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Scn1a missense mutation impairs $GABA_A$ receptor-mediated synaptic transmission in the rat hippocampus

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ABSTRACT

Mutations of the $Na_v1.1$ channel subunit SCN1A have been implicated in the pathogenesis of human febrile seizures (FS). We have recently developed hyperthermia-induced seizure-susceptible (Hiss) rat, a novel rat model of FS, which carries a missense mutation (N1417H) in *Scn1a* [1]. Here, we conducted electrophysiological studies to clarify the influences of the *Scn1a* mutation on the hippocampal synaptic transmission, specifically focusing on the GABAergic system. Hippocampal slices were prepared from Hiss or F344 (control) rats and maintained in artificial cerebrospinal fluid saturated with 95% O_2 and 5% CO_2 *in vitro*. Single neuron activity was recorded from CA1 pyramidal neurons and their responses to the test (unconditioned) or paired pulse (PP) stimulation of the Schaffer collateral/commissural fibers were evaluated. Hiss rats were first tested for pentylenetetrazole-induced seizures and confirmed to show high seizure susceptibility to the blockade of $GAGA_A$ receptors. The *Scn1a* mutation in Hiss rats did not directly affect spike generation (i.e., number of evoked spikes and firing threshold) of the CA1 pyramidal neurons elicited by the Schaffer collateral/commissural stimulation. However, $GABA_A$ receptor-mediated inhibition of pyramidal neurons by the PP stimulation was significantly disrupted in Hiss rats, yielding a significant increase in the number of PP-induced firings at PP intervals of 32–256 ms. The present study shows that the *Scn1a* missense mutation preferentially impairs $GABA_A$ receptor-mediated synaptic transmission without directly altering the excitability of the pyramidal neurons in the hippocampus, which may be linked to the pathogenesis of FS.

1. Introduction

Human febrile seizures (FS) are the most common type of seizures in childhood. Most FS are generally benign, but, about one-third of patients exhibit recurrent seizures and have a potential risk for secondary epilepsy (e.g., temporal lobe epilepsy) [2–5]. Although the pathophysiological mechanisms underlying FS are not fully elucidated, $Na_v1.1$ channels have been implicated in the etiology of FS [6–8]. Specifically, more than 200 mutations of the $Na_v1.1$ channel α subunit SCN1A have been reported in patients of generalized epilepsy with febrile seizures plus (GEFS+) and severe myoclonic epilepsy in infancy (SMEI) [6–8]. Furthermore, recent studies have shown that the truncated mutations of $Na_v1.1$ channels caused hypersusceptibility to hyperthermia-induced seizures in mice, which accompanied a marked reduction in sodium currents in the GABAergic neurons [9–11]. Nonetheless, due to the diversity of *Scn1a* mutations and/or the complexity in functional changes of $Na_v1.1$ channels in FS patients [6,8], the precise mechanisms underlying the pathogenesis of FS remain to be clarified.

We have recently developed a novel rat model which carries a missense mutation (N1417H) in the third pore-forming region of *Scn1a* using *N*-ethyl-*N*-nitrosourea mutagenesis [1]. Since these animals at a very young age (~5 week old) exhibited markedly high susceptibility to hyperthermia-induced seizures, we designated them hyperthermia-induced seizure-susceptible (Hiss) rats. Besides the vulnerability to hyperthermic seizures, Hiss rats were also very sensitive to seizures induced by pentylenetetrazole (PTZ, a blocker of $GABA_A$ receptors), suggesting that the *Scn1a* missense mutation impairs the GABAergic functions [1]. In addition, electrophysiological analyses using mutated $Na_v1.1$ channels or dissociated hippocampal neurons revealed that the N1417H missense mutation causes multiple changes in $Na_v1.1$ channel properties in GABA neurons, including a hyperpolarized shift in the voltage-dependency of $Na_v1.1$ inactivation, an increase in persistent leak currents and a reduced amplitude of evoked spikes [1]. However, influences of the *Scn1a* mutation on the GABAergic

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synaptic transmission and/or the hippocampal neural network remain to be clarified.

In the present study, therefore, we conducted electrophysiological studies in Hiss rats to clarify the effects of the *Scn1a* missense mutation on the hippocampal synaptic transmission, specifically focusing on the GABAergic inhibitory control of the CA1 pyramidal neurons. Our results show that the *Scn1a* missense mutation impairs $GABA_A$ receptor-mediated synaptic transmission in the hippocampus, supporting the notion that the *Scn1a* missense mutations play an important role in the pathogenesis of FS by disrupting the inhibitory GABAergic neurotransmissions.

2. Materials and methods

2.1. Animals

Hiss rats (F344-*Scn1a*$^{Kyo811/Kyo811}$) were obtained from the National BioResource Project for the Rat (NBRPR#0455) in Japan. As reported previously [1], Hiss rats carry homozygous *Scn1a*-$^{Kyo81/Kyo8111}$ alleles, which have the missense mutation N1417H located in the third pore-forming region of the *Scn1a* gene (Fig. 1A). Hiss rats were backcrossed more than 5 generations against F344/NSlc inbred background to eliminate mutations in chromosomal regions other than the *Scn1a* locus, and F344/NSlc (F344) rats were used as the control. The animals were kept in air-conditioned rooms under a 12-h light/dark cycle and allowed *ad libitum* access to food and water. The housing conditions and the animal care methods complied with NIH guide for the care and use of laboratory animals. The experimental protocols of this study were approved by the Experimental Animal Research Committee at Osaka University of Pharmaceutical Sciences.

2.2. Induction of PTZ-induced seizures

Male Hiss or F344 rats (8 weeks old) were used. Animals were cumulatively injected with an increasing dose of PTZ at 10, 20 or 30 mg/kg (i.p.) with a 30-min intervals. Immediately after each dosing of PTZ, animals were placed in an observation cage ($28 \times 45 \times 20$ cm) and the incidence of excitation behaviors and seizures were continuously monitored for 10 min using the following scores, (0) no change, (1) recurrent head twitches or wet dog shakes, (2) myoclonic jerk of forepaws and/or upper trunk, (3) recurrent and marked myoclonic jerk of forepaws and/or upper trunk, (4) clonic seizures, (5) marked clonic seizures with falling dawn.

2.3. Electrophysiology using hippocampal slices

Male Hiss or F344 rats (6–8 weeks old) were used. Experiments were carried out as reported previously with slight modifications [12–14]. After decapitation, the brain was immediately removed from the skull and chilled in ice-cold artificial cerebrospinal fluid (ACF) saturated with a gas mixture of 95% O_2 and 5% CO_2. A tissue block containing the dorsal hippocampus (-3.24 to -3.96 mm posterior to the bregma) [15] was then dissected out and cut into slices at a thickness of 400 μm using a Microslicer (Dosaka EM, DSK-3000, Kyoto, Japan) (Fig. 2A). The hippocampal slice was completely submerged in the recording chamber which was continuously perfused with ACF at a flow rate of about 1.5 ml/min. ACF contained (in millimolar): NaCl 116.4, KCl 5.4, $MgSO_4$ 1.3, NaH_2PO_4 0.92, $CaCl_2$ 2.5, $NaHCO_3$ 26.2, glucose 11.0) and was continuously bubbled with 95% O_2 and 5% CO_2. The temperature of the ACF was maintained at 29–30 °C.

Single neuron activity was extracellularly recorded from the CA1 pyramidal neurons using a glass microelectrode which was filled with 3 M NaCl and had an electrical resistance of 4–8 MΩ. For the stimulation of the Schaffer collateral/commissural fibers, a bipolar stimulating electrode was inserted into the stratum radiatum in the CA1 field (Fig. 2). Stimuli (0.1 ms duration) with various intensities were applied every 10 s to measure the threshold for spike generation of CA1 pyramidal neurons. To measure the PP-induced responses, the intensity of the stimulation was adjusted to about 120% of the threshold level and the PP stimulation was applied with various PP intervals ranging from 16 to 512 ms. The recorded signals were amplified (Microelectrode Amplifier MEZ-8301, Nihon Kohden, Tokyo, Japan), monitored and stored in a computer (PowerLab ML4/36, AD Instruments, Bella Vista, Australia). Ten to 20 successive responses of neurons to the Test (unconditioned single pulse) or PP stimulation were usually recorded to calculate the mean spike number and latency in each treatment. In the experiments using the receptor antagonists, either bicuculline (a selective $GABA_A$ antagonist, 10 μM), saclofen (a selective $GABA_B$ antagonist, 100 μM) or 2-amino-5-phosphonovaleric acid (APV: a selective NMDA antagonist, 30 μM) was dissolved in ACF and added to the perfusion medium. This concentration of each antagonist was reportedly sufficient to block the respective receptor *in vitro* [14,16]. The spike generation of pyramidal neurons by the Test or PP stimulation were recorded immediately before and 10 min after the drug application.

2.4. GAD67-immunofluorescence histochemistry

Experiments were carried out as reported previously with slight modifications [17]. Briefly, sections (4 μm) of 4% formaldehyde-fixed, paraffin-embedded brains from Hiss or F344 rats (6–8 weeks old) were deparaffinized, rehydrated and autoclaved for 10 min in 10 mM citrate buffer (pH 6.0). Endogenous peroxidase was quenched by incubation with 3% hydrogen peroxidase in PBS. After incubating the slides with blocking solution (10% normal rabbit serum) for 30 min at room temperature, sections were incubated with a mouse anti-GAD67 antibody (Santa Cruz Biotech., CA) in a humidified chamber for 12 h at 4 °C. The sections were then incubated with a TRITC conjugated rabbit anti-mouse IgG secondary antibody (Sigma–Aldrich, St. Louis, MO). Immunofluorescence images was taken with a confocal laser scanning microscope (LSM510 Ver.3.2, Carl Zeiss Japan, Tokyo, Japan) and processed with instrumental image software.

2.5. Drugs

The drugs used in this study were as follows: PTZ hydrochloride (Sigma–Aldrich), bicuculline hydrochloride (Sigma–Aldrich), saclofen (Sigma–Aldrich) and D,L-APV (Tocris, Bristol, UK). All other reagents were obtained from commercial sources. PTZ hydrochloride was dissolved in saline and intraperitoneally injected to the animals at a volume of 5 ml/kg.

2.6. Statistical analysis

Data are expressed as the mean ± SEM. Statistical significance of differences in the behavioral scores (two groups comparison) was performed using Mann–Whitney's *U*-test. Comparison of differences in the stimulus threshold and the number of spikes between two groups (Hiss and F344 rats) was determined by the Student's *t*-test, while the comparison among multiple groups was determined by one-way ANOVA followed by Tukey's *post hoc* multiple comparison test.

3. Results

3.1. Susceptibility to PTZ-induced seizures

To assess the seizure susceptibility to the $GABA_A$ receptor/Cl^- channel blocker PTZ, we treated the animals with an increasing dose of PTZ (10, 20 and 30 mg/kg, i.p.) in a cumulative fashion.

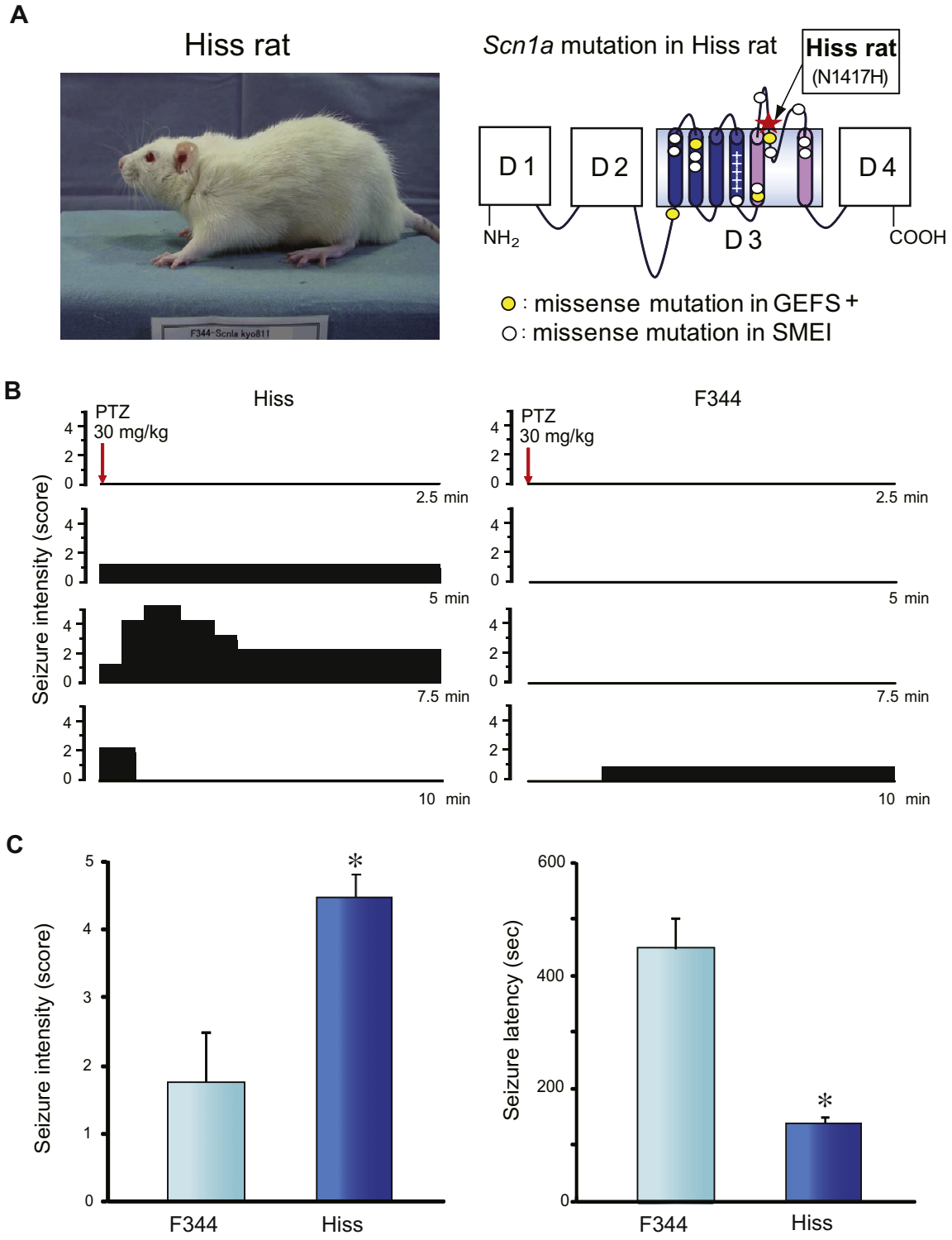

Fig. 1. Susceptibility of Hiss rats to pentylenetetrazole (PTZ)-induced seizures. (A) A photograph of a Hiss rat and a schematic drawing illustrating the location of the Scn1a missense (N1417H) mutation in Hiss rats. Scn1a consists of 4 homologous domains (D1–D4), each containing 6 transmembrane regions. The N1417H mutation is located in the pore-forming region of the third domain (D3). As a reference, missense mutation sites in D3 reported in GEFS + and SMEI are also shown (Meisler and Kearney, 2005). (B) A typical time-course of the PTZ-induced excitatory behaviors and seizures. Hiss or F344 rats were treated with an increasing dose of PTZ (10, 20 and 30 mg/kg, i.p.) in a cumulative fashion. At 30 mg/kg (i.p.), PTZ caused abnormal excitation and clonic seizures in Hiss rats while it usually induced only wet dog shakes (score 1) in F344 rats. (C) Comparison of the seizure intensity (seizure score) and the seizure latency (the time for seizure score 1) between Hiss and F344 rats. Each column represents the mean ± SEM of 5 rats. * $P < 0.05$; Significantly different from the control (F344) rats.

Neither Hiss nor F344 rats showed any abnormal behaviors following the treatments with 10 and 20 mg/kg PTZ. In the subsequent treatment with 30 mg/kg (i.p.), however, PTZ caused abnormal excitatory behaviors (e.g., head twitches and/or wet dog shakes) leading to marked myoclonic jerks and generalized clonic seizures in all Hiss examined ($N = 5$) (Fig. 1B). In contrast, PTZ-induced behaviors in F344 rats were very mild, in that PTZ (30 mg/kg, i.p.) induced only head twitches and/or wet dog shakes (score = 1)

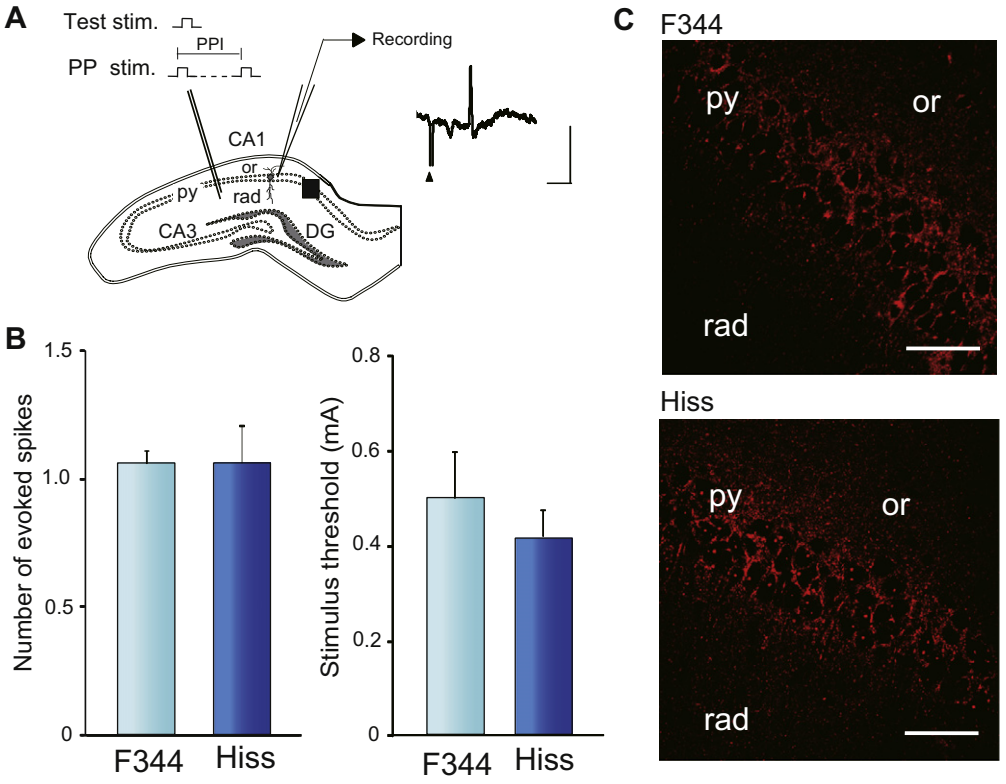

Fig. 2. Spike generation of hippocampal CA1 pyramidal neurons induced by stimulation of the Schaffer collateral/commissural fibers. (A) Schematic illustrations of the hippocampal slices and the recording of the spike generation in the CA1 field. A typical spike trace is also shown in the right panel. PPI: Paired pulse (PP) interval. or: stratum oriens, py: pyramidal layer, st: stratum radiatum. Calibration: 1 mV and 5 ms. (B) Comparison of the stimulus threshold and the spike number between Hiss and F344 rats. Each column represents the mean ± SEM of 9 or 13 neurons recorded in separate experiments. (C) Representative photograph illustrating GAD67-immunostaining of the CA1 field. The position of the photograph is shown as a closed square in schema A. GAD67-immunoreactivity (Red) is located around the cell body of the hippocampal CA1 pyramidal neurons similarly in Hiss and F344 rats. Calibration: 100 μm. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

without causing any convulsive seizures in F344 rats, except for one which exhibited a transient (duration: <5 s) clonic seizure (score = 4) ($N = 5$). The seizure intensity (behavioral score) and latency (the time until score 1) in Hiss rats were significantly higher and shorter, respectively, than in F344 rats (Fig. 1C).

3.2. Electrophysiological responses of hippocampal pyramidal neurons

It is known that the hippocampal CA1 pyramidal neurons receive both $GABA_A$ receptor-inhibitory and NMDA receptor-mediated excitatory regulations upon PP stimulation [18,19]. We therefore evaluated the changes in hippocampal synaptic transmission in Hiss rats using the PP paradigm. Action potentials were recorded from CA1 pyramidal neurons and the test or PP stimulation was applied to the Schaffer collateral/commissural fibers in the stratum radiatum (Fig. 2A).

We first compared the threshold for spike generation in CA1 pyramidal neurons between Hiss and F344 rats. Stimulation of the stratum radiatum consistently elicited spike generation in CA1 pyramidal neurons with a mean spike latency of 8.64 ± 0.81 ($N = 9$) and 7.31 ± 0.81 ($N = 13$) msec in Hiss and F344 rats, respectively. The number of spikes evoked by the stimulation was nearly unit both in Hiss and F344 rats (Fig. 2B). In addition, the stimulus threshold for the spike generation was also equal between Hiss and F344 rats, suggesting that the synaptic neurotransmission from the Schaffer collateral/commissural fibers or the excitability of CA1 pyramidal neurons per se remained unaltered in Hiss rats (Fig. 2B).

After assessment of the stimulation threshold for spike generation, Test (unconditioned single pulse) or PP stimulation was delivered to the stratum radiatum at various PP intervals ranging from 16 to 512 ms in each neuron. Under these conditions, the PP stimulation (the 2nd stimulation) induced a slight facilitation of spike generation in F344 rats (Figs. 3 and 4), which was completely abolished in the presence of the NMDA antagonist APV (30 μM) (Fig. 4). However, the PP-induced facilitation of the spike generation was markedly augmented in Hiss rats (Figs. 3 and 4). The number of PP-elicited spikes was significantly higher in Hiss rats than in F344 rats at the PP intervals between 32 and 256 ms. In addition, bath application of the $GABA_A$ antagonist bicuculline (10 μM) also increased the PP-elicited spike generation to an extent similar to that in Hiss rats, whereas the $GABA_B$ antagonist saclofen (100 μM) showed no effects (Fig. 4).

Using GAD67-immunofluorescence staining, we also confirmed the distribution of GABAergic nerve terminals in the CA1 field. As shown in Fig. 2C, the GABAergic nerve terminals were mainly located around the cell bodies (somata) of the CA1 pyramidal neurons, and no apparent differences were found in the intensity of GAD67-immunoreactivity between Hiss and F344 rats.

4. Discussion

Previous studies have shown that homozygous *Scn1a* (−/−) knockout mice developed motor ataxia and die very early after birth (age of 2–3 weeks) [9]. Heterozygous *Scn1a* (+/−) mice also showed sporadic death after the age of 3 weeks concomitantly with spontaneous seizure induction [10]. In addition, knock-in mice with insertion of a loss-of-function nonsense mutation of SMEI into the *Scn1a* gene also developed an ataxic gait, spontaneous seizures and premature death [20]. All these animals exhibited a marked reduction in sodium currents in inhibitory GABAergic

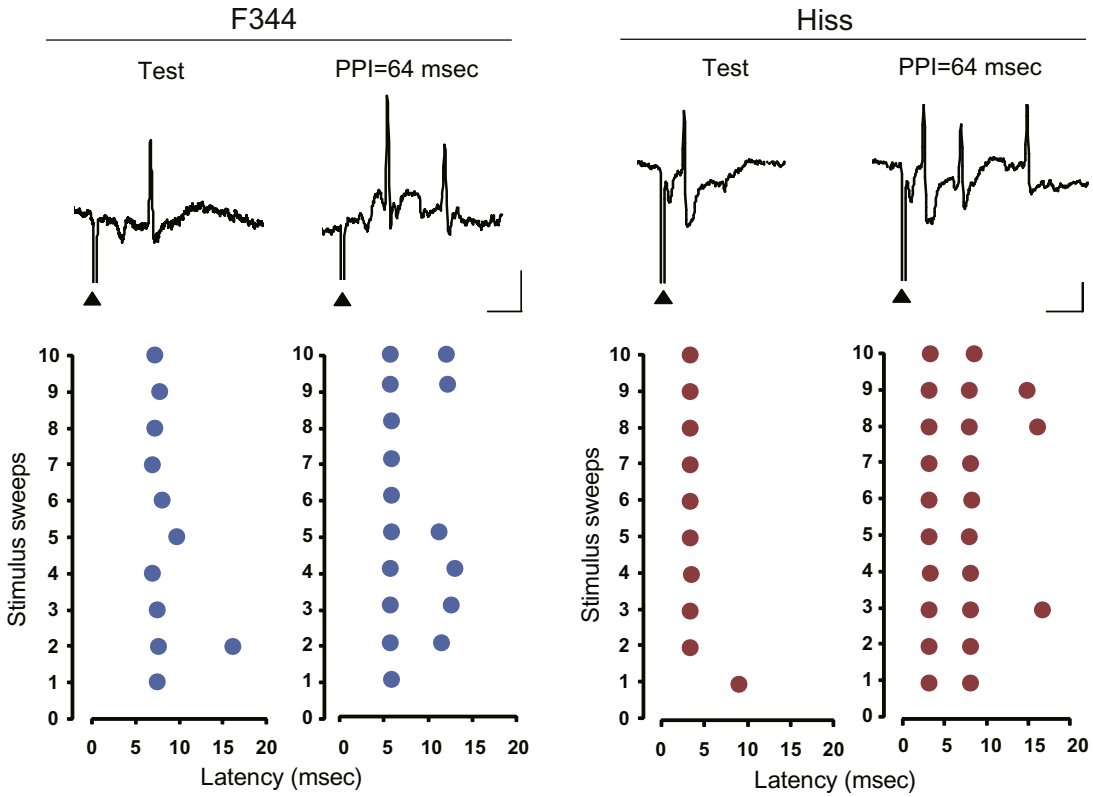

Fig. 3. Typical responses of hipocampal CA1 pyramidal neurons induced by the paired pulse (PP) stimulation. Test (unconditioned single pulse) or PP stimulation at a PP interval of 64 ms (PPI = 64 ms) was applied to the stratum radiatum, and the stimulus-evoked spike generation was compared between Hiss and F344 rats. The graph shows the poststimulus-latency plots of the evoked spikes, where each spike evoked by 10 successive stimulations was plotted according to its latency. Actual traces of the evoked spikes are shown on the top. Calibration: 1 mV, 5 ms.

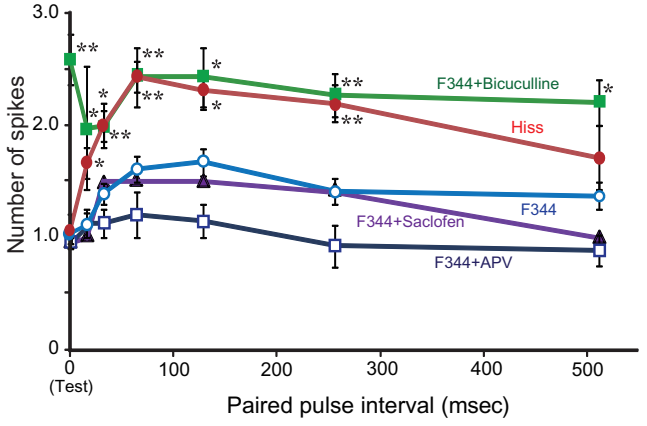

Fig. 4. Paired pulse (PP)-induced responses of hippocampal CA1 pyramidal neurons at various PP intervals in Hiss (closed circle) and F344 (open circle) rats. In the experiments using the receptor antagonists, either bicuculline (10 μM, closed square), saclofen (100 μM, closed triangle) or APV (30 μM, open square) was added to the perfusion medium, and the spike generation by the test (single pulse) or PP stimulation were recorded immediately before and 10 min after the drug application. Each column represents the mean ± SEM of 4–18 neurons recorded in separate experiments, except for saclofen ($N = 2$). $^{*}P < 0.05$, $^{**}P < 0.01$; Significantly different from F344 rats without treatment (F344).

interneurons, suggesting that the truncated mutations of *Scn1a* inhibit GABAergic activity to confer the seizure vulnerability. Nonetheless, it is known that the missense mutations of *Scn1a* gene reported in FS patients produce a variety changes in the channel functions such as gain-of-function changes (e.g., increased persistent leak current, depolarized shift in voltage-dependence of inactivation, hyperpolarized shift in voltage-dependence of activation), loss-of-function changes (e.g., hyperpolarized shift in voltage-dependence of inactivation and depolarized shift in voltage-dependence of activation) and mixed patterns of the above changes [6,8,21–25]. Due to the diversity of these functional alterations, influences of the *Scn1a* missense mutations on the GABAergic neural network or synaptic transmission remain to be clarified.

In the present study, we first confirmed that Hiss rats at an adult age continue to exhibit high susceptibility to PTZ-induced seizures. Electrophysiological evaluations using hippocampal slices from Hiss rats revealed that responses of the CA1 pyramidal neurons to the stimulation of the stratum radiatum were normal, suggesting that either the synaptic transmission from the Schaffer collateral/commissural fibers or the excitability of pyramidal neurons per se remained unaltered with the N1417H mutation. In contrast, the PP-induced facilitation of the firing of pyramidal neurons was markedly augmented in Hiss rats. In addition, a similar augmentation of the firing was also obtained in the presence of bicuculline (a selective $GABA_A$ receptor antagonist), but not saclofen (a selective $GABA_B$ receptor antagonist). Our results are consistent with previous findings [18] that the paired pulse facilitation of the field EPSP amplitude in the CA1 field was markedly enhanced by the targeted disruption of $GABA_A$ receptors, indicating that the CA1 pyramidal neurons receive $GABA_A$ receptor-mediated inhibition control with PP stimulation. It is therefore conceivable that the N1417H mutation in Hiss rats impairs $GABA_A$ receptor-mediated inhibitory synaptic transmission without significantly altering the excitability of the pyramidal neurons in the hippocampus. In addition, since GABAergic nerve terminals labeled by GAD67-immunostaining in the CA1 field were similarly observed both in Hiss and F344 rats, the disrupted GABAergic transmission in Hiss rats may not result from altered innervation density of GABAergic terminals, but possibly reflect their reduced activities. The prefer-

ential impairment of GABAergic functions rather than that of pyramidal neurons was consistent with our previous findings that the changes in channel properties with the N1417H mutation were observed only in hippocampal bipolar interneurons, but not in pyramidal neurons [1], which was probably due to specific expression of $Na_v1.1$ channels in GABAergic neurons [9,20].

The present study demonstrated for the first time that the *Scn1a* missense mutation impairs $GABA_A$ receptor-mediated synaptic transmission in the hippocampus. The disruption of GABAergic neurotransmission in Hiss rats can account for their vulnerability to PTZ-induced seizures [1]. More importantly, since hyperthermia per se is known to suppress the GABAergic neurotransmissions in the hippocampus [26–30], the impairment of GABAergic functions by the *Scn1a* mutation seems to be directly linked to the vulnerability to hyperthermic seizures.

The N1417H mutation in Hiss rats caused loss-of-function changes in the $Na_v1.1$ channel properties, a hyperpolarized shift in the voltage-dependency of the channel inactivation and a reduced amplitude of the evoked spikes in GABAergic neurons [1]. However, since the N1417H mutation did not cause a significant reduction in the $Na_v1.1$ sodium currents, these changes in $Na_v1.1$ channel properties were considered to be milder as compared to those reported for the truncated mutations [9,20]. Even though, Hiss rats were distinct from the control animals, exhibiting a significant inhibition in GABAergic neurotransmission as well as a high susceptibility to FS or PTZ seizures. Our results support the notion [8] that even mild loss-of-function mutations in $Na_v1.1$ channels can cause a significant vulnerability to FS by disrupting the GABAergic neurotransmissions. This may be due to the following possibilities. (1) N1417H mutation inhibited the GABAergic activity by reducing axonal excitability (i.e., propagation of action potentials), since recent analysis revealed that *Scn1a* are expressed not only in the somata of GABAergic neurons, but also in their proximal and distal axons [20]. (2) *In vivo* influences of *Scn1a* mutations may turn out to be more intense by the multiple and convergent projections of GABAergic neurons onto the pyramidal neurons, leading to synchronized or magnified influences of GABAergic impairment. Finally, as described previously, (3) hyperthermic stimuli per se inhibit hippocampal GABAergic neurotransmission [26–30].

In conclusion, the present study demonstrated that the N1417H missense mutation in Hiss rats preferentially impairs $GABA_A$ receptor-mediated inhibitory synaptic transmission without significantly altering the excitability of the pyramidal neurons in the hippocampus. Since hyperthermia per se is known to inhibit GABAergic neurotransmission [26–30], the present results strongly suggest that the missense mutations of *Scn1a* gene play a crucial role in the pathogenesis of human FS by disrupting the hippocampal GABAergic neurotransmission.

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References

[1] T. Mashimo, I. Ohmori, M. Ouchida, Y. Ohno, T. Tsurumi, T. Miki, M. Wakamori, S. Ishihara, T. Yoshida, A. Takizawa, M. Kato, M. Hirabayashi, M. Sasa, Y. Mori, T. Serikawa, A missense mutation of the gene encoding voltage-dependent sodium channel ($Na_v1.1$) confers susceptibility to febrile seizures in rats, J. Neurosci. 30 (2010) 5744–5753.
[2] S. Shinnar, Febrile seizures and mesial temporal sclerosis, Epilepsy Cur. 3 (2003) 115–118.
[3] S. Baulac, I. Gourfinkel-An, R. Nabbout, G. Huberfeld, J. Serratosa, E. Leguern, M. Baulac, Fever genes and epilepsy, Lancet Neurol. 3 (2004) 421–430.
[4] A. Fetveit, Assessment of febrile seizures in children, Eur. J. Pediatr. 167 (2008) 17–27.
[5] M.H. Scantlebury, J.G. Heida, Febrile seizures and temporal lobe epileptogenesis, Epilepsy Res. 89 (2010) 27–33.
[6] M.H. Meisler, J.A. Kearney, Sodium channel mutations in epilepsy and other neurological disorders, J. Clin. Invest. 115 (2005) 2010–2017.
[7] D.S. Ragsdale, How do mutant $Na_v1.1$ sodium channels cause epilepsy?, Brain Res Rev. 58 (2008) 149–159.
[8] W.A. Catterall, F. Kalume, J.C. Oakley, $Na_v1.1$ channels and epilepsy, J. Physiol. 588 (Pt11) (2010) 1849–1859.
[9] F.H. Yu, M. Mantegazza, R.E. Westenbroek, C.A. Robbins, F. Kalume, K.A. Burton, W.J. Spain, G.S. McKnight, T. Scheuer, W.A. Catterall, Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy, Nat. Neurosci. 9 (2006) 1142–1149.
[10] J.C. Oakley, F. Kalume, F.H. Yu, T. Scheuer, W.A. Catterall, Temperature- and age-dependent seizures in a mouse model of severe myoclonic epilepsy in infancy, Proc. Natl. Acad. Sci. USA 106 (2009) 3994–3999.
[11] M.S. Martin, K. Dutt, L.A. Papale, C.M. Dube, S.B. Dutton, G. de Haan, A. Shankar, S. Tufik, M.H. Meisler, T.Z. Baram, A.L. Goldin, A. Escayg, Altered function of the SCN1A voltage-gated sodium channel leads to gamma-aminobutyric acid-ergic (GABAergic) interneuron abnormalities, J. Biol. Chem. 285 (2010) 9823–9834.
[12] A. Akaike, Y. Ohno, M. Sasa, S. Takaori, Excitatory and inhibitory effects of dopamine on neuronal activity of the caudate nucleus neurons in vitro, Brain Res. 418 (1987) 262–272.
[13] Y. Ohno, J.E. Warnick, Effects of thyrotropin-releasing hormone on phencyclidine- and ketamine-induced spinal depression in neonatal rats, Neuropharmacology 27 (1988) 1013–1018.
[14] Y. Ohno, J.E. Warnick, Selective depression of the segmental polysynaptic reflex by phencyclidine and its analogs in the rat in vitro: interaction with *N*-Methyl-D-Aspartate receptors, J. Pharmacol. Exp. Ther. 252 (1990) 246–252.
[15] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, 6th ed., Elsevier, Massachussets, 2007.
[16] R. Lipowsky, R. Gillessen, C. Alzheimer, Dendritic Na^+ channels amplify EPSPs in hippocampal CA1 pyramidal cells, J. Neurophysiol. 76 (1996) 2181–2191.
[17] Y. Ohno, S. Ishihara, R. Terada, M. Kikuta, N. Sofue, Y. Kawai, T. Serikawa, M. Sasa, Preferential increase in the hippocampal synaptic vesicle protein 2A (SV2A) by pentylenetetrazole kindling, Biochem. Biophys. Res. Commun. 390 (2009) 415–420.
[18] N. Collinson, F.M. Kuenzi, W. Jarolimek, K.A. Maubach, R. Cothliff, C. Sur, A. Smith, F.M. Otu, O. Howell, J.R. Atack, R.M. McKernan, G.R. Seabrook, G.R. Dawson, P.J. Whiting, T.W. Rosahl, Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor, J. Neurosci. 22 (2002) 5572–5580.
[19] W.R. Proctor, L. Diao, R.K. Freund, M.D. Browning, P.H. Wu, Synaptic GABAergic and glutamatergic mechanisms underlying alcohol sensitivity in mouse hippocampal neurons, J. Physiol. 575 (2006) 145–159.
[20] I. Ogiwara, H. Miyamoto, N. Morita, N. Atapour, E. Mazaki, I. Inoue, T. Takeuchi, S. Itohara, Y. Yanagawa, K. Obata, T. Furuichi, T.K. Hensch, K. Yamakawa, $Na_v1.1$ localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an *Scn1a* gene mutation, J. Neurosci. 27 (2007) 5903–5914.
[21] C. Lossin, D.W. Wang, T.H. Rhodes, C.G. Vanoye, A.L. George Jr., Molecular basis of an inherited epilepsy, Neuron 34 (2002) 877–884.
[22] P. Cossette, A. Loukas, R.G. Lafrenière, D. Rochefort, E. Harvey-Girard, D.S. Ragsdale, R.J. Dunn, G.A. Rouleau, Functional characterization of the D188V mutation in neuronal voltage-gated sodium channel causing generalized epilepsy with febrile seizures plus (GEFS), Epilepsy Res. 53 (2003) 107–117.
[23] C. Lossin, T.H. Rhodes, R.R. Desai, C.G. Vanoye, D. Wang, S. Carniciu, O. Devinsky, A.L. George Jr., Epilepsy-associated dysfunction in the voltage-gated neuronal sodium channel SCN1A, J. Neurosci. 23 (2003) 11289–11295.
[24] T.H. Rhodes, C. Lossin, C.G. Vanoye, D.W. Wang, A.L. George Jr., Noninactivating voltage-gated sodium channels in severe myoclonic epilepsy of infancy, Proc. Natl. Acad. Sci. USA 101 (2004) 11147–11152.
[25] J. Spampanato, J.A. Kearney, G. de Haan, D.P. McEwen, A. Escayg, I. Aradi, B.T. MacDonald, S.I. Levin, I. Soltesz, P. Benna, E. Montalenti, L.L. Isom, A.L. Goldin, M.H. Meisler, A novel epilepsy mutation in the sodium channel SCN1A identifies a cytoplasmic domain for beta subunit interaction, J. Neurosci. 24 (2004) 10022–10034.
[26] J. Wu, S.P. Javedan, K. Ellsworth, K. Smith, R.S. Fisher, Gamma oscillation underlies hyperthermia-induced epileptiform-like spikes in immature rat hippocampal slices, BMC Neurosci. 2 (2001) 18.
[27] J.Q. Kang, W. Shen, R.L. Macdonald, Why does fever trigger febrile seizures? $GABA_A$ receptor gamma2 subunit mutations associated with idiopathic generalized epilepsies have temperature-dependent trafficking deficiencies, J. Neurosci. 26 (2006) 2590–2597.
[28] L. Qu, X. Liu, C. Wu, L.S. Leung, Hyperthermia decreases GABAergic synaptic transmission in hippocampal neurons of immature rats, Neurobiol. Dis. 27 (2007) 320–327.
[29] L. Qu, L.S. Leung, Mechanisms of hyperthermia-induced depression of GABAergic synaptic transmission in the immature rat hippocampus, J. Neurochem. 106 (2008) 2158–2169.
[30] L. Qu, L.S. Leung, Effects of temperature elevation on neuronal inhibition in hippocampal neurons of immature and mature rats, J. Neurosci. Res. 87 (2009) 2773–2785.