in hippocampal and cortical neurons

We focused our electrophysiological studies on neurons from the hippocampal CA1 and neocortical neurons and observed major differences in membrane properties and excitability between the mutant and control mice (Fig. 1).

**FIG. 1.** Major difference in rheobase between the mutant and wild-type neurons. **A:** voltage traces with evoked action potentials in hippocampal CA1 and cortical neurons from the Na⁺/H⁺ exchanger 1 (NHE1) null mutant (mutant) and wild-type brains. **B:** mean values of rheobase (means ± SE). In the neurons of wild-type CA1, wild-type and mutant cortex, evoked action potentials were collected in the current-clamp mode with 10 depolarizing currents, starting with 10 pA and using 10-pA increments. For mutant CA1 neurons, evoked action potentials were collected with 10 depolarizing currents, starting with 5 pA and using 5-pA increments. Voltage traces start at the bottom and follow upward incrementally. Note that both hippocampal and cortical neurons had a lower rheobase than in wild-type, especially the former, suggesting increased excitability in the mutant neurons.
NEURONAL PROPERTIES AND MEMBRANE EXCITABILITY OF CA1 NEURONS. We used HEPES-containing solutions in this study, with $V_m$ around $-40$ mV (in bicarbonate solutions, $V_m$ was in the range of $-50$ mV). We observed that $V_m$ and $R_m$ in hippocampal CA1 neurons of the wild-type were similar to those of the NHE null mutant ($V_m$ was $-39 \pm 4$ mV, $n = 14$ and $-39 \pm 2$ mV, $n = 23$; and $R_m$ was $617 \pm 301$ M$\Omega$, $n = 12$ and $511 \pm 92$ M$\Omega$, $n = 21$ for the wild-type and the mutant, respectively). However, CA1 neurons in the NHE null mutant were much more excitable than wild-type CA1 neurons in spite of the lack of difference in $V_m$ and $R_m$. The rheobase in the wild-type neurons, for example, was more than double that of the NHE null neurons ($53.3 \pm 14.7$ pA, $n = 14$ for the wild-type neurons vs. $23.6 \pm 7.6$ pA, $n = 12$ for the NHE null neurons, $P = 0.05$; Fig. 1).

NEURONAL PROPERTIES AND MEMBRANE EXCITABILITY OF CORTICAL NEURONS. In HEPES solutions, wild-type cortical neurons were more depolarized than NHE1 null mutant neurons ($-27 \pm 4$ mV, $n = 9$ for wild-type and $-38 \pm 3$ mV, $n = 16$ for mutant, $P = 0.01$). Both groups had variable $R_m$ ($724 \pm 218$ M$\Omega$, $n = 9$ and $1,035 \pm 230$ M$\Omega$, $n = 16$ for wild-type and mutant, respectively, $P = 0.19$). Cortical neurons in the NHE null mutant were, however, as in the CA1 neurons, more excitable than wild-type neurons (Fig. 1). The rheobase in the wild-type neurons was significantly higher than that of NHE1 mutant neurons ($55.0 \pm 14.4$ pA, $n = 13$ for the wild-type neurons vs. $32.6 \pm 5.9$ pA, $n = 28$ for the NHE null neurons, $P < 0.05$).

**Increased Na$^+$ current density in hippocampal and cortical neurons**

Because we found major differences in excitability between mutant and wild-type neurons without difference in $V_m$ or $R_m$, we next investigated whether there is any difference in Na$^+$ current density between the mutant and control mice (Fig. 2).

**MAJOR INCREASE IN NA$^+$ CURRENT DENSITY IN HIPPOCAMPAL CA1 NEURONS.** In hippocampal CA1 neurons, the peak Na$^+$ current in the NHE null neurons was much larger compared with that of wild-type neurons ($5.3 \pm 0.9$ nA, $n = 23$ for the NHE null neurons and $2.7 \pm 0.7$ nA, $n = 15$ for the wild type, $P = 0.02$). Similarly, the current density was also significantly larger in the mutant than wild type ($0.65 \pm 0.08$ nA/pF, $n = 23$ for the NHE null neurons and $0.44 \pm 0.08$ nA/pF, $n = 14$ for the wild-type neurons, $P = 0.04$).

![FIG. 2. Major differences in Na$^+$ currents between the mutant and wild-type neurons. A: Na$^+$ current trace in the cortex of the mutant and wild-type mice. B: quantitative changes in peak Na$^+$ currents and Na$^+$ current density (means ± SE). Na$^+$ currents were recorded in voltage clamp. The current density was obtained by normalized peak Na$^+$ currents to whole cell capacitance. Note that Na$^+$ current size and density were higher in the NHE1 null mutant neurons than in wild-type neurons.](image-url)
INCREASED Na\(^+\) CURRENT DENSITY IN NEOCORTICAL NEURONS.

The peak Na\(^+\) current of NHE null cortical neurons was significantly larger, compared with that of wild-type neurons (1.2 ± 0.1 nA, \(n = 29\) for the NHE null neurons and 0.77 ± 0.1 nA, \(n = 16\) for the wild types, \(P < 0.02\)). The current density of the NHE null neurons was also significantly larger (0.4 ± 0.3 nA/pF, \(n = 27\) for the NHE null neurons and 0.3 ± 0.04 nA/pF, \(n = 16\) for the wild-type neurons, \(P = 0.04\)).

Selective upregulation of Na\(^+\) channel density in hippocampus and cortex

Because Na\(^+\) channel expression is a major factor that determines Na\(^+\) current density, we measured the STX binding density to compare Na\(^+\) channel expression between the mutant and wild-type mice and found that Na\(^+\) channel expression was selectively upregulated in cortical and hippocampal regions (Figs. 3 and 4).

NA\(^+\) CHANNEL UP-REGULATION IN CORTICAL AND HIPPOCAMPAL REGIONS. STX binding density increased in most cortical regions of NHE1 null mice (Fig. 3A), but the increase was heterogeneous. For example, STX binding density increased by 17% \((n = 7, P < 0.05)\) in the striate cortex and by 11% \((n = 7, P < 0.05)\) in the temporal cortex of NHE1 null mutant mice as compared with that in wild-type. In contrast, other regions such as the frontoparietal cortex and entorhinal cortex of the mutant mice had virtually no increase in STX binding density. In the hippocampus, only the pyramidal cell layer of the CA1 region showed an increase of 12% \((n = 7, P < 0.001)\) in the mutant as compared with control mice (Fig. 3A). The quantitative changes in major cortical and hippocampal regions are shown in Fig. 4A.

It is noteworthy that the upregulation of Na\(^+\) channel expression was not consistent with that of Na\(^+\) current density (Fig. 5). In CA1 region, for instance, Na\(^+\) current density increased by >45% although Na\(^+\) channel expression increased by only 11%. In the cortical region, however, Na\(^+\) current density increased by ~30% in spite of a larger increase in Na\(^+\) channel density (+17%).

NO MAJOR CHANGE IN STX BINDING DENSITY WITHIN MOST SUBCORTICAL AND OTHER REGIONS. In most subcortical regions, STX binding density had no appreciable change in the NHE1 mutant brains as documented in Figs. 3 and 4B. In the thalamus, STX binding density tended to increase only in the medial part of the ventroposterior thalamic nucleus (VP, 14%), but this change was not statistically significant \((P > 0.05, n = 7)\) in the midbrain, the only region showing an upregulation of Na\(^+\) channel expression was the superior colliculus (SC, 13%, \(n = 7, P < 0.05\)). Some brain stem nuclei actually exhibited higher STX binding density. For example, raphe obscurus nucleus (ROb) showed a higher level of STX binding density as compared with wild-type (22%, \(n = 7, P < 0.05\); Figs. 3 and 4B). However, no significant increase in STX binding density was found in most brain stem regions. In other brain regions, like the cerebellum, STX binding density tended even to decrease although the change was not statistically significant.

Differential regulation of Na\(^+\) channel subtypes in the hippocampus and cortex

Because there are three major neuronal subtypes of Na\(^+\) channels in the brain, we further asked which subtype(s) is upregulated in the hippocampus and cortex. To differentiate subtypes I to III, we performed immunoblotting using antibodies against the neuronal isoforms of the Na\(^+\) channel and discovered that distinct subtypes of Na\(^+\) channels contributed to the Na\(^+\) channel upregulation differentially in different regions. Figure 6 shows a significant increase (27%, \(n = 4, P < 0.05\)) of subtype II in the cortex and an increase (18%, \(n = 5, P < 0.05\)) of subtype I in the hippocampus. In addition, Na\(^+\) channel subtype III was expressed at very low level in both cortex and hippocampus and thus no obvious difference was detected between NHE1 null mutants and their wild-type controls. In contrast, other regions, such as cerebellum, tended to decrease both Na\(^+\) channel subtypes I and II in the NHE1 mutant as compared with wild type (data not shown).

DISCUSSION

We have made two important observations in this work. First, Na\(^+\) channel expression is selectively upregulated in the
cortex and hippocampus of the NHE1 mutant mice. Second, the cortical and hippocampal regions, which showed an increased STX binding, also showed an increase in the size of the Na\(^+\)/H\(^+\) current as well as increased membrane excitability. Our data suggest that NHE1 null mutation leads to Na\(^+\)/H\(^+\) channel dysregulation and thus increases neuronal excitability.

A number of factors may alter Na\(^+\) channel expression and function (O’Reilly et al. 1997; Urenjak and Obrenovitch 1996; Xia et al. 2000). In this work, we have shown that the whole cell Na\(^+\) current is upregulated in the CA1 and neocortex in the NHE1 null mutant mice. This increase in the Na\(^+\) current can be a result of increased expression, increased open probability ($P_o$) or increased conductance ($i$). The STX binding studies that showed increased density in the same regions from which the triturated cells were taken, strongly suggest that the increased whole cell current is at least partly due to the increased expression of Na\(^+\) channels. However, this result does not rule out a change in Na\(^+\) channel open probability or conductance. As shown in Fig. 5, the increase in whole cell current is larger than the difference in the upregulation of STX binding density in CA1 and neocortical neurons. These data suggest that the increase in Na\(^+\) current density is not solely dependent on an

FIG. 4. Differences in Na\(^+\) channel density between mutant and wild-type brain regions. - - - , a control level of STX binding density in age-matched wild-type mice. A: cortical and hippocampal regions. FrPa, fronsoparietal cortex; Str, striate cortex; Te, temporal cortex; Ent, entorhinal cortex; CA1 py, hippocampal CA1 field, pyramidal layer; CA1 mol, hippocampal CA1 field, molecular layer; CA3 py, hippocampal CA3 field, pyramidal layer; CA3 mol, hippocampal CA3 field, molecular layer; CA4, hippocampal CA4 field. B: subcortical and caudal regions. CPu, caudate putamen (striatum); GP, globus pallidus; AM, amygdaloid nucleus; VP, ventroposterior thalamic nucleus; Hyp, posterior hypothalamic nucleus; SN, substantia nigra; MG, medial geniculate nucleus; SC, superior colliculus. Data were expressed as means ± SE. *, $P < 0.05$; ***, $P < 0.001$ as compared with wild type.

Note that Na\(^+\) channel density in most regions of the mutant forebrain increased or tended to increase. In contrast, Na\(^+\) channel density in the mutant cerebellum tended to decrease.
increase in Na\(^+\) channel number, other factors may also play a role in the increase of Na\(^+\) currents in mutant neurons. There are multiple Na\(^+\) channel subtypes in the brain (Catterall 1992; Urenjak and Obrenovich 1996). Their regulation under various physiological and pathophysiological conditions is not well understood. Our present work shows that Na\(^+\) channels I and II are differentially upregulated in the hippocampus and cortex, suggesting that different mechanisms underlie the regional upregulation of Na\(^+\) channels in the mutant neurons. Such regionally and differentially regulated Na\(^+\) channel isoforms have been described in other pathophysiological conditions such as hypoxia (Zhang et al. 2001) and nerve cell injury (Waxman et al. 1994).

One important and interesting issue is the relation between Na\(^+\) channels and NHE1. In this work, we show that, in the absence of NHE1, certain Na\(^+\) channel isoforms are upregulated in certain specific cells or regions of the CNS. This raises a number of questions. For instance, we have previously shown that NHE isoform 1 is ubiquitously expressed in the CNS (Ma and Haddad 1997). Hence, one could ask: why is Na\(^+\) channel expression upregulated in certain specific regions and not ubiquitously? Another question is whether there is an interaction, physical or indirect, between NHE1 and the Na\(^+\) channel. At present, we do not have any evidence suggesting a direct interaction. However, it is possible that the lack of NHE1 leads to alterations in other membrane proteins or intracellular signals that, in turn, alter Na\(^+\) channel expression in neurons. This notion of altered Na\(^+\) channel expression may not be surprising as the expression of other membrane proteins is also altered in the same NHE1 null mutant mouse (Xue et al. 2002). It would be interesting to speculate that Na\(^+\) transport is affected in this null mouse because the Na\(^+\) transport-related proteins such as the Na\(^+\)-HCO\(_3\) cotransporter, the Na\(^+\)-H\(^+\) exchanger, and the Na\(^+\) channels, are all affected. Based on our work, we would also like to emphasize that the phenotype in a particular gene knock-out animal may not be related directly to that gene but to the effect of other genes that are influenced by the knock-out of that gene.

In summary, we have observed that Na\(^+\) channel expres-

FIG. 5. Comparison between Na\(^+\) channel expression and Na\(^+\) current density in mutant hippocampal and cortical neurons. Na\(^+\) channel density was assayed with STX binding and autoradiography. Na\(^+\) current density was obtained from voltage-clamp recordings with peak currents normalized to whole cell capacitance. Data were expressed as percentage of control level in wild type. *, \(P < 0.05\) and ***, \(P < 0.001\) as compare with wild-type. Note that Na\(^+\) channel density increased more in the striate cortex than in the hippocampal CA1 neurons, but Na\(^+\) current density increased less in the cortical neurons than in CA1 neurons.

FIG. 6. Differential upregulation of Na\(^+\) channel subtypes I (A) and II (B) in the mutant hippocampus and cortex. Immunoblotting was employed to compare protein levels of Na\(^+\) channel subtypes I and II between NHE1 null mutant (N) and their age-matched wild-type controls (C) in the cerebral cortex and hippocampus. Top: representative immunoblots of Na\(^+\) channel proteins and their corresponding acts. Densitometric analyses of the protein signals. The x axis showed the different brain regions. The y axis depicts the relative level of Na\(^+\) channels, as a ratio of Na\(^+\) channel protein to actin density per 30 \(\mu\)g of total membrane protein. Values were means ± SE (n = 5 for subtype I; n = 4 for subtype II). Statistical significance (\(P < 0.05\), mutant vs. wild-type) was indicated (*). Note that Na\(^+\) channel subtype I increased in the mutant hippocampus and Na\(^+\) channel subtype II increased in the mutant cortex, while actin density was similar in mutant and wild-type.

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