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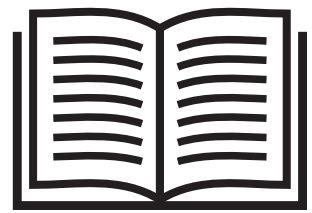KYOTO UNIVERSITY

Title	Methylphenidate improves learning impairments and hyperthermia-induced seizures caused by an Scn1a mutation.
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Methylphenidate improves learning impairments and hyperthermia-induced seizures caused by an *Scn1a* mutation

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SUMMARY

Objective: Developmental disorders including cognitive deficit, hyperkinetic disorder, and autistic behaviors are frequently comorbid in epileptic patients with *SCN1A* mutations. However, the mechanisms underlying these developmental disorders are poorly understood and treatments are currently unavailable. Using a rodent model with an *Scn1a* mutation, we aimed to elucidate the pathophysiologic basis and potential therapeutic treatments for developmental disorders stemming from *Scn1a* mutations.

Methods: We conducted behavioral analyses on rats with the N1417H-*Scn1a* mutation. With high-performance liquid chromatography, we measured dopamine and its metabolites in the frontal cortex, striatum, nucleus accumbens, and midbrain. Methylphenidate was administered intraperitoneally to examine its effects on developmental disorder–like behaviors and hyperthermia-induced seizures.

Results: Behavioral studies revealed that *Scn1a*-mutant rats had repetitive behavior, hyperactivity, anxiety-like behavior, spatial learning impairments, and motor imbalance. Dopamine levels in the striatum and nucleus accumbens in *Scn1a*-mutant rats were significantly lower than those in wild-type rats. In *Scn1a*-mutant rats, methylphenidate, by increasing dopamine levels in the synaptic cleft, improved hyperactivity, anxiety-like behavior, and spatial learning impairments. Surprisingly, methylphenidate also strongly suppressed hyperthermia-induced seizures.

Significance: Dysfunction of the mesolimbic dopamine reward pathway may contribute to the hyperactivity and learning impairments in *Scn1a*-mutant rats. Methylphenidate was effective for treating hyperactivity, learning impairments, and hyperthermia-induced seizures. We propose that methylphenidate treatment may ameliorate not only developmental disorders but also epileptic seizures in patients with *SCN1A* mutations.

KEY WORDS: Methylphenidate, Dopamine, Mesolimbic reward pathway, *Scn1a*, Developmental disorders.

The voltage-gated sodium channel α1 subunit, Nav1.1, encoded by *SCN1A*, is mainly expressed in γ-aminobutyric acid (GABA)ergic interneurons and plays a crucial role in the initiation and propagation of action potentials.[1,2] Mutations of *SCN1A* usually result in haploinsufficiency of Nav1.1 and contribute to intractable epileptic syndromes. *SCN1A* mutations are detected in 70–80% of patients with Dravet syndrome[3–5] and in 3–10% of patients with genetic epilepsy with febrile seizures plus (GEFS+).[6–8] In addition to intractable epilepsy, patients with Dravet syndrome also experience developmental disorders such as attention-deficit/hyperactive disorder (ADHD), severe cognitive deficit, autistic behaviors, and motor imbalance.[9–12] GEFS+ patients with *SCN1A* mutations also exhibit psychiatric disorders including anxiety disorder and Asperger syndrome.[13] However, developmental disorders are not common in GEFS+ patients, and simple febrile seizures is a phenotype that be caused only by an *SCN1A* mutation.[14] Most studies of Dravet syndrome and GEFS+ focus on treating epileptic symptoms, whereas comparatively fewer studies focus on comorbid developmental disorders. As such, a treatment for developmental disorders in these patients is not yet established. Because these developmental disorders profoundly disrupt patients' quality of life, it is important to clarify the pathophysiologic basis of and find an effective treatment for these symptoms.

Animal models are useful tools to elucidate the mechanisms of developmental disorders caused by single gene mutations. We previously generated a GEFS+ rat model with a N1417H-*Scn1a* mutation and characterized its physiologic properties.[15] Using this rat model, the efficacy of many anticonvulsant drugs on hyperthermia-induced seizures (HIS) was comparable to that in patients with GEFS+ and Dravet syndrome.[16]

In this study, using *Scn1a*-mutant rats, we investigated the pathophysiologic basis of developmental disorders and potential therapeutics for *Scn1a* mutation. We found that even without recurring seizures, *Scn1a* mutation leads to neurodevelopmental behavioral disorders. Dopamine (DA) levels in the striatum and nucleus accumbens (NA) in *Scn1a* mutant rats were significantly lower than in wild-type (WT) controls. Methylphenidate (MPH) treatment improved hyperactivity and impaired spatial learning. In addition, MPH strongly suppressed HIS in *Scn1a*-mutant rats.

MATERIALS AND METHODS

Animals

We used male F344/NSlc-*Scn1a*^Kyo811 rats with a homozygous N1417H missense mutation (*Scn1a* mutant rats; National BioResource Project for Rat in Japan, Kyoto University, Japan)[15] and WT littermate male rats. N1417H-*Scn1a* mutation does not represent *SCN1A* mutations found in human patients, which arise from random mutagenesis. Animals were maintained under standard laboratory condition with a 12 h light/dark cycle with food and water available ad libitum. A general health and neurologic screen was conducted before behavioral studies. WT and *Scn1a* homozygous mutant rats were first observed in their home cages for general health, and sensory and motor reflexes were evaluated. Body weight at 5 weeks of age was measured.

All animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of Okayama University.

Behavioral studies

Male rats at 5- to 8-weeks-old were used for all behavioral analyses. All experiments were conducted in dedicated behavioral testing rooms during the standard light phase, usually between 13:00 and 17:00 h. Rats were brought to a holding room in the hallway of the testing area at least 1 h prior to the start of the behavioral test. Testing order was as follows: observation of repetitive self-grooming behavior, open field test, elevated plus-maze test, three-chamber social interaction test, Barnes maze test, and rotarod test. All behavioral tests were performed in the same order and with 1–2 day intervals. All experiments were done blind to genotype and treatment. Detailed procedures were described in Data S1.

Video-EEG monitoring

To confirm whether spontaneous seizures occurred, we recorded video-EEG as previously described.[16] Briefly, at 4 weeks of age, rats were implanted with stainless steel screw electrodes bilaterally into the frontal (anteroposterior [AP]: +0.5 mm; mediolateral [ML]: ±3.0 mm from bregma) and occipital cortices (AP: −7.0 mm; ML: ±3.0 mm from bregma). Starting 1 week after surgery, digital video–electroencephalography (EEG) recordings were carried out: a 15 h session (from 18:00 to 09:00 h) and a 7 h session (from 10:00 to 17:00 h) from 5- to 8-weeks-old. In total, >132 h of video-EEG recordings were analyzed at each week of age from six rats.

High-performance liquid chromatography (HPLC) analysis

Levels of DA and its metabolites were measured using a previously reported method.[17] Briefly, male WT and *Scn1a* mutant rats were sacrificed at 8–weeks of age. Striatum, NA, midbrain, and frontal cortical regions were dissected, frozen in liquid nitrogen, and stored until homogenization with 10 volumes of 200 mM ice-cold perchloric acid with 10 mM ethylene diamine tetraacetic acid (EDTA). Homogenized samples were centrifuged at 11,750 g for 20 min at 4°C, and the supernatant was filtered (0.45 μm) and then injected directly into a high-performance liquid

chromatography (HPLC) with electrochemical detectors (HPLC-ECD; Tosoh, Co., Tokyo, Japan). Regional concentrations of DA and the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured. The HPLC system consisted of a delivery pump (PX-8020; Tosoh, Co.) and an analytical column (EICOM-PAK SC-5ODS, 3.0 mm × 150 mm; Eicom, Co., Kyoto, Japan). An electrochemical detector (EC-8020; Tosoh, Co.) with glassy carbon was used with a voltage setting of 700 mV and an Ag/AgCl reference electrode. A mobile phase containing 0.1 M citrate-sodium acetate buffer (pH 3.5), methanol (17% vol/vol), EDTA-2Na, and sodium 1-octanesulfonate was infused at a flow rate of 0.6 ml/min. Protein concentration was determined with a protein assay kit (Bio-Rad Laboratories, Hercules, CA, U.S.A.).

Histologic assessment

At 8–9 weeks of age, male WT and *Scn1a* mutant rats were deeply anesthetized with an intravenous injection of sodium pentobarbital and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains fixed in 4% paraformaldehyde were embedded in paraffin wax. For histologic assessment, paraffin sections (4 μm thick) were deparaffinized and rehydrated. To visualize brain structure, sections were stained using the Kluver-Barrera method using Luxol fast blue and cresyl violet.

Immunohistochemistry was performed to examine the number of the dopaminergic neurons in the ventral tegmental area (VTA) and substantia nigra (SN). An anti-tyrosine hydroxylase (TH) antibody was used to immunolabel dopaminergic neurons. Coronal sections including the VTA and SN (4 μm thick) were autoclaved at 120°C for 5 min for antigen retrieval. Endogenous peroxidases were quenched by incubation with 0.3% hydrogen peroxide in methanol. After incubating the slides with blocking serum for 1 h at room temperature, sections were incubated with the mouse anti-TH antibody (1:1,000; Millipore, Billerica, MA, U.S.A.) in a humidified chamber overnight at 4°C. The sections were then incubated with a biotinylated mouse polyclonal secondary antibody (Vector Laboratories, Burlingame, CA, U.S.A.). To reveal the staining, we used an avidin-biotinylated peroxidase complex (ABC) kit (Vectastain Elite ABC kit; Vector Laboratories) and diaminobenzidine (DAB) substrate kit (Vector Laboratories). Using a microscope (BH-2; Olympus, Tokyo, Japan), the number of the TH-positive cells in the VTA and SN pars compacta were counted in coronal sections (n = 3 rats, each group).

To assess the expression of Nav1.1 on dopaminergic neurons in the VTA and SN in WT littermate controls and *Scn1a* mutant rats, we performed fluorescent immunohistochemistry. Sagittal and coronal sections including the VTA and SN were dissected and postfixed with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) (pH 7.4) at 4°C. Brains were cryoprotected in PBS containing 30% sucrose prior to cryostat sectioning. Sections (10–20 μm thick) were incubated overnight at 4°C with primary antibodies diluted in PBS. Dopaminergic neurons and Nav1.1 were stained with mouse anti-TH antibody (1:1,000; Millipore) and goat anti-Nav1.1 antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), respectively. Next, sections were incubated with secondary antibodies for 2 h at room temperature. The secondary antibodies were rhodamine-labeled anti-mouse antibody (1:100; Sigma Aldrich, St. Louis, MO, U.S.A.) and fluorescein isothiocyanate (FITC) –labeled anti-goat antibody (1:100; Santa Cruz Biotechnology). Images were obtained with a fluorescence microscope (BIOREVO, BZ-8100; Keyence, Osaka, Japan). Negative controls that were not incubated with primary antibodies showed no staining.

Treatment with MPH for neurologic symptoms and HIS

MPH is currently the most common treatment for ADHD. MPH blocks DA transporters and robustly increases DA release in the NA and dorsal striatum in rats.[18] We wished to examine the effects of MPH on neurologic symptoms. One hour after intraperitoneal administration of saline or 0.5 mg/kg MPH (Sigma Aldrich), behavioral tests were conducted on WT and *Scn1a*-mutant rats. Behavioral studies included grooming, open field test, locomotor activity, rotarod test, and Barnes maze.

Because some psychotropic agents aggravate epileptic seizures, we examined the effect of MPH on HIS. One hour after intraperitoneal administration of saline, 0.5 or 2.0 mg/kg of MPH, HIS were evoked by hot water baths at 45°C as described previously.[16] Briefly, rats at 5 weeks of age were placed in a water bath at 45°C for a maximum of 5 min or until a seizure onset. In this experimental condition, HIS were never evoked in WT controls. The duration of HIS, latency until onset of HIS, and rectal temperature at the end of the hot water bath were assessed. The duration of HIS was measured using video-EEG monitoring as described earlier.

Statistical analyses

Data are presented as average ± standard error of the mean (SEM) and reported as significant at p < 0.05. The data analyses were performed using nonrepeated measures analysis of variance (ANOVA) with Dunnett's post hoc test. The data were compared using the paired *t*-test for cases involving two groups as appropriate.

RESULTS

General health and neurologic screen

Table S1 lists general health conditions and neurologic reflexes of WT and *Scn1a*- mutant rats at 5 weeks of age. Both genotypes did not differ significantly in terms of general health, reflexes, and sensory function.

Behavioral studies

Repetitive self-grooming behavior

The results for repetitive self-grooming are shown in Figure 1A. During the 10-min test, while WT littermate controls spent 47 ± 10 s grooming, *Scn1a* mutant rats groomed for significantly longer (89 ± 15 s) (p = 0.029).

Open field and locomotor activity

Scn1a-mutant rats traveled significantly farther than WT littermate controls (Fig. 1C,D), but spent less time in the center area of the open field during 10 min test sessions (Fig. 1E). *Scn1a*-mutant rats displayed hyperactivity and anxiety-like behavior.

Elevated plus-maze test

Figure 1F illustrates anxiety-like behavior assessed by the elevated plus-maze task. Percentage of time spent in the open arms did not differ significantly between WT littermate controls ($25.2 \pm 3.1\%$) and *Scn1a*-mutant rats ($30.4 \pm 2.9\%$) (p = 0.160).

Three-chamber social interaction test

Both WT littermate controls and *Scn1a*-mutant rats demonstrated a significant preference for spending time in the chamber containing a stranger versus spending time exploring the chamber containing an empty cage (Fig. 1G; WT, stranger vs. empty, 378.8 ± 22.5 s vs. 110.4 ± 15.9 s, p < 0.001; *Scn1a* mutant, 402.7 ± 17.9 s vs. $108.6 \pm$
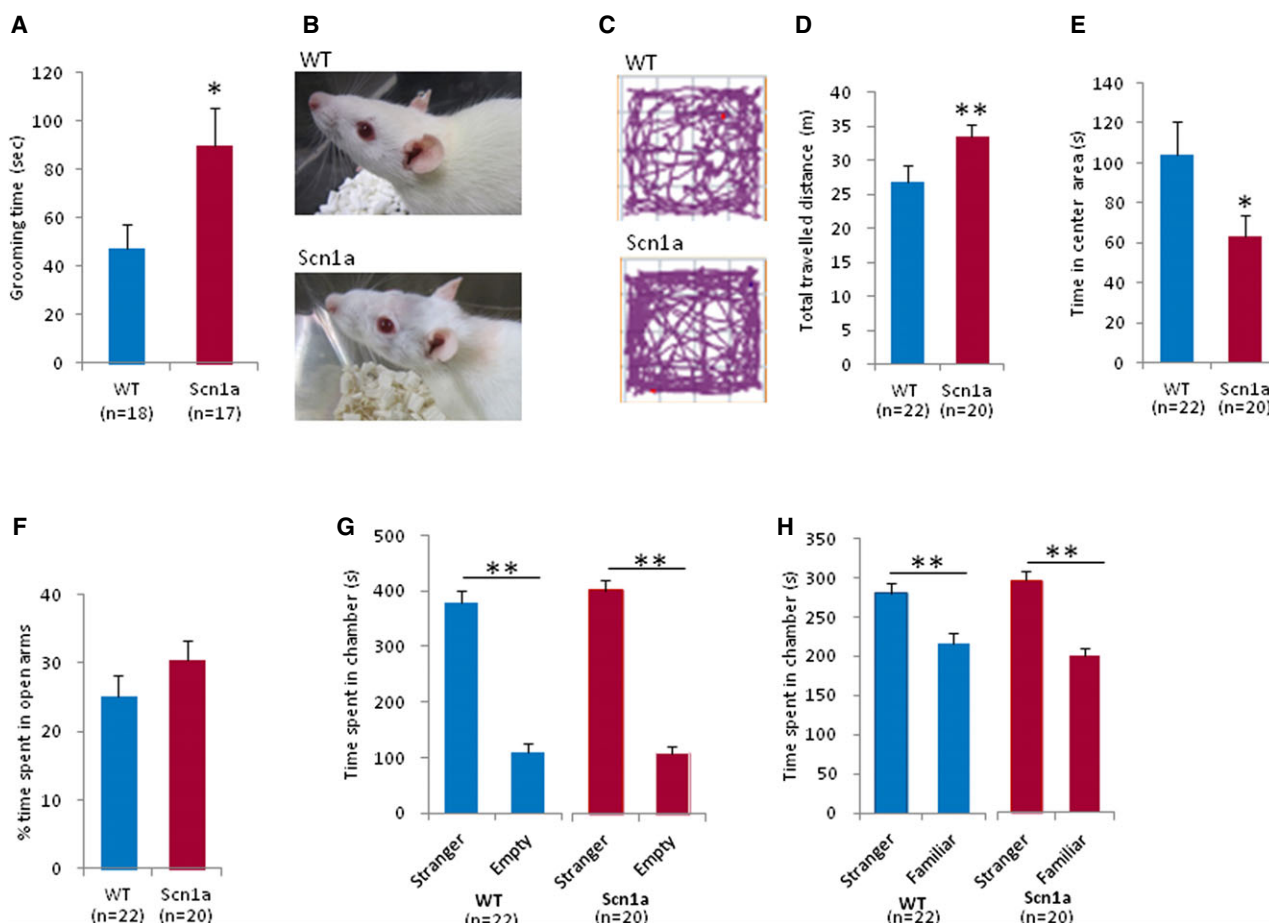

Figure 1.
Scn1a-mutant rats exhibit increased self-grooming, hyperactivity, and anxiety-like behaviors. (**A**) *Scn1a*-mutant rats spent significantly longer time grooming than WT rats. (**B**) *Scn1a*-mutant rats at 5 weeks of age appeared physically normal; however, one of 17 *Scn1a*-mutant rats showed scattered fur on the head at 3 months of age potentially due to over-grooming. (**C**) Representative traces in a novel open field to assess exploratory locomotion. (**D**) *Scn1a*-mutant rats traversed more total distance in the novel open field as compared to WT. (**E**) *Scn1a*-mutant rats spent less time in the center area of the open field than WT. (**F**) No genotypic difference in anxiety-like behaviors on the elevated plus-maze. (**G**) Amount of time spent in the chamber containing the stranger rat in a wire cage versus amount of time spent in the chamber containing an identical but empty wire cage. (**H**) Amount of time spent in the chamber containing the stranger or familiar rat. Both genotypes demonstrated significant sociability and preference for social novelty. There was no difference within genotypes on the three-chamber social interaction test. **p < 0.01, *p < 0.05.
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12.3 s, p < 0.001). In the test of preference for social novelty, both WT littermate controls and *Scn1a*-mutant rats demonstrated a significant novelty preference (Fig. 1H; WT, stranger vs. familiar: 280.0 ± 13.1 s vs. 216.4 ± 12.8 s, p < 0.001; *Scn1a*, 292.4 ± 12.0 s vs. 197.2 ± 9.5 s, p < 0.001). Together, the results from the three-chamber social interaction tests show that WT and *Scn1a*-mutant rats have similar sociability and novelty preference.

Barnes maze

Compared to WT littermate controls, Barnes maze acquisition was significantly slower in *Scn1a*-mutant rats (Fig. 2A–C). Acquisition trials revealed that *Scn1a*-mutant rats committed more errors than WT controls in the first entry into the escape box on days 2, 3, and 5 (Fig. 2A). The distance to reach the target hole in *Scn1a*-mutant rats was longer than that of WT controls on days 2–6 (Fig. 2B). Latency to reach the correct hole above the escape box in *Scn1a* mutant rats was longer than that of WT littermate controls on day 5 (Fig. 2C).

Motor function tests

There were no statistical differences between WT littermate controls and *Scn1a*-mutant rats at a constant rotarod speed of $4/60$ s^{-1} (Fig. 2D; WT, 153.1 ± 22.1 s; *Scn1a*, 106.5 ± 15.9 s, p = 0.100). However, compared with WT littermate controls, *Scn1a*-mutant rats spent significantly less time on the accelerating rotarod (Fig. 2E; WT, 102.3 ± 7.0 s; *Scn1a*, 73.6 ± 7.6 s, p = 0.022).

Long-term video-EEG monitoring

We analyzed >132 h of video-EEG recordings at each week of age from six *Scn1a* mutant rats and observed no spontaneous seizures and no clear epileptiform discharges (Fig. S1).

HPLC analysis

To assess a potential relationship between brain neurochemistry and behavioral disruptions in *Scn1a* mutant rats, we quantified the levels of DA, DOPAC, and HVA in various brain regions (Fig. 3). We compared DA, DOPAC, and HVA levels in the striatum, NA, midbrain, and frontal cortex of WT littermate control and *Scn1a*-mutant rats. Overall, compared to WT controls, we found that *Scn1a* rats had significantly lower DA levels in the striatum (WT, 481.7 ± 14.9 pmol/mg protein; *Scn1a*, 366.7 ± 40.0 pmol/mg protein, p = 0.022) and in the NA (WT, 226.4 ± 21.0 pmol/mg protein; Scn1a, 169.3 ± 11.0 pmol/mg protein, p = 0.034). *Scn1a* rats also had diminished DOPAC levels, but only in the NA (WT, 55.1 ± 5.1 pmol/mg protein; *Scn1a*, 42.0 ± 2.3 pmol/mg protein, p = 0.041).

Histologic assessment

We assessed gross brain structure with Kluver-Barrera's staining to visualize myelin sheaths and neuronal cell bodies. There were no major anatomic abnormalities in the midbrain (Fig. S2A), hippocampus (Fig. S2B), cerebellum (Fig. S2C), or the layering of either the frontal cortex (Fig. S2D) or the cerebellar cortex (Fig. S2E) of *Scn1a* rats. Of interest, the number of TH-positive cells in coronal sections that included the VTA and the SN did not differ between WT and *Scn1a*-mutant rats (Fig. S2F–H; WT, 125.2
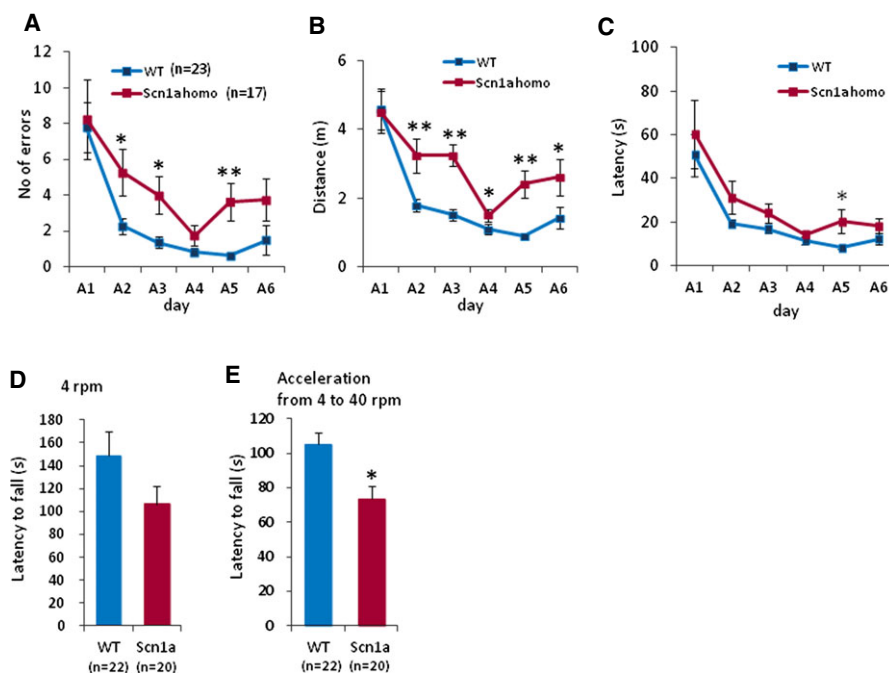

Figure 2.
Scn1a-mutant rats show impaired spatial learning and memory in the Barnes maze. (**A**) Number of errors to the first entry into the escape box. (**B**) Distance to reach the target hole. (**C**) Latency to reach the correct hole above the escape box. (**D,E**) Rotarod test revealed that *Scn1a*-mutant rats have deficits of motor coordination. Motor function was assessed by using Rotarod test at $4/60$ s^{-1}(**D**) and accelerated from 4/60 to $40/60$ s^{-1} (**E**). **p < 0.01, *p < 0.05.
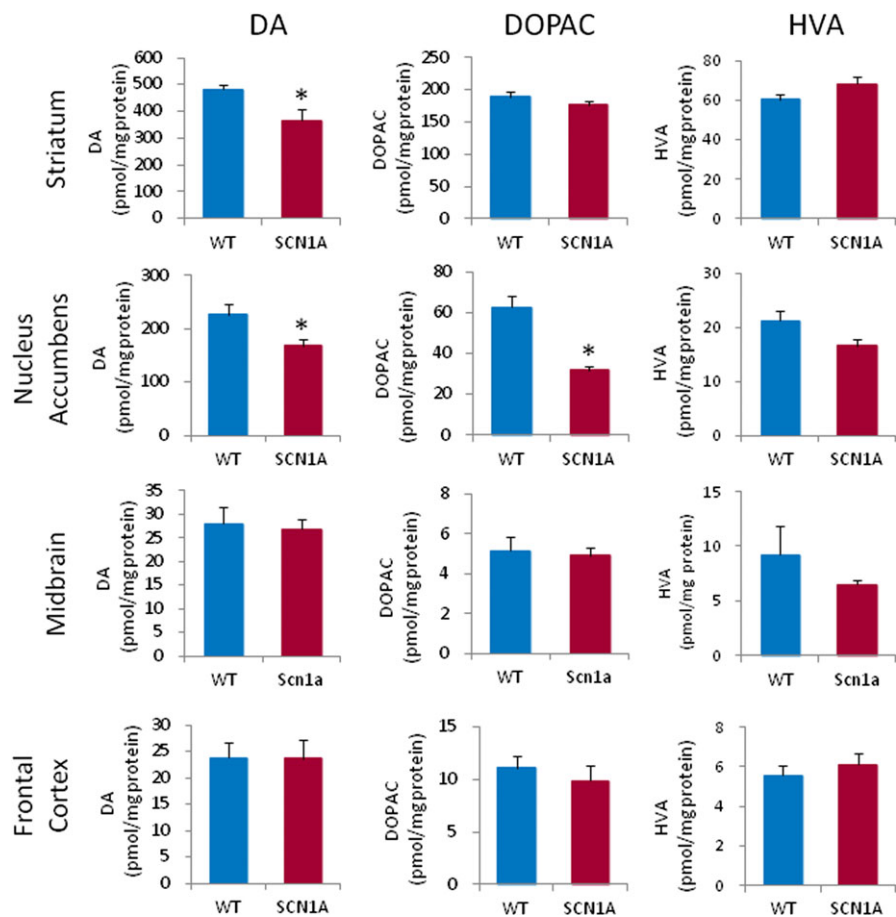*Epilepsia* © ILAE

Figure 3.
Comparison of the levels of DA and its metabolites in different brain structures. Levels of DA, DOPAC, and HVA in the striatum, nucleus accumbens, midbrain, and frontal cortex in *Scn1a*-mutant rats compared with WT controls. The level of DA in the striatum and the levels of DA and DOPAC in the nucleus accumbens from *Scn1a*-mutant rats were significantly lower than those from WT controls. Each group n = 6, *p < 0.05.
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± 7.9 cells/slide; *Scn1a*, 127.9 ± 11.2 cells/slide, p = 0.85, six slides from three rats per genotype).

Next, we used immunofluorescence to study the distribution of Nav1.1 subunits on dopaminergic cells (using TH as a marker) in *Scn1a* rats. Although Nav1.1 staining was also found on cells unlabeled for TH, we found many Nav1.1 puncta located on TH-positive dopaminergic neurons from sagittal sections of the VTA (Fig. 4C–E), as well as coronal sections of the SN (Fig. 4F–H).

MPH treatment for neurologic symptoms of *Scn1a* rats

Next, we wished to determine if the commonly used drug MPH could improve the neurologic deficits exhibited by *Scn1a* rats. Compared to vehicle saline treatment alone, MPH treatment (0.5 mg/kg) shortened the total traveled distance of *Scn1a*-mutant rats in the open field (Fig. 5A; saline, 34.4 ± 3.0 m; MPH, 26.5 ± 2.0 m, p = 0.004). *Scn1a* rats treated with MPH also spent more time in the center area of the open field compared to saline-treated *Scn1a* rats (Fig. 5B; saline 24.4 ± 3.8 s; MPH, 36.2 ± 3.7 s, p = 0.009). These results suggest that MPH reduces anxiety-like behaviors in *Scn1a* rats.

On acquisition trial day 2 in the Barnes maze (Fig. 5C), MPH treatment (0.5 mg/kg) of *Scn1a* rats significantly reduced the number of errors (Fig. 5D; saline, 6.8 ± 1.1

errors; MPH, 2.6 ± 0.6 errors, p = 0.003) and the latency time (Fig. 5F; saline, 41.3 ± 7.3 s; MPH, 23.4 ± 3.4 s, p = 0.039) to reach the target hole. In contrast, WT littermates were unaffected by MPH treatment on both the open field and Barnes maze tests (Fig. 5A,B,G–I). Nevertheless, MPH treatment did not change grooming time and motor balance in both WT and *Scn1a*-mutant rats (Fig. S3). Taken together, MPH improved hyperactivity, anxiety-like behaviors, and spatial learning in *Scn1a*-mutant rats.

Effect of MPH on HIS in *Scn1a*-mutant rats

Figure 6A illustrates representative EEG recordings from the frontal cortex of HIS in *Scn1a*-mutant rats treated with either saline (top) or 0.5 mg/kg of MPH (bottom). Treating *Scn1a* rats with either 0.5 or 2 mg/kg of MPH markedly reduced seizure duration (Fig. 6B; saline, 62.6 ± 12.6 s; 0.5 mg/kg of MPH, 20.5 ± 5.1 s, p = 0.005; 2 mg/kg of MPH, 14.3 ± 3.5 s, p = 0.001). In addition, treatment with a higher concentration of MPH (2 mg/kg) increased HIS threshold in *Scn1a* rats so that HIS were triggered by a significantly longer immersion in hot water (Fig. 6C; saline, 235.6 ± 13.9 s; 2 mg/kg of MPH, 276.1 ± 7.0 s, p = 0.012) and a significantly higher rectal temperature compared with saline-treated rats (Fig. 6D; saline, 43.3 ± 0.1°C; 2 mg/kg of MPH, 43.7 ± 0.1°C, p = 0.040).
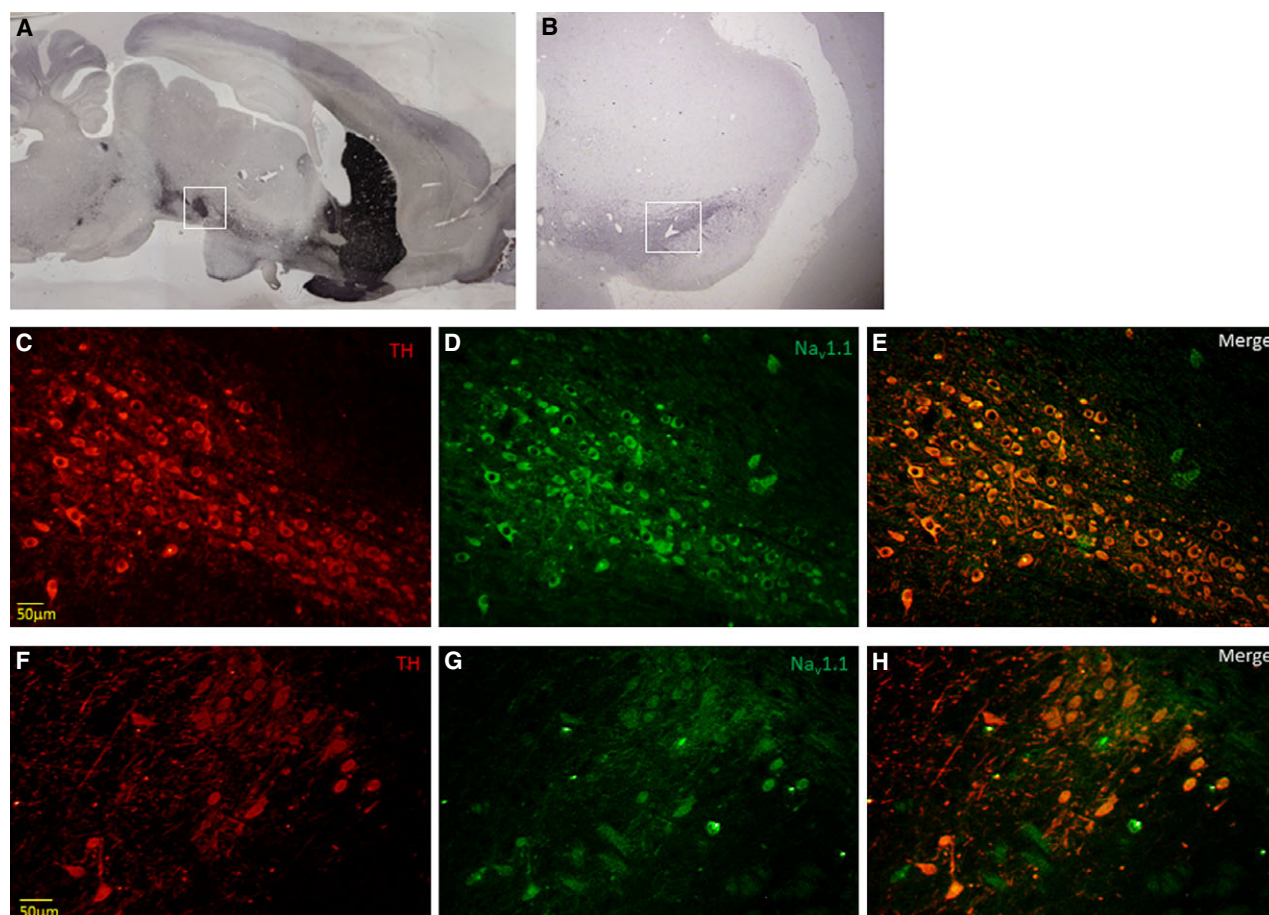
Figure 4.
Nav1.1-containing channels are expressed on dopaminergic neurons in the VTA and SN of *Scn1a*-mutant rats. Immunohistochemistry of TH-positive cells in a sagittal section (**A**) and a coronal section (**B**) including the VTA and SN. Boxed areas (white squares) indicate the observed region of the immunofluorescence. All dopaminergic neurons (red, **C,F**) in the VTA and SN were Nav1.1-positive (green, **D**, **G**). Merged images (**E,H**).
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These results strongly suggest that MPH treatment increases the resistance of *Scn1a* rats to HIS.

DISCUSSION

Developmental disorder–like behaviors in *Scn1a*-mutant rats

Behavioral studies revealed that *Scn1a*-mutant rats have increased grooming time (repetitive behavior), hyperactivity, anxiety-like behaviors, impaired spatial learning, and motor imbalance. Heterozygous Scn1a-knockout (KO) mice also display impaired social behaviors in addition to those behavioral abnormalities found in *Scn1a*-mutant rats.[19,20] The absence of impaired social behaviors in *Scn1a*-mutant rats may depend on the age tested (adult vs. young adult), frequency of spontaneous seizures (some vs. none), or functional properties of mutant Nav1.1-containing channels (null vs. partial function lowering). Hippocampal GABAergic interneurons

from *Scn1a*-mutant rats are less excitable compared with those from WT controls.[15]

Comorbidity of epilepsy and developmental disorders are not rare,[21] and recurrent seizures are associated with later cognitive impairment in rats.[22,23] Heterozygous Scn1a-KO mice with developmental disorder–like behaviors have spontaneous seizures. In this mouse model, it is difficult to evaluate the impact of recurrent seizures on psychomotor development. With long-term EEG recordings, we did not detect spontaneous seizures in *Scn1a*-mutant rats. This finding suggests that *Scn1a* mutation itself can cause neurodevelopmental disorder–like behaviors that include repetitive behavior, hyperactivity, anxiety-like behavior, impaired spatial learning, and motor imbalance.

Pathophysiologic basis of developmental disorder–like behaviors

Dravet syndrome patients with *SCN1A* mutations show hyperactivity in early childhood. *Scn1a* mutant rats are
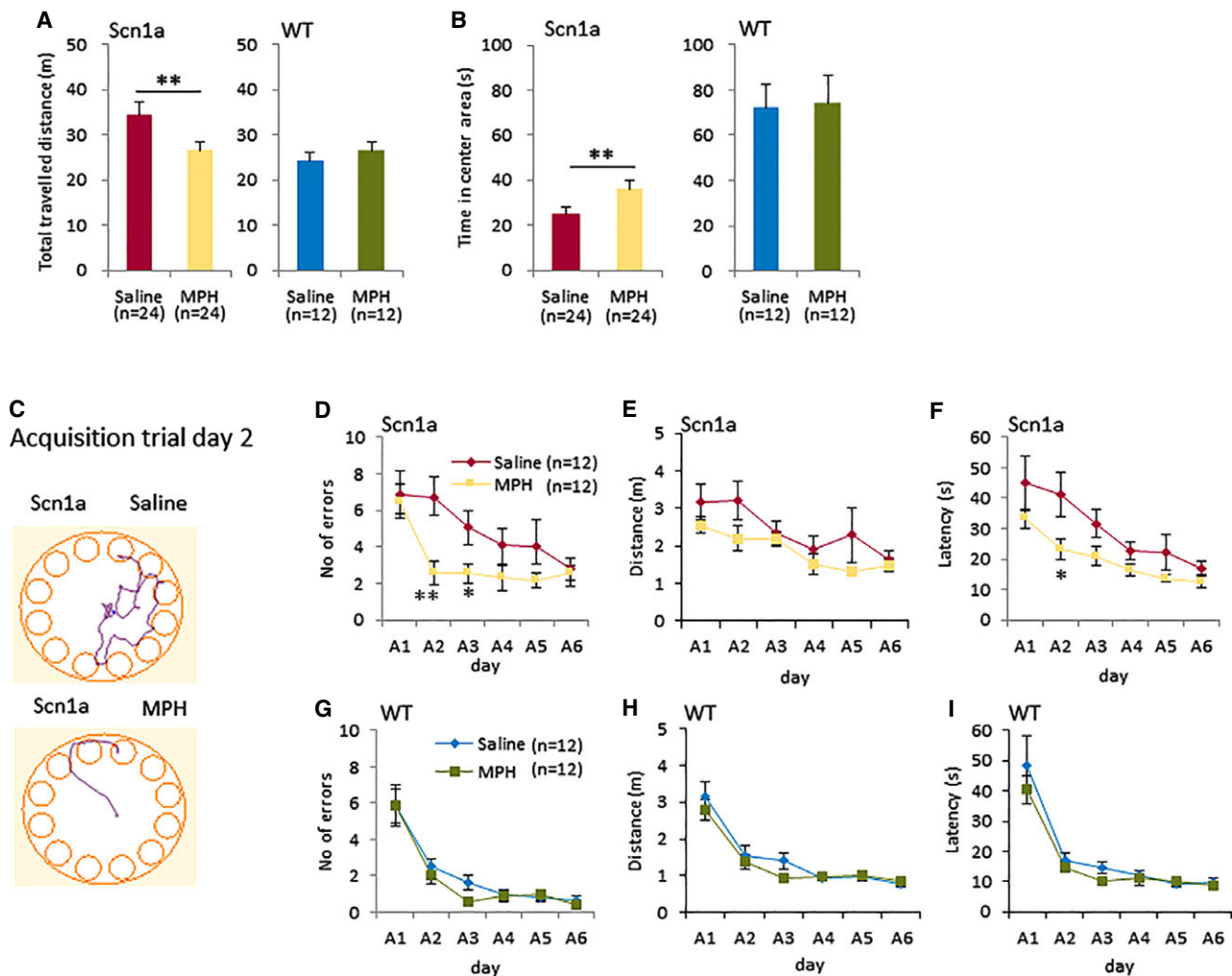
Figure 5.
MPH treatment improved hyperactivity, anxiety-like behavior, and spatial memory in *Scn1a*-mutant rats. Intraperitoneal administration of MPH significantly shortened the total traveled distance (**A**) and increased time spent in the center area (**B**) of the open field in *Scn1a*-mutant rats. (**C**) Representative traces of acquisition trial day 2 in the Barnes maze when *Scn1a*-mutant rats are treated with saline (top) and MPH (bottom). (**D**) The number of errors made by *Scn1a*-mutant rats was markedly decreased on day 2 when treated with MPH as compared with saline. (**E**) Distance and (**F**) time of latency to reach the target hole were also assessed in rats treated with saline and MPH. In contrast, there were no differences between treatments with MPH and saline in WT controls in the open field (**A,B**) and Barnes maze (**G**–**I**). $**p < 0.01$, $*p < 0.05$.
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hyperactive as well. Brain imaging studies of patients with ADHD have shown disrupted neurotransmission of the mesolimbic DA reward pathway (projection from the VTA to the NA).[24,25] Midbrain dopaminergic neurons play a key role in cognition, motivation, reward, and regulation of coordinated movements.[26,27] Therefore, using HPLC, we measured DA and its metabolites in several brain areas of *Scn1a*-mutant rats and discovered decreased DA levels in the NA and the striatum. Lower DA levels in the mesolimbic reward pathway may underlie hyperactivity and the cognitive impairment associated with *Scn1a* mutation.

Why are DA levels in the mesolimbic reward pathway in *Scn1a*-mutant rats significantly lower than in WT littermate

controls? Counting the number of TH-positive dopaminergic neurons in the VTA and SN revealed no difference in the amount of dopaminergic neurons between WT littermate controls and *Scn1a*-mutant rats. Although the number of cells may be comparable, DA content in the dopaminergic neuron–projecting area in *Scn1a*-mutant rats may be lower than that of WT littermate controls. Immunofluorescence revealed that Nav1.1 localized to TH-positive dopaminergic neurons. Dopaminergic neurons in the VTA fire spontaneously in a pacemaker-like manner, and pacemaking is inhibited by blocking voltage-dependent sodium current using tetrodotoxin.[28] Therefore, in the VTA of *Scn1a*-mutant rats, the electrophysiologic properties of the
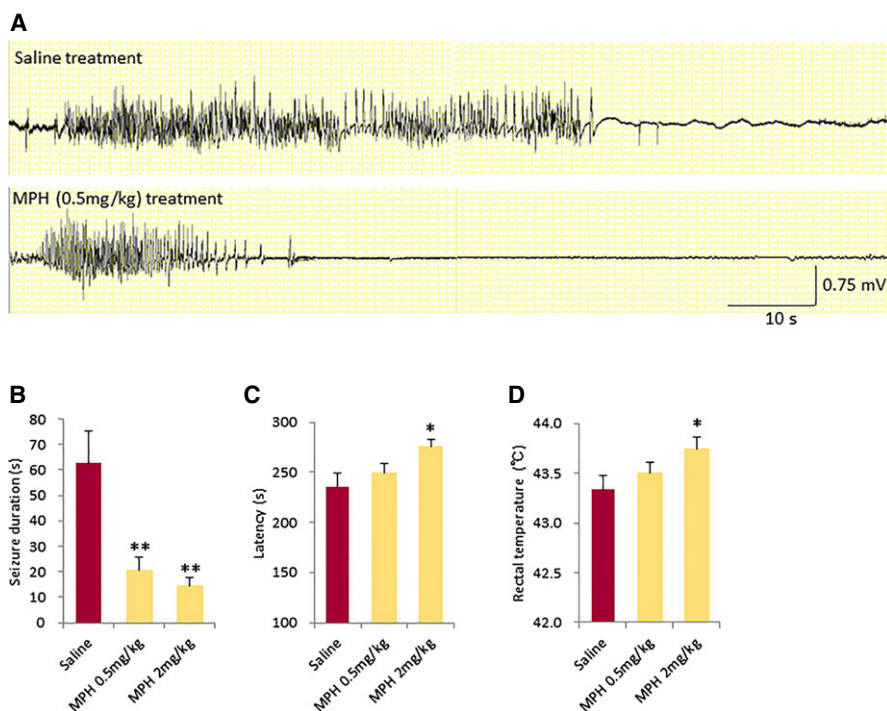
Figure 6.
MPH treatment shortened the duration of HIS and increased seizure threshold with dose dependence in *Scn1a*-mutant rats. (**A**) Representative EEG recordings from the frontal cortex during HIS in *Scn1a* rats pretreated with saline (top) or 0.5 mg/kg of MPH (bottom). (**B**) The mean duration of HIS in *Scn1a* rats pretreated with saline, 0.5 mg/kg of MPH, and 2 mg/kg of MPH. (**C**) Latency to HIS onset. (**D**) Rectal temperature at the onset of HIS. Saline, n = 14; 0.5 mg/kg of MPH, n = 14; 2 mg/kg of MPH, n = 15; **p < 0.01, *p < 0.05.
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sodium current may be altered by the N1417H-*Scn1a* mutation and in turn modify the firing properties of these dopaminergic neurons.

Effects of MPH on developmental disorder–like behaviors and HIS

MPH is the most common treatment for patients with ADHD. MPH increases brain DA and norepinephrine levels by blocking presynaptic reuptake transporters. Because *Scn1a*-mutant rats exhibit hyperactivity and low levels of DA in the NA, we attempted to rescue DA levels with intraperitoneal administration of MPH. We found that at a dose normally administered to patients with ADHD, MPH improved hyperactivity and spatial learning in *Scn1a*-mutant rats. Treatment with clonazepam, an enhancer of GABA-mediated neurotransmission, improved autistic-like behaviors in Scn1a-KO mice.[19] We propose that MPH can be another candidate to treat developmental disorders associated with *SCN1A* mutation.

How MPH affects epileptic seizures is unclear. Some studies revealed that MPH treatment had no adverse effect on epileptic seizures,[29,30] or might worsen epileptic seizures in ADHD patients with epilepsy.[31] Given that patients with *SCN1A* mutations often show intractable epilepsy, we wanted to determine if MPH can control *Scn1a* mutation–

related seizures. Surprisingly, MPH treatment strongly suppressed HIS in *Scn1a*-mutant rats. It is notable that MPH reduced HIS to an extent similar to the most effective anticonvulsant drugs such as diazepam and potassium bromide in our previous study.[16]

Dysfunction of GABAergic neurons is considered as the pathophysiologic basis of epilepsy caused by *Scn1a* mutations.[1,2,15] Indeed, anticonvulsants such as valproic acid, benzodiazepines, and potassium bromide—drugs that enhance GABA-mediated inhibition—were effective in Dravet syndrome patients with *SCN1A* mutations. Although we did not determine how MPH elevates seizure threshold, MPH may directly suppress brain excitability, enhance GABA-mediated inhibition, or have another unknown target. Further studies to elucidate the anticonvulsant action(s) of MPH in this model may lead to new treatments for epileptic patients with *SCN1A* mutations.

In conclusion, we demonstrated that *Scn1a* mutation can cause developmental disorder–like behaviors without recurring epileptic seizures. Low DA levels in the NA and the striatum may underlie these developmental disorder–like behaviors. MPH treatment improved hyperactivity and learning impairments in *Scn1a*-mutant rats. Unexpectedly, we found that MPH markedly suppressed HIS. Thus, MPH

might be effective to treat neurodevelopmental disorders and epilepsy in patients with Dravet syndrome and GEFS+.

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DISCLOSURE

None of the authors has any conflict to disclosure. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Long-term video-EEG monitoring.

Figure S2. *Scn1a*-mutant rats exhibit no histologic abnormalities of the brain.

Figure S3. MPH treatment did not change grooming time or motor balance in WT and *Scn1a*-mutant rats.

Table S1. General health and neurologic screening.

Data S1. Experimental procedures of behavioral studies.