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Impairments in social novelty recognition and spatial memory in mice with conditional deletion of *Scn1a* in parvalbumin-expressing cells

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ABSTRACT

Loss of function mutations in the *SCN1A* gene, which encodes the voltage-gated sodium channel Nav1.1, have been described in the majority of Dravet syndrome patients presenting with epileptic seizures, hyperactivity, autistic traits, and cognitive decline. We previously reported predominant Nav1.1 expression in parvalbumin-expressing (PV+) inhibitory neurons in juvenile mouse brain and observed epileptic seizures in mice with selective deletion of *Scn1a* in PV+ cells mediated by *PV*-Cre transgene expression (*Scn1a*$^{fl/+}$/*PV*-Cre-TG). Here we investigate the behavior of *Scn1a*$^{fl/+}$/*PV*-Cre-TG mice using a comprehensive battery of behavioral tests. We observed that *Scn1a*$^{fl/+}$/*PV*-Cre-TG mice display hyperactive behavior, impaired social novelty recognition, and altered spatial memory. We also generated *Scn1a*$^{fl/+}$/*SST*-Cre-KI mice with a selective *Scn1a* deletion in somatostatin-expressing (SST+) inhibitory neurons using an *SST*-IRES-Cre knock-in driver line. We observed that *Scn1a*$^{fl/+}$/*SST*-Cre-KI mice display no spontaneous convulsive seizures and that *Scn1a*$^{fl/+}$/*SST*-Cre-KI mice have a lowered threshold temperature for hyperthermia-induced seizures, although their threshold values are much higher than those of *Scn1a*$^{fl/+}$/*PV*-Cre-TG mice. We finally show that *Scn1a*$^{fl/+}$/*SST*-Cre-KI mice exhibited no noticeable behavioral abnormalities. These observations suggest that impaired Nav1.1 function in PV+ interneurons is critically involved in the pathogenesis of hyperactivity, autistic traits, and cognitive decline, as well as epileptic seizures, in Dravet syndrome.

1. Introduction

Dravet syndrome (Mendelian Inheritance in Man #607208) is an intractable infantile epileptic encephalopathy characterized by epileptic seizures, often followed by hyperactivity, autistic traits, and cognitive decline (Cassé-Perrot et al., 2001; Dravet et al., 2005; Wolff et al., 2006; Berg et al., 2010; Chieffo et al., 2011; Nabbout et al., 2013). Major causes of Dravet syndrome include heterozygous loss-of-function mutations in the *SCN1A* gene encoding the voltage-gated sodium channel Nav1.1 (Claes et al., 2001; Sugawara et al., 2002; Fujiwara et al., 2003; Mulley et al., 2006; Harkin et al., 2007; Depienne et al., 2009; Nakayama et al., 2010; Meng et al., 2015). In mice, Nav1.1 haploinsufficiency resulting from *Scn1a* knocking out (*Scn1a*$^{KO/+}$) or knock-in of a pathogenic nonsense p.Arg1407Stop mutation (*Scn1a*$^{RX/+}$) causes early-onset epileptic seizures, lowered threshold for hyperthermia-induced seizures, hyperactivity, altered anxiety-like behavior, lowered sociability, lack of preference for social novelty, spatial learning disability, and impaired spatial memory; these features correspond to the clinical features of Dravet syndrome (Yu et al., 2006; Ogiwara et al., 2007; Oakley et al., 2009; Cao et al., 2012; Han et al., 2012; Ito et al., 2013).

In juvenile mouse brain, Nav1.1 is predominantly localized in the axon initial segments and nodes of Ranvier of parvalbumin-expressing (PV+) neurons and plays critical roles in spike output from these neurons (Yu et al., 2006; Ogiwara et al., 2007; Ogiwara et al., 2013). These features indicate the involvement of Nav1.1 haploinsufficient PV + inhibitory neurons in the pathophysiology of Dravet syndrome. In support of this idea, we reported that selective deletion of the *Scn1a* gene in PV+ cells mediated by *PV*-Cre transgene expression (*PV*-Cre-TG) caused epileptic seizures in mice (Ogiwara et al., 2013). We also reported that another PV+ cell-selective *Scn1a* deletion using a *PV*-Cre knock-in driver line (*PV*-Cre-KI) caused much milder epileptic phenotypes than those produced by *PV*-Cre-TG-mediated *Scn1a* deletion (Ogiwara et al., 2013). Although *PV*-Cre-KI-mediated heterozygous *Scn1a* deletion was recently shown to cause lowered sociability

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(Rubinstein et al., 2015), the effect of *PV*-Cre-TG-mediated one (*Scn1a*$^{fl/+}$/*PV*-Cre-TG) on behavior have not yet been investigated.

The present study aims to determine the involvement of PV+ inhibitory neurons in shaping psychiatric phenotypes of Dravet syndrome using *Scn1a* conditional knockout mice. While *Scn1a*$^{fl/+}$/*PV*-Cre-TG mice have hyperactive behavior, lack of social novelty preference and impaired spatial memory, *Scn1a*$^{fl/+}$/*Somatostatin* (*SST*)-Cre-KI mice display no discernible behavioral abnormalities. Our results support the hypothesis that functional impairment in PV+ inhibitory neurons is the cellular basis of hyperactivity, autistic traits and cognitive decline.

2. Materials and methods

2.1. Mice

Mice were handled in accordance with the guidelines of the Animal Experiment Committee of RIKEN Brain Science Institute. *Scn1a* floxed mice and *PV*-Cre recombinase bacterial artificial chromosome transgenic mice (*PV*-Cre-TG) were described previously (Tanahira et al., 2009; Ogiwara et al., 2013) and backcrossed into a C57BL/6J background for > 10 generations. *Scn1a* floxed heterozygotes (*Scn1a*$^{fl/+}$) and *PV*-Cre-TG hemizygotes, both on a congenic C57BL/6J background, were crossed to generate *Scn1a*$^{fl/+}$/*PV*-Cre-TG mice. *Somatostatin*-IRES-Cre knock-in mice (*SST*-Cre-KI) on a mixed C57BL/6J × 129 background, whose generations have been described previously (Taniguchi et al., 2011), were purchased from the Jackson Laboratory (JAX #013044). *SST*-Cre-KI mice were backcrossed into a C57BL/6J background for > 10 generations and therefore maintained in the heterozygous state. *Scn1a*$^{fl/+}$ mice and *SST*-Cre-KI heterozygotes, both on a congenic C57BL/6J background, were crossed to generate *Scn1a*$^{fl/+}$/*SST*-Cre-KI mice. *Scn1a*$^{fl/+}$/*SST*-Cre-KI mice were then crossed with *Scn1a*$^{fl/+}$ mice to obtain *Scn1a*$^{fl/fl}$/*SST*-Cre-KI mice. Ai14 mice on a congenic C57BL/6J background, which harbor a targeted mutation of the Gt(ROSA)26Sor locus with a loxP-flanked STOP cassette to prevent transcription of a CAG promoter-driven red fluorescent protein variant (tdTomato) (Madisen et al., 2010), were purchased from the Jackson Laboratory (JAX #007914). Ai14 homozygotes were maintained by brother–sister inbreeding. To visualize *PV*-Cre-TG-mediated recombination, *PV*-Cre-TG hemizygotes were bred to Ai14 homozygotes. Food and water were available *ad libitum*, and cages were kept at 23 ± 2 °C and 55 ± 10% humidity on a 12-hour light/dark cycle with the lights off at 20:00.

Only male mice were included in the behavioral tests to avoid possible variability arising due to hormonal fluctuations over the estrous cycle in female mice. The behavioral tests were started at the age of 8 weeks, after the peak period of epileptic seizures and sporadic sudden death in *Scn1a*$^{fl/+}$/*PV*-Cre mice (Ogiwara et al., 2013). The mice employed in the behavioral tests apparently had no seizures during the periods of habituation and testing. All mice were housed in groups of 2 to 5 individuals per cage until administration of the novel open field test, after which the mice were individually housed. All tests were conducted during the light phase of the light/dark cycle. The mice were habituated to the behavioral testing room for at least 30 min before starting the experiment. The behavioral tests were performed blindly to genotype.

Scn1a$^{fl/+}$/*PV*-Cre-TG (n = 13) males and their age-matched *Scn1a*$^{+/+}$ (n = 11), *Scn1a*$^{+/+}$/*PV*-Cre-TG (n = 11) and *Scn1a*$^{fl/+}$ (n = 10) control littermate males were assessed in a battery of tests in the following order: the novel open field test (8 weeks old), elevated-plus maze test (8 to 9 weeks old), three-chambered social interaction test (11 to 13 weeks old), Barnes maze test (18 to 20 weeks old), and buried food test (28 weeks old). One *Scn1a*$^{fl/+}$/*PV*-Cre-TG mouse that suffered premature sudden death 3 days after the elevated plus maze test was not included in the three-chambered social interaction, Barnes maze, or buried food tests. One *Scn1a*$^{fl/+}$/*PV*-Cre-TG mouse that escaped from the three-chambered apparatus was excluded from the three-chambered social interaction test. One *Scn1a*$^{+/+}$ mouse that was found injured at the beginning of the Barnes maze test was removed from the Barnes maze and buried food tests.

Scn1a$^{fl/+}$/*SST*-Cre-KI (n = 12) males and their age-matched *Scn1a*$^{+/+}$ (n = 11), *Scn1a*$^{+/+}$/*SST*-Cre-KI (n = 10), and *Scn1a*$^{fl/+}$ (n = 11) control littermate males were assessed in a battery of tests in the following order: the open field test (8 weeks old), elevated-plus maze test (9 weeks old), and three-chamber social interaction test (12 weeks old).

2.2. Novel open field test

The novel open field test was performed as previously described (Ito et al., 2013) with slight modifications. The open field (60 × 60 × 40 cm) was made from non-transparent, gray plates and illuminated at 70 lx. The exploratory activities of 8-week-old males in the novel open field were analyzed every 10 min during a period of 30 min (O'Hara & Co., Tokyo, Japan). The total distance traveled and time spent in the center of the open field (area > 12 cm from the walls) were determined using a video tracking system. The rearing counts were measured by counting the number of beam breaks.

2.3. Elevated-plus maze test

The elevated-plus maze test was performed as previously described (Ito et al., 2013) with slight modifications. The elevated-plus maze consisted of two orthogonal closed and open arms (25 cm long) forming a cross, with a quadrangular area located at the intersection (O'Hara & Co.). The arms and quadrangular area were made from gray plates and were elevated 60 cm above the floor. The walls of the closed arms were made from transparent, colorless plates. The open arms were surrounded by small ledges to prevent the mice from falling from the maze. The light intensity was 70 lx at the center field of the maze. Each 8- to 9-week-old male was placed in the center section of the maze facing one of the closed arms and allowed to explore the maze freely for 10 min. The time spent in the open and closed arms and center field and the number of entries into the open and closed arms were determined using a video tracking system.

2.4. Three-chambered social interaction test

The apparatus, a rectangular, three-chambered box made from non-transparent, gray plates, was placed in a soundproof outer box (Nakatani et al., 2009) (O'Hara & Co.). Two transparent, colorless partitions divided the box into three identical 20-cm-long chambers. Square openings in the bottom of the partitions allowed mice to move between the three chambers. Light intensity was 70 lx at the center field of the box. Wire cages with the shape of a quarter cylinder were used to enclose stranger mice that were 7- to 8-week-old C57BL/6J males that had been habituated to placement in the same cage for 10 min per the test. The social behavior test was performed as described previously (Nakatani et al., 2009). Each 11- to 13-week-old male was placed in the center chamber of the three-chambered box and allowed to explore the entire test box for 10 min for habituation. Each of the side chambers contained an empty wire, quarter cylinder shaped cage placed on the corner. After the habituation period, the test mouse was put in the center chamber, and the empty wire cage placed on the corner of one of the side chamber was replaced with the wire cage containing a never-before-met, unfamiliar mouse (stranger 1) whose placement in the left or right side chamber was counterbalanced within each genotype group in pseudorandom fashion. The test mouse then was allowed to explore the entire test box for 10 min (sociability test). At the end of the 10-minute session, the empty cage was replaced with the wire cage containing a new mouse (stranger 2) that originated from a home cage different from that of stranger 1 and had never had any physical contact with either stranger 1 or the test mouse. The test mouse was again