Mutations in the SCN2A gene encoding a voltage-gated sodium channel Nav1.2 are associated with epilepsies, intellectual disability, and autism. SCN2A gain-of-function mutations cause early-onset severe epilepsies, while loss-of-function mutations cause autism with milder and/or later-onset epilepsies. Here we show that both heterozygous Scn2a-knockout and knock-in mice harboring a patient-derived nonsense mutation exhibit ethosuximide-sensitive absence-like seizures associated with spike-and-wave discharges at adult stages. Unexpectedly, identical seizures are reproduced and even more prominent in mice with heterozygous Scn2a deletion specifically in dorsal-telencephalic (e.g., neocortical and hippocampal) excitatory neurons, but are undetected in mice with selective Scn2a deletion in inhibitory neurons. In adult cerebral cortex of wild-type mice, most Nav1.2 is expressed in excitatory neurons with a steady increase and redistribution from proximal (i.e., axon initial segments) to distal axons. These results indicate a pivotal role of Nav1.2 haplodeficiency in excitatory neurons in epilepsies of patients with SCN2A loss-of-function mutations.
Voltage-gated sodium channels in neurons play essential roles in the generation and propagation of action potentials. These channels consist of one pore-forming α subunit and one or two accessory β subunits. In the mammalian brain, four α subunits, namely, Nav1.1, 1.2, 1.3, and 1.6 encoded by SCN1A, 2A, 3A, and 8A, respectively, are expressed at high levels.

Mutations in voltage-gated sodium channel genes have been described in patients with a wide spectrum of neurological disorders including epilepsy. The first mutation in SCN2A was discovered in a patient with atypical generalized epilepsy with febrile seizures plus. Subsequently, inherited SCN2A mutations were found in families with benign familial neonatal-infantile seizures. We further reported a nonsense mutation SCN2A-R102X (RX) in a patient with epileptic encephalopathy, autism spectrum disorder (ASD) and intellectual disability, which was the first report of a de novo SCN2A mutation in a patient with ASD or intellectual disability. Subsequently, we and others reported a number of de novo SCN2A mutations in patients with neurological disorders such as epileptic encephalopathy including Ohtahara syndrome, West syndrome, Lennox Gastaut syndrome, and schizophrenia. Recent large-scale whole exome sequencing studies further revealed that SCN2A is the gene showing the most frequent and common de novo mutations among these patients.

Although mutations of SCN1A have also been described in patients with epileptic encephalopathy, intellectual disability, and ASD, the distributions of Nav1.1 and Nav1.2 are highly distinct from each other in brain. In neocortex, hippocampus and cerebellum, Nav1.1 is dominantly expressed in parvalbumin-positive GABAergic neurons. In neocortex, hippocampus and cerebellum, Nav1.1 is dominantly expressed in parvalbumin-positive GABAergic neurons such as fast-spiking (FS) basket cells and Purkinje cells in their axonal features. While Nav1.2 is robustly expressed in glutamatergic neurons including most neocortical pyramidal cells at their axon initial segments. Nav1.2 is densely expressed in unmyelinated axons of neurons in hippocampal dentate and cerebellar granule cells, although in striatum Nav1.2 is present at unmyelinated axons of GABAergic medium spiny neurons. We recently reported that Nav1.1 and Nav1.2 are expressed in a mutually exclusive manner not only in neocortex, hippocampus, and cerebellum, but also in striatum, where medium spiny neurons are Nav1.2-positive and presumed FS inhibitory interneurons are Nav1.1-positive. In globus pallidus, all GABAergic neurons are Nav1.1-positive and the dense Nav1.2 signals are derived from axonal fibers of striatal medium spiny neurons. Li and colleagues reported that Nav1.2 is expressed in neocortical somatostatin-positive inhibitory neurons but not in parvalbumin-positive neurons. However, we found that Nav1.2 is expressed in caudal ganglionic eminence-derived vasoactive intestinal peptide-positive or reelin-positive/somatostatin-negative inhibitory neurons in neocortex and hippocampus, but is not expressed in parvalbumin or somatostatin-positive neurons, which are medial ganglionic eminence-derived inhibitory neurons.

Contrary to SCN1A loss-of-function mutations in patients with severe epilepsies such as Dravet syndrome, SCN2A gain-of-function (increased or accelerated, but not toxic) has recently been recognized as a cause of early infantile-onset severe epileptic encephalopathies such as Ohtahara syndrome, whereas loss-of-function SCN2A mutations underlie ASD or intellectual disability with later-onset mild epilepsy or without epilepsy. Given that the predominant expression of Nav1.1 is in inhibitory neurons and that of Nav1.2 is in excitatory neocortical/hippocampal neurons, it seems reasonable that SCN1A loss- or SCN2A gain-of-function mutations lead to epilepsies. However, it still remains unclear why SCN2A loss-of-function mutations also cause epilepsies.

A mouse transgenic line Scn2aQ54 exhibiting partial seizures originating in hippocampus has long been used as a model for diseases caused by SCN2A mutations. This mouse model harbors a GAL879-881IQQQ Scn2a gain-of-function mutation and the mutant protein is ectopically expressed under the control of the rat promoter for a neuron-specific enolase gene, while intrinsic Scn2a genes remain intact. In mice with genuine Scn2a deficiency, no epileptic seizures have been described so far.

In this study, we discovered that Scn2a haploinsufficient mice show a mild spontaneous epileptic phenotype of absence-like seizures. Contrary to the previous proposal that loss-of-function Scn2a mutations may reduce excitability of Nav1.2 expressing inhibitory neurons and thereby lead to epileptic seizures, we show here that the epileptic phenotypes in mice with Scn2a deficiency depend on Nav1.2 deficiency in excitatory neurons, suggesting critical contributions of impaired functions of excitatory neurons to the pathophysiology of epileptic seizures associated with SCN2A mutations.

Results

Absence-like seizures in Nav1.2 haploinsufficient mice. We have previously suggested that a truncated non-functional peptide (Nav1.2-RX) consisting of the N-terminal 101 amino acid residues might cause dominant negative Nav1.2 suppression leading to intractable seizures in a patient with the Scn2a-RX mutation. In order to test this hypothesis, we generated knock-in mice carrying the RX mutation (Fig. 1a and Supplementary Fig. 1a-c) and compared their phenotypes with those of previously reported Scn2a-knockout (KO) mice carrying the disrupted exon 1 with an insertion of neo cassette. Western blot analyses of whole brain lysate at postnatal day (P) 0.5, using a newly-generated anti-N-terminal-Nav1.2 antibody (EM1) and an anti-internal (ASC-002) Nav1.2 antibody showed that wild-type Nav1.2 was highly expressed in wild-type (Scn2a+/+) mice, moderately expressed in heterozygote (Scn2aRX/+ mice and negligibly expressed in homozygote (Scn2aRX/RX) mice (Fig. 1b and Supplementary Fig. 1d). Meanwhile, truncated Nav1.2-RX was undetected in Scn2aRX/+ and Scn2aRX/RX mice (Fig. 1c), suggesting that the mutated Scn2a allele was inactivated, presumably by nonsense-mediated mRNA decay. Western blot analyses using the anti-pan Nav1 antibody (SP19) also showed reduced expression levels of Nav1 (total voltage-gated sodium channel alpha-subunits) in Scn2aRX/RX mice, compared with Scn2aRX/+ mice (Supplementary Fig. 1e), consistent with inactivation of the Scn2a mutated allele. Like homozygous Scn2a KO (Scn2aKO/KO) mice, Scn2aRX/RX pups were born in the approximately expected Mendelian ratios, but all died within two days after birth (Fig. 1d). Scn2aRX/+ mice were viable and fertile, and had normal life spans, as observed in Scn2aKO/RX mice.

Although visual inspection did not detect behavioral seizures in Scn2aRX/+ mice, long-term electrocorticography (ECOG)-electromyography (EMG) recordings at 6–11 weeks of age revealed frequent abnormal ECOG patterns, typically <1 s bursts of high-amplitude bilateral spike-and-wave discharges (SWDs) associated with EMG suppression indicating behavioral arrest (Fig. 2a). These features of Scn2aRX/+ mice closely resembled epileptiform discharges observed in rodent models of absence epilepsy, except that the duration of SWD episodes in Scn2aRX/+ mice (mean ± SEM, 0.71 ± 0.04 sec, 70 ECOG discharges) was much shorter than those (usually more than 2 s) in other rodent models. 24-h ECOG-EMG recordings showed that Scn2aRX/+ mice had a higher prevalence and a greater incidence of epileptiform discharges than Scn2aRX/+ mice (prevalence rate: Fisher’s exact test, *P = 0.0455, hourly incidence: Mann–Whitney test, U = 0, **P = 0.0025, Fig. 2b). ECOG–EMG recordings also detected one prolonged
null non-convulsive seizure with duration of 50 s in 1 out of 7 Scn2αKO/+ mice examined (Fig. 2c). Analysis of susceptibility to induced seizures by a chemoconvulsant, a GABA A receptor antagonist pentylentetrazol (PTZ, 50 mg per kg or 25 mg per kg), revealed a shorter latency to the appearance of absence seizure-like sudden immobility in Scn2αKO/+ than in Scn2α+/+ mice (Fig. 2d, e).

We next investigated whether Scn2αKO/+ mice also have absence-like seizures. While visual inspections did not reveal any discernible behavioral seizures in Scn2αKO/+ mice, consistent with a previous study 38, ECoG-EMG recordings from Scn2αKO/+ mice at 10–27 weeks of age detected absence-like seizures with SWDs associated with EMG suppression (Fig. 3a) as observed in Scn2αRX/+ mice. ECoG monitoring further revealed two prolonged non-convulsive seizures with duration of 30–45 s in 2 out of 6 Scn2αKO/+ mice examined (Fig. 3b). 3-h ECoG-multisite local field potential (LFP) recordings showed that Scn2αKO/+ mice had a greater incidence of ECoG SWDs than Scn2α+/+ mice (ECoG on somatosensory cortex, prevalence rate: Fisher’s exact test, \( P = 0.1942 \), hourly incidence: Mann–Whitney test, \( U = 0, \) \( P = 0.2826 \)) (Fig. 3c, d). Moreover, ECoG-multisite LFP recordings revealed the predominant appearance of LFP epileptiform discharges in medial prefrontal cortex (mPFC) and caudate putamen (CPU) (Fig. 3c, d). While the incidence and duration of epileptiform discharges did not significantly differ between Scn2αRX/+ and Scn2αKO/+ mice (hourly incidence: Scn2αRX/+, 1.018 ± 0.552; Scn2αKO/+, 1.417 ± 0.417; Mann–Whitney test, \( U = 4, P = 0.0606, 95.76\% \) confidence interval [−2.292, 3.292], duration: Scn2αRX/+, 0.707 ± 0.044 sec; Scn2αKO/+, 0.578 ± 0.046 sec; Mann–Whitney test, \( U = 1134, P = 0.0982, 95.04\% \) confidence interval [−0.016, 0.226], Scn2αRX/+, \( N = 7 \), 70 SWD episodes; Scn2αKO/+, \( N = 4, 40 \) SWD episodes), the maximum amplitude and spike numbers during a SWD episode were larger in Scn2αRX/+ mice than in Scn2αKO/+ mice (maximum amplitude: Scn2αRX/+, 0.568 ± 0.019 mV; Scn2αKO/+, 0.453 ± 0.014 mV; Mann–Whitney test, \( U = 736.5, *** P < 0.0001, 95.04\% \) confidence interval [−0.053, 0.142], number of spikes: Scn2αRX/+, 5.33 ± 0.27; Scn2αKO/+, 4.20 ± 0.21; Mann–Whitney test, \( U = 968, P = 0.0057, 95.04\% \) confidence interval [0.00, 1.00], Scn2αRX/+, \( N = 7 \), 70 SWD episodes; Scn2αKO/+, \( N = 4, 40 \) SWD episodes). Given that incidence and duration of SWD episodes were 1.018 ± 0.552 episodes per hour and 0.71 ± 0.04 sec in Scn2αRX/+ mice and 1.417 ± 0.417 episodes per hour and 0.58 ± 0.05 sec in Scn2αKO/+ mice, the percentage of total recording period displaying seizures was calculated to be around 0.02% for both lines. The susceptibility to hyperthermia-induced seizure was not altered in Scn2αKO/+ compared to Scn2α+/+ mice (Fig. 3e). Analysis of susceptibility to PTZ (50 mg per kg or 25 mg per kg) induced seizures showed a shorter latency to the appearance of absence seizure-like sudden immobility, myoclonus, and clonic convulsion in Scn2αKO/+ than in Scn2α+/+ mice (Fig. 3f, g). Nav1.2 haplosufficiency in Scn2αKO/+ did not alter
the basal expression levels of other sodium channel subunits (Fig. 4). Taken together, these results suggest that Nav1.2 haploinsufficiency is the pathological basis for the absence-like seizures in these mice.

**Seizures in mice with Nav1.2 deletion in excitatory neurons.** Nav1.2 is expressed in caudal ganglionic eminence-derived inhibitory neurons, such as vasoactive intestinal peptide-positive or reelin-positive/somatostatin-negative inhibitory neurons, and in pyramidal neurons. In order to evaluate the relative impact of Nav1.2 haploinsufficiency in excitatory and inhibitory neurons on the absence-like seizures observed in Scn2a-knock-in and KO heterozygous mice, we examined a series of Scn2a conditional KO mice. At first, we generated a mouse line with a floxed Scn2a allele containing two loxP cassettes placed on either side of coding exon 2 (Supplementary Fig. 2a-c). Mice homozygous for the floxed allele (Scn2a<sup>fl/fl</sup>) were viable, showed no obvious abnormal phenotypes, and expressed normal levels of Nav1.2 (Supplementary Fig. 2d). Next, we generated a constitutively deleted Scn2a allele by crossing Scn2a<sup>fl/+</sup> with EIIa-Cre.
Fig. 3 Heterozygous Scn2a knockout mice showed absence-like seizures with SWDs. a Representative traces of ECoG/EMG recordings from 10–27 weeks-old Scn2a+/KO (KO/+) mice (N = 6). Black arrowheads indicate the onset of SWD. Gray arrowheads indicate the onset and end of EMG suppression. b A representative trace of prolonged non-convulsive seizure. ECoG recordings detected 2 episodes of prolonged non-convulsive seizure with duration of 30–45 s in 2 out of 6 Scn2a+/KO mice, which were neither accompanied by convulsions, nor followed by post-ictal depression. c Representative ECoG/EMG/LFP recordings during an SWD episode in Scn2a+/KO mice. Epileptiform discharges with large amplitudes are seen in mPFC and CPu. Positivity was plotted up (a, b, c). d Quantification of ECoG SWDs and LFP epileptiform discharges [3-hour recording, light period, Scn2a+/KO, Scn2a+/KO (N = 4, each genotype)]. Mann-Whitney test, medial prefrontal cortex: U = 0, *P = 0.0286; visual cortex: U = 8, P > 0.9999; basolateral amygdala: U = 3, P = 0.2571; hippocampus CA1: U = 4, P = 0.4286; caudate putamen: U = 0, *P = 0.0286; ventroposterior thalamus: U = 7.5, P > 0.9999. e Thresholds of body temperature for hyperthermia-induced seizures did not differ between Scn2a+/KO and Scn2a+/KO mice (4-week-old, N = 10, each genotype) [unpaired t-test, t(18) = 1.149, P = 0.2659]. f, g Increased seizure susceptibility to PTZ in 10-week-old Scn2a+/KO mice. Latencies to the first appearance of sudden immobility, myoclonus, clonic convulsion, and tonic-clonic convulsion after administrating of PTZ at doses of 50 (f, N = 20, each genotype) or 25 (g, N = 12, each genotype) mg per kg body weight. The latencies to the appearance of sudden immobility, myoclonus and clonic convulsion were shorter in Scn2a+/KO mice than in Scn2a+/+ mice (Mann-Whitney test, 50 mg per kg, sudden immobility, U = 69.5, ***P = 0.0002, myoclonus, U = 110.5, **P = 0.0145, clonic convulsion, U = 119, *P = 0.0278, tonic-clonic convulsion, U = 188.5, P = 0.7624; 25 mg per kg, sudden immobility, U = 26.5, ***P = 0.0071). Data represent means ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001. Scale bars: (a-c) vertical 0.5 mV; horizontal 1 s.
line, in which the Cre-loxP recombination occurs in germline cells42. Nav1.2 expression levels in whole brain were high, moderate and undetectable in wild-type (Scn2a+/+), heterozygous (Scn2a+/del+) and homozygous mice (Scn2a(del/del)), respectively (Supplementary Fig. 2e). Scn2a(del/del) was viable and fertile, while Scn2a(del/del) died within two days after birth (Supplementary Fig. 2f), similarly to those of Scn2a-knock-in and -KO mice.

We then crossed Scn2a(fl/fl) with an Emx1-Cre driver line43, in which Cre recombines is expressed in excitatory neurons of dorsal telencephalon consisting of neocortex, hippocampus, amygdala, piriform cortex, entorhinal cortex and olfactory bulb, and Cre-mediated recombination is detectable at embryonic day 10. We also deleted Scn2a gene selectively in global inhibitory neurons by crossing Scn2a(fl/fl) mice with a Vgat-Cre driver line25. PCR analyses of DNAs from Scn2a(fl/fl)/Emx1-Cre and Scn2a(fl/fl)/Vgat-Cre whole brains at P0.5 verified Cre-dependent recombination of the floxed Scn2a allele (Supplementary Fig. 3a).

Western blot analyses of Nav1.2 expression in P0.5 whole brain showed a ~30% reduction in Scn2a(fl/fl)/Emx1-Cre and a ~60% reduction in Scn2a(fl/fl)/Vgat-Cre, compared with Scn2a(fl/fl) mice (Supplementary Fig. 3b, c), suggesting that Emx1-Cre or Vgat-Cre-mediated recombination effectively occurs in the perinatal period. Immunohistochemistry showed that Nav1.2-immunoreactive fibers and puncta scattered in cortical plate and hippocampal stratum pyramidale and radiatum, presumably corresponding to axon initial segments (AISs) of excitatory neurons26–29, were strongly detected in P0.5 Scn2a(fl/fl) controls, but virtually undetectable in P0.5 Scn2a(fl/fl)/Emx1-Cre mice (Supplementary Fig. 3d-i). Immunohistochemistry furthermore showed that Nav1.2-immunoreactive fibers in neocortical layer I and hippocampal stratum lacunosum-molecular, which putatively correspond to AISs of reelin-positive/somatostatin-negative inhibitory neurons29, were clearly observed in P9.5 Scn2a(fl/fl) controls, but almost absent in P9.5 Scn2a(fl/fl)/Vgat-Cre mice (Supplementary Fig. 3j-o). Like homozygous Scn2a-deficient mice, Scn2a(fl/fl)/Emx1-Cre and Scn2a(fl/fl)/Vgat-Cre mice were born, while all (n = 6) Scn2a(fl/fl)/Emx1-Cre and most (4 out of 5) Scn2a(fl/fl)/Vgat-Cre mice died within two days after birth (Fig. 5a). Scn2a(fl/fl)/Emx1-Cre mice were viable and fertile and showed no obvious phenotypic abnormalities, while Scn2a(fl/fl)/Vgat-Cre mice were viable but approximately one-third (9 out of 30) suffered sudden death for unknown reasons between P18 and 25 (Fig. 5b).

ECG-EMG recordings at 6–8 weeks of age detected absence-like seizures with SWDs with EMG suppression in Scn2a(fl/fl)/Emx1-Cre, but not in Scn2a(fl/fl)/Vgat-Cre mice (Mann–Whitney test, Cntl vs. Scn2a(fl/fl)/Emx1-Cre: U = 0, ***P = 0.0075) (Fig. 5c, d). Absence-like seizures appeared ~10 x more frequently in Scn2a(fl/fl)/Emx1-Cre than in Scn2a(KO/X+/

Redistribution of Nav1.2 during brain development. Western blot analyses showed that Nav1.2 expression was already detectable on embryonic day 14.5 and steeply up-regulated during postnatal development (Supplementary Fig. 4). At 6–8 weeks of age, Nav1.2 expression in 6-week-old neocortex and hippocampus showed ~50% reduction in Scn2a(fl/fl)/Emx1-Cre and no significant alterations in Scn2a(fl/fl)/Vgat-Cre, compared with Scn2a(fl/fl) mice (Fig. 6), indicating that most Nav1.2 is expressed in excitatory neurons in neocortex and hippocampus at adult stages. We further investigated the temporal and spatial expression pattern of Nav1.2 by immunohistochemistry. Although two commercial (ASC-002, G-20) and one original (EM-1) Nav1.2 antibodies displayed similar staining patterns (Supplementary Fig. 5) and their immunosignals disappeared in Scn2a(KO/KO) when compared with Scn2a(fl/fl) mice (Supplementary Fig. 6), the goat antibody G-20 gave the clear signal and allowed double staining with rabbit or mouse antibodies, and was selected for subsequent immunohistochemical analyses. At P0.5, immunosignals for ankyrinG were detected in both Scn2a(KO/KO) and Scn2a(fl/fl) mice, suggesting that AIS structures in Scn2a(KO/KO) mice remain largely intact (Supplementary Fig. 7). Immunohistochemistry of Nav1.2 in wild-type mice detected immunosignals throughout the central nervous system with drastic changes in signal intensity and subcellular distribution from neonatal period to adulthood (Fig. 7 and Supplementary Fig. 4b and 8). During this period, the intensity of Nav1.2-immunosignals grew in unmyelinated fibers, including mossy fibers of hippocampal dentate granule cells (Fig. 7p–t) and cerebellar parallel fibers (Supplementary Fig. 8a-d). Nav1.2 signals also appeared and grew more intense in unmyelinated AIS segments of neocortical and hippocampal pyramidal cell axons (Fig. 7i–j, p–t) and nodes of Ranvier (Fig. 7k–o). At P15.5 in neocortex and hippocampus, Nav1.2 was strongly observed at the ankyrinG-positive AISs of excitatory neurons (Fig. 8). Consistent with previous studies6,33, the AISs of P15.5 neocortical pyramidal cells expressed Nav1.2-immunoreactivity in their proximal part and Nav1.6-immunoreactivity in
their distal part (Supplementary Fig. 9). It is noteworthy that diffuse Nav1.2-immunoreactivity assumed to be distal axonal features throughout neocortex and hippocampus continued to become stronger until adulthood (Fig. 7), while such diffuse signals of Nav1.6 were not observed (Supplementary Fig. 10). These observations suggest that Nav1.2, rather than Nav1.6, is the major voltage-gated sodium channel at more distal axonal sites of excitatory neurons. To localize Nav1.2 in the cerebral cortex at adult stage, we employed pre-embedding silver-enhanced immunogold electron microscopy (Supplementary Fig. 11). Most metal particles binding Nav1.2 were detected on the cell membrane of thin processes, presumably distal unmyelinated portions of preterminal axon. By contrast, only a few particles were detected in axon terminals, and none at myelinated portions of axons.

Reduced action potential amplitude in excitatory cells. Our previous study of voltage-clamp analysis of cultured Scn2aKO/+ hippocampal pyramidal neurons after 5–9 days in vitro showed a ~45% reduction in maximum sodium conductance density. Here we further investigated the properties of action potentials of

Fig. 5 Scn2a deletion in dorsal telencephalic excitatory but not global inhibitory neurons triggered SWDs in mice. a Survival rates at P2.5 of Scn2afl/fl (N = 7), Scn2afl/fl/Emx1-Cre (N = 6) and Scn2afl/fl/Vgat-Cre mice (N = 5). All Scn2afl/fl/Emx1-Cre and all but one Scn2afl/fl/Vgat-Cre mice died before P2.5. One Scn2afl/fl/Vgat-Cre survivor died at P8.5. b Survival curves during P3–30 of Scn2afl/fl/Emx1-Cre (N = 39), Scn2afl/fl/Vgat-Cre (N = 30) and Scn2afl/+ mice (N = 95). About 30% of Scn2afl/fl/Vgat-Cre mice suffered premature death between P16 and P25. c Representative ECoG/EMG/LFP traces in Scn2afl/fl/Emx1-Cre mice. SWDs during waking were often associated with behavioral arrest (right). Black arrowheads indicate the onset of SWDs. Gray arrowheads indicate the onset and end of behavioral arrest. d, e Frequencies of SWDs during 24 h ECoG recordings in Scn2afl/fl/Emx1-Cre (N = 5) and littermate controls (Cntl) (2 Scn2afl/+; 3 Scn2afl*/Emx1-Cre, N = 5), Scn2afl/fl/Vgat-Cre (N = 4) and littermate Cntl (2 Scn2afl/+; 1 Scn2afl*/Vgat-Cre, N = 3), and Scn2afl/+ mice (N = 7). All recorded mice were over 8 weeks of age. f Ethosuximide (33.3 mg mL⁻¹ in saline, 200 mg per kg, i.p.) efficiently suppressed SWDs in Scn2afl/fl/Emx1-Cre mice (N = 6). g HFP recordings from 3–6-month-old Scn2afl/fl/Emx1-Cre (N = 4) and littermate Cntl (2 Scn2afl/+; 3 Scn2afl*/Emx1-Cre, N = 7). h Representative ECoG/EMG/LFPs traces in Scn2afl/fl/Emx1-Cre mice. h Epileptiform discharges were predominantly detected in medial prefrontal cortex and caudate putamen of Scn2afl/fl/Emx1-Cre mice (Mann–Whitney test, ECoG, somatosensory cortex: U = 0, *P = 0.0286; LFP, medial prefrontal cortex: U = 0, *P = 0.0286; visual cortex: U = 6, P > 0.999; basolateral amygdala: U = 0, *P = 0.0268; hippocampus CA1: U = 4, P = 0.4286; caudate putamen: U = 0, *P = 0.0268; ventroposterior thalamus: U = 0, *P = 0.0286). Data represent means ± SEM, *P < 0.05, **P < 0.01. Scale bars: (c, g) vertical 0.5 mV; horizontal 1 s

### Fig. 5

**Scn2a deletion in dorsal telencephalic excitatory but not global inhibitory neurons triggered SWDs in mice.**

- **a** Survival rates at P2.5 of Scn2afl/fl (N = 7), Scn2afl/fl/Emx1-Cre (N = 6) and Scn2afl/fl/Vgat-Cre mice (N = 5). All Scn2afl/fl/Emx1-Cre and all but one Scn2afl/fl/Vgat-Cre mice died before P2.5. One Scn2afl/fl/Vgat-Cre survivor died at P8.5.
- **b** Survival curves during P3–30 of Scn2afl/fl/Emx1-Cre (N = 39), Scn2afl/fl/Vgat-Cre (N = 30) and Scn2afl/+ mice (N = 95). About 30% of Scn2afl/fl/Vgat-Cre mice suffered premature death between P16 and P25.
- **c** Representative ECoG/EMG/LFP traces in Scn2afl/fl/Emx1-Cre mice. SWDs during waking were often associated with behavioral arrest (right). Black arrowheads indicate the onset of SWDs. Gray arrowheads indicate the onset and end of behavioral arrest.
- **d** Frequencies of SWDs during 24 h ECoG recordings in Scn2afl/fl/Emx1-Cre (N = 5) and littermate controls (Cntl) (2 Scn2afl/+; 3 Scn2afl*/Emx1-Cre, N = 5), Scn2afl/fl/Vgat-Cre (N = 4) and littermate Cntl (2 Scn2afl/+; 1 Scn2afl*/Vgat-Cre, N = 3), and Scn2afl/+ mice (N = 7). All recorded mice were over 8 weeks of age.
- **f** Ethosuximide (33.3 mg mL⁻¹ in saline, 200 mg per kg, i.p.) efficiently suppressed SWDs in Scn2afl/fl/Emx1-Cre mice (N = 6).
- **g** HFP recordings from 3–6-month-old Scn2afl/fl/Emx1-Cre (N = 4) and littermate Cntl (2 Scn2afl/+; 3 Scn2afl*/Emx1-Cre, N = 7).
- **h** Representative ECoG/EMG/LFPs traces in Scn2afl/fl/Emx1-Cre mice. Epileptiform discharges were predominantly detected in medial prefrontal cortex and caudate putamen of Scn2afl/fl/Emx1-Cre mice (Mann–Whitney test, ECoG, somatosensory cortex: U = 0, *P = 0.0286; LFP, medial prefrontal cortex: U = 0, *P = 0.0286; visual cortex: U = 6, P > 0.999; basolateral amygdala: U = 0, *P = 0.0268; hippocampus CA1: U = 4, P = 0.4286; caudate putamen: U = 0, *P = 0.0268; ventroposterior thalamus: U = 0, *P = 0.0286). Data represent means ± SEM, *P < 0.05, **P < 0.01. Scale bars: (c, g) vertical 0.5 mV; horizontal 1 s.
**Fig. 6** Scn2a haploinsufficiency in dorsal telencephalic excitatory but in those in global inhibitory neurons reduced neocortical and hippocampal Nav1.2 expression levels. Western blot analyses of 6-weeks neocortex or hippocampus for Scn2a+/+/Emx1-Cre, Scn2a+/−/Vgat-Cre and Scn2a+/+ controls (N = 3, each genotype). Unpaired t-test, Scn2a+/+/ vs. Scn2a+/−+/Emx1-Cre, neocortex: t(4) = 5.91, *P* = 0.0041; hippocampus: t(4) = 5.74, **P** = 0.0046; Scn2a+/+ vs. Scn2a+/−/Vgat-Cre, neocortex: t(4) = 1.413, *P* = 0.2305; hippocampus: t(4) = 0.489, *P* = 0.6503. Nav1.2 protein was normalized by β-tubulin. Mean Nav1.2 expression levels are presented as percentages relative to the level of Scn2a+/+ control littermates (100%). Data represent means ± SEM, **P** < 0.01.

**Scn2a KO/+** excitatory and inhibitory neurons in neocortical layer II/III and hippocampal CA1 region using current-clamp recordings of acutely isolated brain slices. To discern between excitatory and inhibitory neurons, we crossed Scn2a KO/+ with a Vgat-Venus line that expresses green fluorescent proteins selectively in inhibitory neurons44.

In cortical pyramidal neurons at early postnatal ages (P7–8), peak amplitudes in single action potentials and spike trains were lower in Scn2a KO/+ than in Scn2a+/+ mice (Fig. 9a, b, Supplementary Fig. 12a, b and Supplementary Table 1). Half widths in single action potentials and spike trains were broader in Scn2a KO/+ than in Scn2a+/+ mice at P7–8 (Fig. 9a, c and Supplementary Table 1). Maximum rates of rise of action potential upstroke at threshold in response to different holding membrane potentials were lower in Scn2a KO/+ than in Scn2a+/+ mice at P7–8 (Fig. 9d), consistent with the lowered voltage-gated sodium channel current density in hippocampal cultured neurons from Scn2a KO/+ mice38,45. At P15–22, peak amplitudes in cortical pyramidal neurons were similar between the genotypes (Fig. 9e, f, Supplementary Fig. 12c, d and Supplementary Table 1), whereas half width was again broader in Scn2a KO/+ than in Scn2a+/+ (Fig. 9e, g and Supplementary Table 1). In hippocampal pyramidal cells, peak amplitudes were lower in Scn2a KO/+ than Scn2a+/+ mice at P7–8 but not at P15–20 (Supplementary Table 1). Half widths in single action potentials and spike trains were similar among the genotypes at both age groups (Supplementary Table 1). In contrast to these excitatory neurons, neocortical and hippocampal FS inhibitory neurons showed no significant differences in the electrophysiological properties between the genotypes at any age groups examined (Fig. 9h–m, Supplementary Fig. 13 and Supplementary Table 1). These results suggest that excitation neural activity is primarily impaired whereas the FS inhibitory activity remains unchanged in Scn2a KO/+ mice.

**Discussion**

Here we demonstrated ethosuximide-sensitive absence-like seizures with bilateral SWDs in mice heterozygous for a patient-derived Scn2a-RX nonsense knock-in mutation originally described in a patient with epileptic encephalopathy, intellectual disability, and ASD4. These same phenotypes were also observed in this study in a previously generated KO mutant mouse38. We further revealed that the RX mutation did not produce a truncated Nav1.2-RX peptide, but rather inactivated the mutated Scn2a allele, leading to Nav1.2 haploinsufficiency in Scn2a RX/+ and presumably in the patient. Although we previously suggested a dominant-negative effect of Nav1.2-RX truncated protein4 and actually SWDs were rather prominent in Scn2a RX/+ mice compared to Scn2a KO/+ mice and itself may suggest a modifying effect of a minor amount of the Nav1.2 truncated protein which was not detectable in our western blot analysis, the incidence and duration of epileptiform discharges still did not significantly differ between Scn2a RX/+ and Scn2a KO/+ mice. This indicates that Nav1.2 haploinsufficiency is the major underlying basis for epileptic seizures. We further showed that a selective Scn2a deletion in dorsal-telencephalic excitatory neurons in mice (Scn2a fl/+/Emx1-Cre) reproduced the absence-like seizures, whereas mice with a global Scn2a deletion in inhibitory neurons (Scn2a fl/+/Vgat-Cre) showed no discernable epileptic abnormalities. Contrary to the suggestion that loss of functional Nav1.2 in inhibitory neurons may contribute to the pathogenesis of epileptic seizures in patients with SCN2A mutations33, our findings indicate that Nav1.2 haploinsufficiency in excitatory neurons causes epilepsy.

Scn2a fl/+/Emx1-Cre and Scn2a fl/+/Vgat-Cre mice both died prematurely, suggesting that expression of Nav1.2 in either excitatory or inhibitory neurons is essential for postnatal viability. Nav1.2 expression in Emx1-Cre-positive excitatory neurons is robust in brain subregions, such as cerebral cortex, olfactory bulb and hippocampus29, and is estimated to explain ~30% of the total Nav1.2 amount in whole brain at P0.5. Similarly, the amount of Nav1.2 expressed in inhibitory neurons at P0.5 is estimated to be ~60% of the whole-brain Nav1.2 amount. We have shown that Nav1.2 is localized at AISs in vasoactive intestinal peptide-positive or reelin-positive/somatostatin-negative inhibitory neurons in neocortex and hippocampus, and distributed in unmyelinated axons of GABAergic medium spiny neurons in striatum29,32. Although the reason(s) for the premature death in these mice is unknown, given that perinatal death in Scn2a KO/KO seems to be associated with severe hypoxia and massive neuronal apoptosis in brainstem38, Nav1.2 deficiency in cortico-brainstem projections or local inhibitory circuits in cerebral cortex and brainstem presumably contributes to premature death.

SCN2A mutations have been described in patients with a wide spectrum of epilepsies, intellectual disability and ASD. SCN2A mutations in patients with the severe end of epilepsies such as early- infantile epileptic encephalopathy, Ohtahara syndrome and West syndrome are almost exclusively missense, while nonsense,
frameshift and splice site mutations are dominant in patients with ASD and intellectual disability associated with milder, later-onset epilepsy or without epilepsy (reviewed in Yamakawa22). Recent patch-clamp analyses confirmed that Nav1.2 channels with missense mutations found in patients with early-infantile severe epilepsies had gain-of-function effects, while mutations found in patients with ASDs or late-onset epilepsies had loss-of-function effects35,36. Although the epileptic phenotype of the patient with SCN2A-R102* mutation who showed intellectual disability and ASD was rather severe4, it was milder and later-onset compared to those of early-infantile epileptic encephalopathy. These observations suggest that Scn2a-deicient mice are models for ASD and intellectual disability with milder epilepsies rather than early-infantile epileptic encephalopathy, and that Scn2a knock-in mice with gain-of-function missense mutations are models for early-infantile epileptic encephalopathy. In fact, Scn2aRX+/+ and Scn2aKO/+ mice did not show spontaneous convulsive seizures but only mild absence-like seizures with SWDs. Similarly, the patient carrying the SCN2A-R102* nonsense mutation showed absence and atonic seizures4. The patient with ASD and intellectual disability harboring the splice site mutation, putatively SCN2A-K90Vfs*9, was also reported to have behavioral episodes characterized by a stone faced expression and limp posture, suggestive of absence seizures 11. Absence or absence-like epilepsies in patients with ASD and intellectual disability is consistent with absence-like seizures in mice with Scn2a-haploinsufficiencies.

The Scn2aQ54 mouse, long considered a model of diseases with SCN2A gain-of-function mutations, showed partial seizures originating from hippocampus37. In contrast, our ECoG recordings of Scn2aRX+/-, Scn2aKO/+/-, and Scn2aRX+/-/Emx1-Cre mice all showed absence-like seizures with SWDs. It is plausible that loss-of-function and gain-of-function Scn2a mutations culminate in different seizure phenotypes with specific patterns of epileptiform discharges. However, Nav1.2 expression in the Scn2aQ54 mouse was driven by the neuron-specific enolase promoter and it may lead to ectopic expression and other epistatic effects. Direct comparisons with knock-in mice bearing Scn2a missense mutations...
mutations found in patients with early-infantile epileptic encephalopathies under the control of intrinsic SCN2A promoters may provide a more accurate model.

Febrile seizures are highly unusual for patients with SCN2A mutations\(^{22-23}\), despite the proposed role of Nav1.2 in febrile seizure generation\(^{16,47}\). We showed that Scn2a\(^{−/−}\) mice exhibit normal susceptibility to hyperthermia-induced seizures that seems reminiscent of the temperature-independent nature of the epilepsies in a major proportion of the patient population. This contrasts with the temperature-sensitivity of epilepsies in patients with SCN1A mutations\(^{48,49}\) and Scn1a-deficient mice\(^{50,51}\). Such disparate temperature-sensitivities of seizures in patients and animal models harboring SCN1A and SCN2A mutations may be accounted for by the distinct cellular and regional distributions for Nav1.1 and Nav1.2 rather than by distinct kinetics of the proteins\(^{22,29}\).

Our immunohistochemistry data showed that at unmyelinated fibers such as hippocampal mossy fibers, axons of striatal medium spiny neurons and parallel fibers of cerebellar granule cells, Nav1.2 is expressed and increased through development. In contrast, Nav1.2 at AISs and nodes of Ranvier in myelinated fibers in neocortex and hippocampus became intense at ~P15 where Nav1.2 and Nav1.6 were co-expressed at proximal and distal AISs respectively. Nav1.2 density in these areas gradually decreased in later stages and was replaced with Nav1.6, consistent with previous studies\(^{52-54}\). However, we found that diffuse Nav1.2 signals in neocortex and hippocampus (presumably in synaptic terminals) continued to increase through development. Of note, Nav1.2 was suggested to be abundantly expressed in synaptic terminals of cerebellar parallel fibers\(^{54}\). Contrary to the increase of the diffuse Nav1.2 signals, Nav1.6 did not show such increase in the corresponding brain regions, suggesting that Nav1.2 is a major voltage-gated sodium channel in axon terminals responsible for synaptic transmission at adult stages. Because of rather late onset of epilepsies in patients with SCN2A loss-of-function mutations and the reproduction of absence seizures in mice with dorsal-telecephalic excitatory neuron-specific Scn2a deletion, it would be of interest whether the seizures are caused by Nav1.2 deficiency at the distal axons of neocortical or other dorsal-telecephalic excitatory neurons.

Our whole-cell current-clamp recordings showed that, at early postnatal stage (P7–8), neocortical and hippocampal Scn2a\(^{−/−}\) excitatory pyramidial neurons displayed decreased action potential peak amplitudes by ~10–15%. Nevertheless, our previous study using voltage-clamp recordings showed a ~45% reduction in maximum sodium conductance density in dissociated hippocampal neurons from Scn2a\(^{−/−}\)-newborn mice after ~5–9 days culture in vitro\(^{38}\). Similarly, acutely dissociated guinea-pig hippocampal neurons treated with a moderate dose of tetrodotoxin, a sodium channel blocker, reduced sodium current to less than half, whereas action potential amplitude was only slightly affected, suggesting a surplus of sodium channels in neurons for action potential firing\(^{55}\). However, the study showed that such surplus was required for repetitive action potential firings, and this could also be the case in Scn2a\(^{−/−}\)- mice. The present current-clamp recordings further showed that action potential peak amplitude recorded from neocortical and hippocampal Scn2a\(^{−/−}\) excitatory neurons reached normal levels at P15–22, raising a possibility that Nav1.2 may not be needed to generate action potentials at later postnatal stage, consistent with the developmental changes in subcellular localization and distribution of Nav1.2. Alternatively, Nav1.2 haploinsufficiency may be compensated by other subtypes of voltage-gated sodium channel. No significant changes in mRNA expression levels of the other sodium channel subunit genes were observed in Scn2a\(^{−/−}\)-brains, excluding dosage compensation of loss of Nav1.2. Instead, Nav1.6 co-expression with Nav1.2 in the AISs of pyramidal neurons may compensate for loss of Nav1.2. Steep up-regulation of Nav1.6 expression in the AISs of pyramidal neuron between P7.5 and P15.5 could account for the significant decrement of action potential peak amplitudes in Scn2a\(^{−/−}\)-pyramidal neurons at P7–8, but not at P15–22. Despite the possible functional compensation for loss of Nav1.2 at AISs and nodes of Ranvier, Nav1.6 may not have sufficient compensatory effects on absence-like seizures at adult stages, presumably due to the limited expression of Nav1.6 at synaptic terminals. It has been reported that impairing voltage-gated sodium channels function in FS parvalbumin-positive inhibitory neurons in mice led to epileptic seizures\(^{25}\). However, no differences were detected in the responses of neocortical FS interneurons to current injections between Scn2a\(^{−/−}\) and wild-type mice.
Possible mechanism is abnormal activity in thalamocortical telencephalic excitatory neurons cause epileptic seizures? One
communication biology | (2018) 1:96 | doi: 10.1038/s42003-018-0099-2 | www.nature.com/commsbio

How does the Nav1.2 haploinsufficiency in dorsal-telencephalic excitatory neurons cause epileptic seizures? One possible mechanism is abnormal activity in thalamocortical circuits, which has long been proposed as a basis for absence epilepsy\(^\text{36,37}\). Impaired firing properties in cortical excitatory neurons may impact their downstream input onto inhibitory neurons in the thalamic reticular nucleus, which in turn fails to suppress excitatory thalamocortical relay neurons. The excited thalamocortical neurons may then provide feedback inhibition through the thalamic reticular nucleus inhibitory neurons and generate thalamocortical hyper-synchronous oscillations that result in absence seizures\(^\text{37}\). P/Q-type calcium channel gene CACNA1A mutations have been described in patients with absence epilepsy\(^\text{38}\), and Cacna1a KO mice have absence epilepsies\(^\text{11}\). Recently, we reported that a selective Cacna1a gene deletion in cortical layer VI pyramidal cells, which innervate thalamic relay neurons and reticular thalamic neurons, caused upregulation of T-type calcium current in thalamic relay neurons and resulted in absence epilepsy in mice\(^\text{39}\), suggesting that impaired cortical excitatory input to thalamic regions may cause rebound burst of thalamocortical relay neurons and leads to thalamocortical hyper-synchronous oscillations as may be the case for ethosuximide-sensitive absence-like seizures in Scn2a-deficient mice. A second plausible mechanism considers a rebound hyper-excitability within neocortex. In neocortex, excitatory, and inhibitory neurons form reciprocal and highly complex networks. Impaired excitatory inputs into inhibitory neurons may even disinhibit the downstream excitatory neurons and the consequent epileptic discharges widely spread to the corticothalamic circuit. Meer en and colleagues\(^\text{40}\) actually reported that somatosensory cortex is the initiation site of epileptic activity in a rat model of absence epilepsy. Thirdly, the predominant appearance of epileptiform discharges in CPu of Scn2aKO/− mice and Scn2a\(^\text{fl/fl}\)/Emx1-Cre mice may implicate CPu in the pathology of absence-like seizures. CPu is the largest compartment of

circuits, which has long been proposed as a basis for absence epilepsy\(^\text{36,37}\). Impaired firing properties in cortical excitatory neurons may impact their downstream input onto inhibitory neurons in the thalamic reticular nucleus, which in turn fails to suppress excitatory thalamocortical relay neurons. The excited thalamocortical neurons may then provide feedback inhibition through the thalamic reticular nucleus inhibitory neurons and generate thalamocortical hyper-synchronous oscillations that result in absence seizures\(^\text{37}\). P/Q-type calcium channel gene CACNA1A mutations have been described in patients with absence epilepsy\(^\text{38}\), and Cacna1a KO mice have absence epilepsies\(^\text{11}\). Recently, we reported that a selective Cacna1a gene deletion in cortical layer VI pyramidal cells, which innervate thalamic relay neurons and reticular thalamic neurons, caused upregulation of T-type calcium current in thalamic relay neurons and resulted in absence epilepsy in mice\(^\text{39}\), suggesting that impaired cortical excitatory input to thalamic regions may cause rebound burst of thalamocortical relay neurons and leads to thalamocortical hyper-synchronous oscillations as may be the case for ethosuximide-sensitive absence-like seizures in Scn2a-deficient mice. A second plausible mechanism considers a rebound hyper-excitability within neocortex. In neocortex, excitatory, and inhibitory neurons form reciprocal and highly complex networks. Impaired excitatory inputs into inhibitory neurons may even disinhibit the downstream excitatory neurons and the consequent epileptic discharges widely spread to the corticothalamic circuit. Meer en and colleagues\(^\text{40}\) actually reported that somatosensory cortex is the initiation site of epileptic activity in a rat model of absence epilepsy. Thirdly, the predominant appearance of epileptiform discharges in CPu of Scn2aKO/− mice and Scn2a\(^\text{fl/fl}\)/Emx1-Cre mice may implicate CPu in the pathology of absence-like seizures. CPu is the largest compartment of
basal ganglia that receive excitatory inputs from cortex and thalamus and send output back to the thalamus, and cortex via thalamus. Basal ganglia were suggested to modulate the occurrence of SWDs generated in the thalamocortical circuits61. The present ECoG recordings also revealed an increased incidence of SWDs in Scn2a+/−/Emx1-Cre mice, compared to Scn2a-haploinsufficient mice. We surmise that the remaining Nav1.2 expression in non-dorsal telencephalic regions such as Cfu5 of Scn2a+/−/Emx1-Cre mice may have aggravating effects on absence-like seizures. Alternatively, given Nav1.2 expression in caudal ganglionic eminence-derived vasoactive intestinal peptide-or reelin-positive inhibitory neurons of neocortex and hippocampus66, selective Nav1.2 elimination in excitatory neurons may shift an excitation/inhibition balance toward inhibition, which enhances hyperpolarization of neurons and low threshold rebound burst-firing via de-inactivation of T-type calcium currents, increasing the risk of SWD generation. Furthermore, the decreased neuronal activity caused by the Nav1.2 deficit affects network formation during development. Congenital Scn2a mutans may impair or affect maturation, migration, or innervation of inhibitory or excitatory neurons and alter the development of brain network excitability. Thus, the circuit basis for the development of absence-like seizures in mice with Scn2a-deficiency requires further study.

In summary, we showed that Scn2a-haploinsufficiency in mice gives rise to absence-like seizures in a dorsal telencephalic excitatory neuron-glia network. Our findings should contribute to understanding of the pathomechanisms of epilepsies in patients with SCN2A mutations and absence epilepsy itself, the mechanism of which is still not fully elucidated.

Methods

Animals. All animal experimental protocols were approved by the Animal Experiment Committees of RIKEN Institute and Shizuoka Institute of Epilepsy and Neurological Disorders.

Generation of knock-in Scn2a mice with the R102* mutation. We isolated the PAC clones 348A2 and 386F4 by screening a pooled mouse genomic PAC library (BACPAC Resource Center, Oakland, CA, USA) with dot blot hybridization using [α-32P]dCTP-labeled DNA corresponding to the genomic fragment containing exon 2 of the mouse 

transgenic mice in a C57BL/6J background32 to remove the neo cassette. The absence of the neo cassette in F3 Scn2aRX+/−/CAG-Flpe mice was verified by PCR. F3 Scn2aRX+/−/CAG-Flpe mice were then crossed with C57BL/6J mice to obtain Flpe-lacking F4 Scn2aRX+/−/ mice. Homozygous mice were obtained by interbreeding F4 Scn2aRX+/−/ mice. No phenotypic differences were observed among the mice derived from the two ES cell clones. Scn2aRX+/− mice were thereafter maintained on a C57BL/6J background. The C57BL/6J background for more than 10 backcross generations were subjected to ECoG-EMG recordings. Genotyping of Scn2a knock-in mice was performed by PCR with the specific primers (forward: 5′-TGT CTC AGA TTC CCT ATT GCT-3′, reverse: 5′-CTT GAT AAC TTT GGA GAG TGA GTC-3′) that detect the wild-type allele (269 bp) and the targeted knock-in allele (484 bp) (Supplementary Fig. 1c). Unprocessed original scans of blots/gels are shown in Supplementary Fig. 14.

Ella-Cre, Emx1-Cre, Vgat-Cre, and Vgat-Venus mouse lines. The Ella-Cre transgenic line, in which the Cre-loxP recombination occurs in germline cells, was previously generated by injection of the Cre cassette under the control of the adenovirus EIIa promoter into mouse zygotes42, and was maintained on a C57BL/6J background. The targeting vector harboring the floxed allele was verified by restriction enzyme digestion and DNA sequencing.

The targeting vector harboring the floxed allele was digested with SacI for linearization and transfected into E14 ES cells with a Gene-Pulser (Bio-Rad, Hercules, CA, USA) at 3 µF and 600 V. Transfected ES cells were placed on neomycin-resistant, mitomycin C-treated C57BL/6J blastocysts to produce male chimeras with greater than 50% agouti coat color. The targeting vector was digested with EcoR1 and SacI sites of pfrt-PGK/gb2-neo/frt-loxP to generate pfrt-PGK/gb2-neo/-frt-loxP−exon2. Next, a genomic fragment containing downstream of exon 2 was amplified by PCR with primers having SacI sites of pfrt-PGK/gb2-neo/kan-frtloxP to generate pfrt-PGK/gb2-neo/kan-frtloxP−exon2. The resultant genomic DNA was isolated from Xhol sites at their 5′-ends, whose nucleotide sequences were as follows: 5′-CCG AAC GCT CTT CCT TTG TGA AGG TGA GTG A-3′ and 5′-CCC AAA GGC AAC TGT TCC ACT TTA GTG AGA TGG C-3′. The resultant amplicons were digested with HindIII and inserted into the HindIII site of pft-PGK/gb2-neo/kan-frtloxP to generate pfrt-PGK/gb2-neo/kan-frtloxP−exon2. The resultant plasmid DNA was then amplified by PCR with primers 5′-TCT TCT GCA GCA TTC AAA G A3′ and 5′-CAT CAT AAA GCT GAA ACA C-3′. The resultant PCR products were used for transformation of an Escherichia coli strain, containing the targeting vector carrying the R102* allele and pSC101-BAD-gbaA (Gene Bridges). The resulting targeting vector harboring the floxed allele was verified by restriction enzyme digestion and DNA sequencing.

The targeting vector harboring the floxed allele was digested with SacI for linearization and transfected into E14 ES cells with a Gene-Pulser (Bio-Rad). The ES cells from two correctly targeted clones (282 and 31H2) were injected into C57BL/6J blastocysts to obtain male chimeras that were subsequently bred to C57BL/6J females to generate F1 mice heterozygous for the targeted allele. F1 heterozygotes were then crossed with CAG-Flpe transgenic mice on a C57BL/6J background42 to generate N2 mice heterozygous (Scn2aRX+/−/CAG-Flpe) for the floxed allele and lacking the neomycin cassette. Absence of the neo-cassette was verified by PCR analysis. Scn2aRX+/−/CAG-Flpe mice were subsequently crossed with C57BL/6J mice to obtain N3 Scn2aRX+/− mice without the CAG-Flpe transgene. The resultant Flpe mice were then crossed with C57BL/6J mice to obtain N4 Scn2aRX+/− mice, which were thereafter maintained by crossing with C57BL/6J mice. Homozygous (Scn2aRX−/−) mice were obtained by interbreeding Scn2aRX+/− mice. No phenotypic differences were observed among the mice derived from the two ES cell clones. Genotyping of Scn2a conditional KO mice was performed by PCR with the specific primers (forward: 5′-TGT CTC AGA TTC CCT ATT GCT-3′, reverse: 5′-CCG ACA GAC CAT AAA GCT GAA ACA C-3′) that detect the wild-type allele (925 bp), the targeted floxed allele (1,162 bp) and the deleted allele (284 bp) (Supplementary Figs. 2c and 3a). Unprocessed original scans of blots/gels are shown in Supplementary Fig. 14.

Ella-Cre, Emx1-Cre, Vgat-Cre, and Vgat-Venus mouse lines. The Ella-Cre transgenic line, in which the Cre-loxP recombination occurs in germline cells, was previously generated by injection of the Cre cassette under the control of the adenovirus EIIa promoter into mouse zygotes42, and was maintained on a C57BL/6J background. Ella-Cre mice were cross-mated with Scn2aRX+/− mice, and heterozygous (Scn2aRX+/−/Ella-Cre) offspring were subsequently backcrossed with C57BL/6J mice to obtain Scn2aRX+/−/Ella-Cre mice. The Ella-Cre transgene was verified by PCR analysis. Homozygous (Scn2aRX−/−/Ella-Cre) mice were obtained by interbreeding Scn2aRX−/−/ mice and Emx1-Cre mice, and subsequently backcrossed with Scn2aRX−/− mice to obtain Scn2aRX−/−/Emx1-Cre mice.

Vascular GABA transporter (Vgat, also known as vascular inhibitory amino acid transporter, Viat)-Cre mouse line. The Viat-Cre transgenic line was previously generated by pronuclear injection of the Cre cassette under the control of the mouse Vgat promoter25, and maintained on a C57BL/6J background. Heterozygous (Scn2aRX+/−/Viat-Cre) mice...
Electrocorticography-electromyography recordings. Adult mice (6–11-week-old Scn2aKO/KO and Scn2a+/+ littermate mice: 10–27-week-olds Scn2aKO/KO and Scn2a+/+ littermate mice 6–8-week-old Scn2aKO/KO/Emx1-Cre, Scn2aKO/KO and control littermates: both sex, C57BL/6j congenic background) were used in this study. The ECoG electrodes were implanted using 1.5% halothane anesthesia with a body temperature threshold in which seizure was induced was made. We gradually elevated the body temperature by 0.5 °C per 2 min. When a seizure appeared, the mouse was promptly rescued by cooling with ice cubes. Measurement of the body temperature threshold in which seizure was induced was made.

Voltage and LFP signals with 3 or more high-amplitude spikes that were over twice the level of the background signal in a 1-s window, were considered to be epileptic discharges and included in the analysis.

Quantitative RT-PCR. Brains were obtained from mice (P14.5 Scn2aKO/KO mice and Scn2a+/+ littermates, both sexes, C57BL/6j congenic background) and total RNA was isolated and purified from brain using the RNeasy Midi Kit (Qiagen, Valencia, CA, USA), and reverse transcribed using the Super Script III First Strand Synthesis System (Thermo Fisher Scientific). The resultant CDNA was then amplified using the Applied Biosystems 7300 Real-Time PCR System (Thermo Fisher Scientific) with pre-designed TaqMan assays for mouse Scn1a, 2a, 2s, 5a, 6a, and 11–16 as well as the internal standard 18 S rRNA or GAPD (Thermo Fisher Scientific). Relative gene expression was determined using the 2−ΔΔCt method. Briefly, the cycle threshold value (Ct) on each PCR amplicon curve was determined, and the ΔCt was calculated by subtracting the Ct value of the internal standard from the Ct value of sodium channel 1b. The ΔCt was then calculated by subtracting the ΔCt value for each individual from the mean ΔCt value of controls. Relative gene expression was calculated as 2−ΔΔCt and represented as percentages, relative to mean expression level of Scn2a+/+ littermate controls (100%).

In vitro electrophysiological recording. Current-clamp recordings were made from pyramidal or FS cells in visual cortex (P7–23) or hippocampus (P7–23) of brains from Scn2aKO/KO or Scn2a+/+ littermates, both sexes, C57BL/6j congenic background. Mice were deeply anesthetized with isoflurane. After decapitation, coronal slices (350 μm) from mice were cut in ice-cold dissection buffer.
buffer containing (in mM): 225 sucrose, 2.5 KCl, 1.25 NaH2PO4, 10 MgSO4, 0.5 CaCl2, 26 NaHCO3, 10 glucose, bubbled with 95% O2, 5% CO2 (pH 7.4). Slices were incubated in artificial cerebrospinal fluid (ACSF) in mM: 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 1 M MgSO4, 0.5 CaCl2, 26 NaHCO3, 10 glucose, 3 sodium pyruvate and 1 ascorbate, at least for 1 h at 35°C before recording. Recordings were made in a chamber superfused at 3 ml/min with the same ACSF maintained at 30–31°C. Whole-cell patch-clamp recordings were obtained from layer II/III pyramidal cells or FS interneurons and using electrodes (4–5 MΩ) filled with an internal solution containing (in mM): 126 K-glutamate, 8 KCl, 2 NaCl, 0.2 EGTA, 20 HEPES, 0.3 Na3GTP, 14 phosphocreatine, and 0.1 Alexa Fluor 594 hydrazide. To visualize interneurons, we crossed Scn2a mice with Vglut-Venus transgenic mice and select all types based on their high frequency firing rate. Data were filtered (2 kHz), digitized (10 kHz), stored, and analyzed using pCLAMP 10 and Origin 8.5 software. To assess membrane properties at resting potentials, pyramidal cells or FS-interneurons were injected with negative square-wave current pulses (300 ms) repeated five times at 10 ms intervals. To assess the properties of single action potential, cells were hyperpolarized to −120 mV from resting potential for 500 msec to induce maximal inactivation state for sodium channels, and then stepped square pulses every 4 μA were injected every 15 sec for triggering a single action potential. Firing frequency and adaptation were evaluated as multiples of threshold intensity for triggering a single action potential. Wide-band potential −120 to −60 mV, cells were first held at −50 mV, and then hyperpolarized at each potential for 1 s. Immediately after the end of hyperpolarization, cells were depolarized to generate action potentials by applying the current injection.

**Immunohistochemistry and immunofluorescence histochemistry.** Immunohistochemistry was carried out as previously described. Briefly, mice (P0.5, 2.5, 7.5 and 15.5 and 8-week-old C57BL mice: P0.5 Scn2a+/+; E10.5-Cre - and Scn2a−/− mice: P0.5 Scn2a+/+; and Scn2a−/− littermates: both sex, C57BL/6J and 129 mixed background) were deeply anesthetized and perfused transcardially with periodate-lysine-4% paraformaldehyde. The brains were removed, and embedded in paraffin. The brains were removed, and embedded in paraffin. Sections were subsequently incubated with the mouse anti-ankyrinG (1:250; SC-12719, Santa Cruz Biotechnology), the goat anti-rabbit antibody (1:500; II-2)23. Endogenous peroxidases were quenched by incubation in 3% H2O2 and the NovaRed substrate kit (SK-4800, Vector Laboratories). Detection of antibody −/− was accomplished using the Vectastain Elite ABC kit (PK-6100, Vector Laboratories).

For immunofluorescence histochemistry, the sections were incubated with the rabbit anti-internal-region Nav1.2 (1:500; SC-31371, G-20, Santa Cruz Biotechnology), and the rabbit anti-nav1.6 antibody (1:500; II-2)23. Endogenous peroxidases were quenched by incubation in 0.3% hydrogen peroxide in phosphate-buffered saline. The sections were further incubated with biotinylated goat polyclonal secondary antibody (1:200; BA-9500, Vector Laboratories). Detection of antibody−antigen complexes was accomplished using the Vectastain Elite ABC kit (PK-6100, Vector Laboratories) and the NovaRed substrate kit (SK-4800, Vector Laboratories).

## References

1. Sugawara, T. et al. A missense mutation of the Na+ channel all subunit gene Nav1.2 in a patient with febrile and afebrile seizures causes channel dysfunction. *Proceedings of the National Academy of Science USA*, 98, 6384–6389 (2001).
2. Heron, S. E. et al. Sodium-channel defects in benign familial neonatal-infantile seizures. *Lancet*, 360, 851–852 (2002).
3. Berkovic, S. F. et al. Benign familial neonatal-infantile seizures: characteristics of a new sodium channelopathy. *Annals of Neurology* 55, 550–557 (2004).
4. Kamiya, K. et al. A nonsense mutation of the sodium channel gene SCN2A in a patient with intractable epilepsy and mental decline. *The Journal of Neuroscience* 24, 2690–2698 (2004).
5. Ogwara, I. et al. De novo mutations of voltage-gated sodium channel alpha1 gene SCN2A in intractable epilepsies. *Neurology*, 73, 1046–1053 (2009).
6. Touma, M. et al. Whole genome sequencing identifies SCN2A mutation in monozygotic twins with Ohtahara syndrome and unique neuropathologic findings. *Epilepsia*, 54, e81–e85 (2013).
7. Nakamura, K. et al. Clinical spectrum of SCN2A mutations expanding to Ohtahara syndrome. *Neurology*, 81, 992–999 (2013).
8. Epileptic Syndrome Project; Epilepsy Phenome/Genome Project, Allen, A. S. et al. De novo mutations in epileptic encephalopathies. *Nature*, 501, 217–221 (2013).
9. Carvill, G. L. et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. *Nature Genetics*, 45, 825–830 (2013).
10. Buxbaum, J. D. et al. The autism sequencing consortium: large-scale, high-throughput sequencing in autism spectrum disorders. *Neuron*, 76, 1052–1056 (2012).
11. Tavassoli, T. et al. De novo SCN2A splice site mutation in a boy with autism spectrum disorder. *BMJ Medical Genetics*, 15, 35 (2014).
12. Rauch, A. et al. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet*, 380, 1674–1682 (2012).
13. de Ligt, J. et al. Diagnostic exome sequencing in persons with severe intellectual disability. *New England Journal of Medicine*, 367, 1921–1929 (2012).
14. Freamer, M. et al. De novo mutations in schizophrenia implicate synaptic networks. *Nature*, 506, 179–184 (2014).
15. Carroll, L. S. et al. Mutation screening of neurodevelopmental disease genes by discovery of new mutations. *Nature*, 480, 506–509 (2011).
16. Sanders, S. J. et al. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature*, 485, 237–241 (2012).
17. Jiang, Y. H. et al. Detection of clinically relevant genetic variants in autism spectrum disorder by whole-genome sequencing. *American Journal of Human Genetics*, 93, 249–263 (2013).
18. Höschen, A., Krumm, N., & Eichler, E. E. Prioritization of neurodevelopmental disease genes by discovery of new mutations. *Nature Neuroscience*, 17, 764–772 (2014).
19. Johnson, M. R. et al. Systems genetics identifies a convergent gene network for cognition and neurodevelopmental disease. *Nature Neuroscience*, 19, 223–232 (2016).
20. Li, J. et al. Genes with de novo mutations are shared by four neuropsychiatric disorders discovered from NPDnovo database. *Molecular Psychiatry*, 21, 290–297 (2016).
21. Claes, L. et al. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. American Journal of Human Genetics 68, 1332–1338 (2001).
22. Yamakawa, K. in Synaptic dysfunction in autism spectrum disorder and intellectual disability (eds Sara, C. & Verpelli, C.) (Elsevier, Amsterdam, Netherlands, 2016).
23. Ogawa, I. et al. Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an ScaN1a gene mutation. Journal of Neuroscience 27, 5993–5914 (2007).
24. Lorincz, A., & Nusser, Z. Cell-type-dependent molecular composition of the axon initial segment. Journal of Neuroscience 28, 14329–14340 (2008).
25. Ogiwara, I. et al. Nav1.1 haploinsufficiency in excitatory neurons ameliorates seizure-associated sudden death in a mouse model of Dravet syndrome. Human Molecular Genetics 22, 4784–4803 (2013).
26. Hu, W. et al. Distinct contributions of Nav1.6 and Nav1.2 in action potential initiation and backpropagation. Nature Neuroscience 12, 996–1002 (2009).
27. Liao, Y. et al. Molecular correlates of age-dependent seizures in an inherited neonatal-infantile epilepsy. Brain 133, 1403–1414 (2010).
28. Tian, C., Wang, K., Ke, W.-C., Guo, H., & Shi, Y. Molecular identity of axonal sodium channels in human cortical pyramidal cells. Frontiers in Cellular Neuroscience 8, 297 (2014).
29. Yamagata, T., Ogawa, I., Mazaki, E., Yanagawa, Y., & Yamakawa, K. Nav1.2 is expressed in caudal ganglionic eminence-derived disinhibitory interneuronal mutants: mutually exclusive distributions of Nav1.1 and Nav1.2. Biochemical and Biophysical Research Communications 491, 1070–1076 (2017).
30. Westenbroek, R. E., Merrick, D. K. & Catterall, W. A. Differential subcellular localization of the RI and RII Na+ channel subtypes in central neurons. Neuron 3, 695–704 (1989).
31. Gong, B., Rhodes, K. J., Bekele-Arcuri, Z., & Trimmer, J. S. Type I and type II N-type channel alpha-subunits polyadenylate exhibit distinct spatial and temporal patterning, and association with auxiliary subunits in rat brain. The Journal of Comparative Neurology 412, 342–352 (1999).
32. Miyazaki, H. et al. Singular localization of sodium channel beta4 subunit in unmyelinated fibers and its role in the striatum. Communications Biology 5, 5225 (2014).
33. Li, T. et al. Action potential initiation in neocortical inhibitory interneurons. PLoS Biology 12, e1001944 (2014).
34. Sugawara, T. et al. Nav1.1 channels with mutations of severe myoclonic epilepsy in infancy display attenuated currents. Epilepsy Research 54, 201–207 (2003).
35. Ben-Shalom, R. et al. Opposing effects on Nav1.2 function underlie differences between SCN2A variants observed in individuals with autism spectrum disorder or infantile seizures. Biological Psychiatry 82, 224–232 (2017).
36. Wolfl, M. et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. Brain 140, 1316–1337 (2017).
37. Kearney, J. A. et al. A gain-of-function mutation in the sodium channel gene SCN8A results in seizures and behavioral abnormalities. Neuroscience 102, 307–317 (2001).
38. Planells-Cases, R. et al. Neuronal death and perinatal lethality in voltage-gated sodium channel alpha-II-deficient mice. Biophysical Journal 78, 2878–2891 (2000).
39. Bertaso, F. et al. PICK1 uncoupling from mGluR7a causes absence-like seizures. Nature Neuroscience 11, 940–948 (2008).
40. Papale, L. A. et al. Heterozygous mutations of the voltage-gated sodium channel SCN8A are associated with spike-wave discharges and absence epilepsy in mice. Human Molecular Genetics 18, 1633–1641 (2009).
41. Maheshwari, A., & Noebels, J. L. Monogenic models of absence epilepsy: windows into the complex balance between inhibition and excitation in thalamocortical microcircuits. Progress in Brain Research 213, 223–252 (2014).
42. Lakso, M. et al. Efficient in vivo manipulation of mouse genomic sequences at the zygote stage. Proceedings of the National Academy of Sciences USA 93, 5860–5863 (1996).
43. Iwata, T. et al. Cortical adenylyl cyclase 1 is required for thalamocortical synapse maturation and aspects of layer IV barrel development. The Journal of Neuroscience 28, 5931–5943 (2008).
44. Wang, Y. et al. Fluorescent labeling of both GABAergic and glycinergic neurons in vesicular GABA transporter (VGAT)-venus transgenic mouse. Neuroscience 164, 1031–1043 (2009).
45. Kole, M. H. et al. Action potential generation requires a high sodium channel density in the axon initial segment. Nature Neuroscience 11, 178–186 (2008).
46. Thomas, E. A. et al. Heat opens axon initial segment sodium channels: a febrile seizure mechanism? Annals of Neurology 66, 219–226 (2009).
47. Ye, M. et al. Differential roles of NaV1.2 and NaV1.6 in regulating neuronal excitability at febrile temperature and distinct contributions to febrile seizures. Scientific Reports 8, 753 (2018).
48. Escayg, A. et al. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFs−. Nature Genetics 24, 343–345 (2000).
49. Fujitani, T. et al. Voltage-gated sodium channel alpha subunit type 1 (SCN1A) in intractable childhood seizures with frequent generalized tonic-clonic seizures. Brain 126, 531–546 (2003).
50. Oakley, J. C., Kalume, F., Yu, F. H., Scheuer, T., & Catterall, W. A. Temperature- and age-dependent seizures in a mouse model of severe myoclonic epilepsy in infancy. Proceedings of the National Academy of Sciences USA 106, 3994–3999 (2009).
51. Cao, D. et al. Efficacy of stiripentol in hyperthermia-induced seizures in a mouse model of Dravet syndrome. Epilepsia 53, 1140–1145 (2012).
52. Masmho, T. et al. A missense mutation of the gene encoding voltage-dependent sodium channel (Nav1.1) confers susceptibility to febrile seizures in rats. The Journal of Neuroscience 30, 5744–5753 (2010).
53. Boiko, T. et al. Compact myelin dictates the differential targeting of two sodium channel isoforms in the same axon. Neuron 30, 91–104 (2001).
54. Martinez-Hernandez, J. et al. Polarisated localisation of the voltage-gated sodium channel Nav1.2 in cerebellar granule cells. Cerebellum 12, 16–26 (2013).
55. Madeja, M. Do neurons have a reserve of sodium channels for the generation of action potentials? A study on acutely isolated CA1 neurons from the guinea-pig hippocampus. European Journal of Neuroscience 12, 1–7 (2000).
56. Huguenard, J. R., & McCormick, D. A. Thalamic synchrony and dynamic regulation of global forebrain oscillations. Trends in Neuroscience 30, 350–356 (2007).
57. Halpern, T. et al. A new mode of corticalothalamic transmission revealed in the Gria4− model of absence epilepsy. Nature Neuroscience 14, 1167–1173 (2011).
58. Noebels, J. L. In Jasper’s basic mechanisms of the epilepsies, 4th edition (eds. Noebels, J. L., Avoli, M., Rogawski, M. A., Olsen, R. W. & Delgado-Escueta, A. V.) (Oxford University Press, USA, 2012).
59. Bomben, V. C. et al. Isolated P/Q calcium channel deletion in layer VI corticothalamic neurons generates absence epilepsy. The Journal of Neuroscience 36, 405–418 (2016).
60. Meeran, H. K., Pijn, J. P., van Luijtelaar, L. E., Goen, A. M., & Lopes da Silva, F. H. Cortical focus drives widespread corticalothalamic networks during spontaneous absence seizures in rats. The Journal of Neuroscience 22, 4191–4195 (2002).
61. Paz, J. T., Chavez, M., Saelit, S., Deniu, J. M., & Charpier, S. Activity of ventral medial thalamic neurons during absence seizures and modulation of cortical paroxysms by the nigothalamic pathway. The Journal of Neuroscience 27, 929–941 (2007).
62. Kanki, H., Suzuki, H., & Itohara, S. High-efficiency CAG-FLPe deleter mice in C57BL/6J background. Experimental Animals 55, 137–146 (2006).
63. Miyamoto, H. et al. Potentiation of excitatory synaptic transmission ameliorates aggression in mice with Stxlp1 haploinsufficiency. Human Molecular Genetics 26, 4961–4974 (2017).
64. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25, 402–408 (2001).
65. Bullone, A. et al. T-brain-1: a homolog of Brachyury whose expression defines molecularly distinct domains within the cerebral cortex. Neuron 15, 63–78 (1995).
66. Hevner, R. F. et al. Tbr1 regulates differentiation of the preplate and layer 6. Neuron 29, 353–366 (2001).
67. Shibata, S. et al. Immuno-electron microscopy and electron microscopic in situ hybridization for visualizing piRNA biogenesis bodies in Drosophila ovaries. Methods in Molecular Biology 1328, 163–178 (2015).
T.Y., T.N., E.Miura, E.Mazaki, S.J.E., D.C., H.O., M.Y., Y.L., and J.L.N. performed genetic, histological, and biochemical analyses. I.O., H.M., T.T., T.Y., T.N., M.M., T.K.H., J.L.N., and K.Y. wrote the paper.

Additional information
Supplementary information accompanies this paper at https://doi.org/10.1038/s42003-018-0099-2.

Competing interests: The authors declare no competing interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ikuko Ogiwara1,2, Hiroyuki Miyamoto1,3,4, Tetsuya Tatsukawa1, Tetsushi Yamagata1, Tojo Nakayama1,5,18, Nafiseh Atapour1,3,19, Eriko Miura6, Emi Mazaki1, Sara J. Ernst7,8, Dezhi Cao9,20, Hideyuki Ohtani9, Shigeyoshi Itohara10,11, Yuchio Yanagawa12,13, Mauricio Montal14, Michisuke Yuzaki6, Yushi Inoue9, Takao K. Hensch3,15,16, Jeffrey L. Noebels7,8,17 & Kazuhiro Yamakawa1

1Laboratory for Neurogenetics, RIKEN Center for Brain Science, Wako, Saitama 351-0198, Japan. 2Department of Physiology, Nippon Medical School, Tokyo 113-8602, Japan. 3Laboratory for Neuronal Circuit Development, RIKEN Center for Brain Science, Wako, Saitama 351-0198, Japan. 4PRESTO, Japan Science and Technology Agency, Saitama 332-0012, Japan. 5Department of Pediatrics, Tohoku University School of Medicine, Sendai 980-8574, Japan. 6Department of Physiology, School of Medicine, Keio University, Tokyo 160-8582, Japan. 7Department of Neurology, Baylor College of Medicine, Houston, TX 77030, USA. 8Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA. 9National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka 420-8688, Japan. 10Laboratory for Behavioral Genetics, RIKEN Center for Brain Science, Wako, Saitama 351-0198, Japan. 11FIRST, Japan Science and Technology Agency, Saitama 332-0012, Japan. 12Department of Genetic and Behavioral Neuroscience, Gunma University Graduate School of Medicine, Maebashi 371-8511, Japan. 13CREST, Japan Science and Technology Agency, Saitama 332-0012, Japan. 14Section of Neurobiology, Division of Biological Sciences, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92039, USA. 15Department of Molecular and Cellular Biology and Center for Brain Science, Harvard University, Cambridge, MA 02138, USA. 16Department of Neurology, FM Kirby Neurobiology Center, Boston Children’s Hospital, Harvard Medical School, Boston, MA 02115, USA. 17Department of Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA. 18Present address: Division of Genetics and Genomics, Boston Children’s Hospital, Harvard Medical School, Boston, MA 02115, USA. 19Present address: Department of Genomic Medicine, Royal Melbourne Hospital, Melbourne, Victoria, Australia. 20Present address: Neurology Department, Shenzhen Children’s Hospital, 518026 Guangdong, China. These authors contributed equally: Ikuko Ogiwara, Hiroyuki Miyamoto, Tetsuya Tatsukawa, Tetsushi Yamagata.