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Autistic behavior in *Scn1a*$^{+/-}$ mice and rescue by enhanced GABAergic transmission

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Abstract

Haploinsufficiency of the *SCN1A* gene encoding voltage-gated sodium channel $Na_V1.1$ causes Dravet Syndrome (DS), a childhood neuropsychiatric disorder including recurrent intractable seizures, cognitive deficit, and autism-spectrum behaviors. The neural mechanisms responsible for cognitive deficit and autism-spectrum behaviors in DS are poorly understood. Here we show that mice with *Scn1a* haploinsufficiency display hyperactivity, stereotyped behaviors, social interaction deficits, and impaired context-dependent spatial memory. Olfactory sensitivity is retained, but novel food odors and social odors are aversive to *Scn1a*$^{+/-}$ mice. GABAergic neurotransmission is specifically impaired by this mutation, and selective deletion of $Na_V1.1$ channels in forebrain interneurons is sufficient to cause these behavioral and cognitive impairments. Remarkably, treatment with low-dose clonazepam, a positive allosteric modulator of $GABA_A$ receptors, completely rescued the abnormal social behaviors and deficits in fear memory in DS mice, demonstrating that they are caused by impaired GABAergic neurotransmission and not by neuronal damage from recurrent seizures. These results demonstrate a critical role for $Na_V1.1$ channels in neuropsychiatric functions and provide a potential therapeutic strategy for cognitive deficit and autism-spectrum behaviors in DS.

Dravet Syndrome (DS), also called Severe Myoclonic Epilepsy of Infancy, is an intractable developmental epilepsy syndrome with seizure onset in the first year of life[1]. However, unlike other generalized epilepsy disorders, it is accompanied by characteristic

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neuropsychiatric comorbidities, including hyperactivity, attention deficit, delayed psychomotor development, sleep disorder, anxiety-like behaviors, impaired social interactions, restricted interests, and severe cognitive deficits[1–6]. These comorbidities in DS overlap with symptoms of autism-spectrum disorders (ASD), and a recent study suggests that DS patients have autism-spectrum behaviors[3]. DS is caused by heterozygous loss-of-function mutations in the *SCN1A* gene[7], which encodes the pore-forming α-subunit of the brain voltage-gated sodium channel type-1 ($Na_V1.1$)[8]. As in DS, mice with heterozygous loss-of-function mutation in *Scn1a* (*Scn1a*$^{+/-}$) have thermally induced and spontaneous seizures, premature death, ataxia and sleep disorder[9–13]. $Na_V1.1$ channels are expressed in cell bodies and axon initial segments of excitatory and inhibitory neurons in the brain[14–16], but deletion of $Na_V1.1$ impairs Na^+ currents and action potential firing of GABAergic interneurons specifically because $Na_V1.1$ is the primary Na^+ channel in those cells[9,15,16]. Specific deletion of $Na_V1.1$ channels in forebrain interneurons using a Cre-LoxP strategy recapitulates the symptoms of DS in mice (Cheah, C. S., Yu, F. H., Westenbroek, R. E., Kalume, F. K., Oakley, J. C., Rubenstein, J. L., Catterall, W. A., *Soc. Neurosci. Abst.* 155.16, 2010)[17], confirming that loss of $Na_V1.1$ in GABAergic interneurons causes this disease. Emerging genetic evidence implicates *SCN1A* in autism[18–22], and there is increasing evidence that dysfunction of GABAergic signaling is associated with ASDs[23–25], leading to the proposal that elevation of excitation/inhibition ratio in neocortical neurons is the primary cause of ASD[26–29]. In this study, we have investigated autism-related behaviors in *Scn1a*$^{+/-}$ mice and shown that they are caused by impaired GABAergic neurotransmission that can be rescued by drug treatment.

Scn1a$^{+/-}$ mice exhibit hyperactivity, anxiety, and excessive stereotyped behaviors

Homozygous *Scn1a*$^{-/-}$ mice developed severe ataxia and died on postnatal day (P) 15, whereas *Scn1a*$^{+/-}$ mice had spontaneous seizures and sporadic deaths beginning after P21[9]. *Scn1a*$^{+/-}$ mice develop multiple behavioral phenotypes, which are phenocopies of comorbidities in DS. During a 10-min open-field test, adult *Scn1a*$^{+/-}$ mice traveled significantly longer than WT (Fig. 1a) but spent less time in the center of the open field (Fig. 1b, Supplementary Fig. 1). In the elevated plus maze, *Scn1a*$^{+/-}$ mice entered open arms less frequently compared with WT (Fig. 1c), and spent less time in the open arms (Fig. 1d, Supplementary Fig. 2). *Scn1a*$^{+/-}$ mice also spent more time self-grooming than WT (Fig. 1e, Supplementary Fig. 3a) and displayed increased circling behavior (Fig. 1f, Supplementary Fig. 3b). These observations indicate that *Scn1a*$^{+/-}$ mice exhibit hyperactivity, increased anxiety, and increased stereotyped behaviors, which are phenocopies of autistic traits in DS. *Scn1a*$^{+/-}$ mice also showed decreased nest-building ability compared to WT (Supplementary Fig. 4), which could indicate deficits in social behavior[30].

Scn1a$^{+/-}$ mice show deficits in social interaction behaviors

We performed behavioral tests to assess deficits in social interaction, a prominent symptom of ASD[31]. A three-chamber experiment showed that *Scn1a*$^{+/-}$ mice displayed profound deficits in social interaction. Both *Scn1a*$^{+/-}$ and WT had no preference for two empty cages, located in the right and the left chambers during a habituation period (Supplementary Fig. 5,

6, 7a). However, when we put a stranger mouse in the cage in one chamber, WT mice spent more time in the mouse-containing chamber than in the empty cage-containing chamber (Fig. 1g, Supplementary Fig. 5), and interacted more extensively with peer mice than with the empty cage (Supplementary Fig. 7b). In contrast, *Scn1a*$^{+/-}$ mice displayed no preference for the stranger mouse (Fig.1g, Supplementary Fig. 5, 7b). When a second stranger mouse was placed in the unoccupied side chamber to assess the discrimination between a novel and a familiar mouse, WT mice showed strong preference for the novel mouse, but *Scn1a*$^{+/-}$ mice did not (Fig. 1h, Supplementary Fig. 5, 7c), even though they have preference for novel objects (see below). We observed similar social deficits of *Scn1a*$^{+/-}$ mice in the social interaction test. *Scn1a*$^{+/-}$ mice interacted significantly less with a caged stranger mouse in an open field compared with WT (Fig. 1i, Supplementary Fig. 8a). When both the inanimate object and the caged stranger mouse were introduced simultaneously, WT mice interacted significantly more with a caged stranger mouse than with an empty cage, whereas *Scn1a*$^{+/-}$ mice displayed no preference for the caged mouse (Supplementary Fig. 9a, b). We also examined reciprocal social interactions of freely moving *Scn1a*$^{+/-}$ and WT littermates with test mice. *Scn1a*$^{+/-}$ mice displayed decreased duration of both non-aggressive and aggressive interactions (Fig. 1j). We observed that *Scn1a*$^{+/-}$ mice displayed increased immobilization behavior when they encountered the caged stranger mouse (Supplementary Fig. 8b). Compared to WT, this immobilization decreased distance traveled (Fig. 1k) and increased immobilization time by 400% (Fig. 1l). Taken together, these results indicate that *Scn1a*$^{+/-}$ mice display profound deficits in social behavior.

In nocturnal rodents, social interaction and olfactory perception are tightly associated[32], and impairment of olfactory perception leads to decreased social interaction[33]. We assessed olfaction in modified three-chamber experiments in which one tightly sealed petri dish containing food pellets and an identical one with holes were placed in the side chambers. Both *Scn1a*$^{+/-}$ and WT mice spent more time in the food-odor chamber, showed a shorter latency to enter it, and entered it more frequently than the odorless chamber (Supplementary Fig. 10a–d). Alternatively, we used bedding from male or female cages as a social odor. WT mice displayed strong preference for the chamber containing bedding, whereas *Scn1a*$^{+/-}$ mice displayed no preference for these social odors (Supplementary Fig. 11a, b, d, e). In close-interaction analysis, *Scn1a*$^{+/-}$ mice avoided interacting with male social cues (Supplementary Fig. 11c), and both WT and *Scn1a*$^{+/-}$ mice displayed strong avoidance to fox urine (Supplementary Fig. 11f). WT displayed strong habituation and dishabituation to odors of banana, male urine, and standard food, whereas *Scn1a*$^{+/-}$ mice gave a normal response to food but failed to display habituation/dishabituation to banana or male urine (Supplementary Fig. 12a). However, *Scn1a*$^{+/-}$ mice displayed greatly increased digging behavior when banana and male urine odors were presented, indicating that they detect these odors (Supplementary Fig. 12b). Moreover, in a Y-maze olfactory choice test, *Scn1a*$^{+/-}$ mice showed strong avoidance to banana and male urine, whereas WT mice displayed strong preference to both (Supplementary Fig. 12c, d). These data indicate that *Scn1a*$^{+/-}$ mice perceive food odors and social olfactory cues, but they have no interest or avoid unfamiliar odors and social odors. These results further establish a deficit in social interaction[34,35] and avoidance of environmental change[36] in *Scn1a*$^{+/-}$ mice, as in ASDs.

Scn1a$^{+/-}$ mice show deficits in context-dependent spatial memory

Both WT and *Scn1a*$^{+/-}$ mice showed a similar ability to recognize a novel object 24 h after training (Fig. 2a, b). In the context-dependent fear-conditioning test, *Scn1a*$^{+/-}$ and WT mice displayed no freezing behavior during the habituation period in context, and both of them showed similar freezing behavior immediately after a mild foot shock (Fig. 2c). However, whereas WT mice displayed sustained freezing behaviors when returned to the shock cage 30 min and 24 h later, *Scn1a*$^{+/-}$ mice displayed substantially reduced freezing behavior (Fig. 2c). The loss of fear-associated freezing behavior was specific because measurements of distance and velocity of movement during the fear-conditioning test did not reveal other fear-associated responses such as panic fleeing (Supplementary Fig. 13).

To assess spatial learning and memory in the absence of fear, we performed the Barnes circular maze test in which mice learn to rapidly escape a brightly lighted circular field by finding a specific dark hole at its periphery. *Scn1a*$^{+/-}$ mice failed to improve their learning performance during four days of training (Fig. 2d, e), and displayed substantially reduced spatial memory during the probe trials at day 5 (Fig. 2f – i). These data, together with the results of the context-dependent fear-conditioning test (Fig. 2c), indicate that *Scn1a*$^{+/-}$ mice have severely impaired spatial learning and memory.

Conditional *Scn1a*$^{+/-}$ mutant mice show autism-related behaviors

To determine whether the autism-related phenotypes of *Scn1a*$^{+/-}$ mice emerge specifically from reduced $Na_V1.1$ activity in forebrain GABAergic neurons, we generated forebrain GABAergic neuron-specific conditional *Scn1a*$^{+/-}$ mutant mice using the *Dlxi12b-Cre* Cre-recombinase mouse line *(Dlx1/2-Cre*; Cheah, C. S., Yu, F. H., Westenbroek, R. E., Kalume, F. K., Oakley, J. C., Rubenstein, J. L., Catterall, W. A., *Soc. Neurosci. Abst.* 155.16, 2010)[17,37]. These mice have a specific reduction of $Na_V1.1$ channels in forebrain GABAergic neurons and have similar epilepsy and premature death as *Scn1a*$^{+/-}$ mice[38]. *Dlx1/2*$^{+}$ *Scn1a* heterozygous mutant mice (*Dlx1/2-Scn1a*$^{+/-}$) recapitulated the autism-related phenotypes and spatial learning deficit of *Scn1a*$^{+/-}$ mice (Fig. 3), whereas control Cre-positive *Scn1a*$^{+/+}$ mice did not (Supplementary Fig. 14). In the open field test, *Dlx1/2-Scn1a*$^{+/-}$ mice traveled more (Fig. 3a), spent less time in the center (Fig. 3b), and displayed increased circling behavior (Fig. 3c) [17]when compared with Cre-negative *Scn1a*$^{loxp/+}$ mice. In the elevated-plus maze test, *Dlx1/2-Scn1a*$^{+/-}$ mice entered the open arms less frequently and spent less time in open arms than Cre-negative *Scn1a*$^{loxp/+}$ mice (Fig. 3d, e). In the social interaction test, *Dlx1/2-Scn1a*$^{+/-}$ mice spent less time interacting with the caged stranger mouse compared to Cre-negative *Scn1a*$^{loxp/+}$ mice (Fig. 3f). In addition, in the three-chamber test Cre-negative *Scn1a*$^{loxp/+}$ mice stayed longer in the mouse chamber vs. the inanimate-object chamber; in contrast, *Dlx1/2-Scn1a*$^{+/-}$ mice showed no preference (Fig. 3g). Finally, in the contextual fear-conditioning test, *Dlx1/2-Scn1a*$^{+/-}$ mice displayed similar freezing behavior in control and training sessions, but significantly less freezing behavior in the 30 min and 24 h after the training compared with *Scn1a*$^{loxp/+}$ mice (Fig. 3h). These results show that *Dlx1/2-Scn1a*$^{+/-}$ mice reproduce hyperactive and anxiety-like behaviors, deficits in social interactions, and impaired context-dependent fear conditioning of global

Scn1a$^{+/-}$ mice. This evidence indicates that the autism-related phenotype emerges from reduced $Na_V1.1$ activity specifically within forebrain GABAergic interneurons.

Deficit of $Na_V1.1$ channels impairs cortical and hippocampal GABAergic interneuron neurotransmission

To test our hypothesis that the autism-related phenotypes and spatial learning deficits in *Scn1a*$^{+/-}$ mice are caused by decreased $Na_V1.1$ activity in GABAergic interneurons in the forebrain, we compared the properties of cortical and hippocampal GABAergic interneurons in WT and *Scn1a*$^{+/-}$ mice. $Na_V1.1$ protein is expressed in adult hippocampal and neocortical interneurons, as assessed by co-immunolabeling of $Na_V1.1$ channels and GABA in the hippocampal CA1 region (Fig. 4a) and prefrontal cortex (Supplementary Fig. 15). The proportion of GABAergic interneurons expressing $Na_V1.1$ in of *Scn1a*$^{+/-}$ mice was decreased 20–50% throughout the cortex and hippocampus (Fig. 4b), whereas there was no reduction in the total number of GABA-stained interneurons (Supplementary Fig. 16, *legend*). The deep layer of prefrontal cortex was the most affected by the *Scn1a* mutation (Fig. 4b), and the intensity of immunostaining for $Na_V1.1$ in GABAergic cells with detectable staining was reduced 50% in the prefrontal cortex (Supplementary Fig. 16).

Some forms of autism are postulated to be caused by an imbalance of synaptic transmission between excitatory and inhibitory circuits[26–29]. *Scn1a*$^{+/-}$ mice have reduced Na^+ currents and impaired action potential firing in hippocampal interneurons and cerebellar Purkinje neurons[9,10], which are GABAergic neurons. When action potentials were blocked with tetrodotoxin (TTX, 1 μM), recordings of miniature inhibitory postsynaptic currents (IPSC) and miniature excitatory postsynaptic current (EPSC) from the hippocampal CA1 region and the prefrontal cortex showed that amplitude and frequency were not altered, indicating normal synaptic function in *Scn1a*$^{+/-}$ slices (Supplementary Fig. 17, 18). Similarly, in the absence of TTX, the amplitudes of spontaneous IPSCs and spontaneous EPSCs were unchanged (Fig. 4c, d, Supplementary Fig. 19, 20), indicating that the postsynaptic response to released neurotransmitter was not altered. In contrast, in the absence of TTX, the frequency of spontaneous IPSCs in hippocampal CA1 and prefrontal cortex slices from *Scn1a*$^{+/-}$ mice was reduced (Fig. 4c, f, Supplementary Fig. 20a, b), and the frequency of spontaneous EPSCs was increased (Fig. 4d, g, Supplementary Fig. 20c, d) compared to WT slices. Because no differences in frequencies of miniature IPSCs or EPSCs were observed when action potentials were blocked by TTX, these changes in frequencies of IPSCs and EPSCs recorded in the absence of TTX must represent differences in action potential-dependent neurotransmission. Therefore, these results indicate that inhibitory synaptic input was decreased because of reduced firing frequency of GABAergic interneurons caused by *Scn1a* haploinsufficiency, whereas excitatory synaptic activity was increased as an indirect consequence of decreased inhibition.

Treatment of autism-related phenotypes in *Scn1a*$^{+/-}$ mice with clonazepam

Given that the autism-related phenotype and spatial-learning deficit in *Scn1a*$^{+/-}$ mice emerge from decreased $Na_V1.1$ activity in GABAergic interneurons, we reasoned that they could be rescued by increasing the strength of GABAergic transmission. To test this idea,

we treated *Scn1a*$^{+/-}$ and WT mice with the benzodiazepine clonazepam, a positive allosteric modulator of the $GABA_A$ receptor. Benzodiazepines do not open the $GABA_A$ receptor chloride channel in the absence of GABA, but instead boost GABA signaling only when presynaptically released GABA binds to the receptor[39]. First, we examined the effects of clonazepam in the open-field and elevated plus-maze tests to avoid potential sedative effects in our behavioral experiments, which depend on locomotor activity. The maximal intraperitoneal dose of clonazepam that did not cause significant sedation or anxiolytic effect in the open field and elevated plus maze tests was 0.0625 mg/kg for *Scn1a*$^{+/-}$ mice (Fig. 5a; Supplementary Fig. 21), 20-fold lower than typical anxiolytic doses[40]. To test the effect of clonazepam on social behavior, we performed three sets of identical trials at one week intervals with same groups of mice. In the first trial, we performed the social interaction test in the open arena and the three-chamber test without any treatment. In a subsequent trial, the same behavioral tests were performed 30 min after i.p. injection of 0.0625 mg/kg clonazepam. In the last trial, the tests were performed 30 min after i.p. injection of vehicle. The data were analyzed as the ratio of the time of interaction with a stranger mouse over the time of interaction with an empty cage. Both in the open arena and in the three-chamber test, clonazepam treatment completely rescued impaired social behaviors of the *Scn1a*$^{+/-}$ mice, and this effect was reversed after the one-week clearing period (Fig. 5b, c; Supplementary Fig. 22, 23). In contrast, low-dose clonazepam had no effect on the social behavior of WT mice. Treatment with low-dose clonazepam 30 min before testing also rescued impaired context-dependent fear conditioning. Whereas WT mice were unaffected by clonazepam (Fig. 5d), *Scn1a*$^{+/-}$ mice displayed a complete reversal of the loss of their 30-min and 24-h contextual fear memory (Fig. 5e). These results indicate that a single low dose of clonazepam can reversibly rescue core autistic traits and cognitive deficit in *Scn1a*$^{+/-}$ mice.

We also tested the effects of clonazepam on GABAergic inhibitory transmission in the hippocampal CA1 region in *Scn1a*$^{+/-}$ mice. As expected, treatment with 10 μM clonazepam increased sIPSC amplitude, but not frequency, in *Scn1a*$^{+/-}$ hippocampal slices (Fig. 5f, Supplementary Fig. 24a). The increased amplitude of spontaneous IPSCs after treatment with 10 μM clonazepam leads to a decrease in frequency of spontaneous EPSCs, without change in amplitude in *Scn1a*$^{+/-}$ hippocampal slices (Fig. 5g, Supplementary Fig. 24b). These results support our hypothesis that behavioral rescue by treatment with clonazepam is associated with increased strength of inhibitory transmission.

Discussion

Despite their adverse impacts on quality of life, the neuropsychiatric comorbidities and cognitive deficit in DS have not previously been studied in an animal model, and the role of the $Na_V1.1$ channel in these deficits in brain functions was unknown. Our results show that mice with heterozygous loss-of-function mutation in $Na_V1.1$ channels display both cognitive deficits and autistic traits, including hyperactivity, anxiety, excessive stereotyped behaviors, and social interaction deficits. Together with previously reported phenotypes of epilepsy[9], premature death[9], thermally induced seizures[11], ataxia[10], and sleep dysfunction[12], these studies show that *Scn1a*$^{+/-}$ mice phenocopy all the major symptoms of DS.

These cognitive and behavioral deficits in *Scn1a*$^{+/-}$ mice are caused by decreased action potential firing in forebrain GABAergic interneurons. Our previous studies show that deletion of $Na_V1.1$ channels causes selective reduction in Na^+ currents and action potential firing of GABAergic interneurons in hippocampus and cerebellum[9,10]. This deficit in action potential firing in interneurons in the hippocampus leads to a selective loss of inhibitory neurotransmission compared to excitatory transmission (Fig. 4). Moreover, *Dlx1/2-Scn1a*$^{+/-}$ mice, which have a specific deficit in $Na_V1.1$ channels in forebrain GABAergic interneurons, reproduce the core autistic features and cognitive deficits (Fig. 3). These results indicate that the autism-related traits in DS mice are caused by decreased inhibitory neurotransmission in GABAergic interneurons as a consequence of *Scn1a* haploinsufficiency.

To further test this hypothesis, we treated *Scn1a*$^{+/-}$ with clonazepam, a benzodiazepine, in order to reverse decreased GABAergic tone. High-dose benzodiazepine has been widely used to alleviate epileptic seizure[13] and anxiety-like behaviors[40], but not for rescuing major autism-related behaviors because of its sedative effects. Remarkably, a single low-dose clonazepam injection completely rescued deficits in social interactions and fear-associated contextual memory without sedative or anxiolytic effects in *Scn1a*$^{+/-}$ mice. The reversible rescue of cognitive deficit and autism-related behaviors by clonazepam at the time of training implies that these comorbidities in DS mice are not caused by recurrent seizure-induced excitotoxicity, but instead are caused by *Scn1a* haploinsufficiency and the resulting reduction of GABAergic transmission. These results suggest that low-dose benzodiazepine treatment could be a potential pharmacological intervention for cognitive deficit and autistic symptoms in DS patients.

Genome-wide association studies identified the chromosome 2q24.3 region, where the *SCN1A* gene is located, as an autism susceptibility locus[18,19]. Sequencing of the genomes of autistic patients identified mutations of *SCN1A* gene in familial autism[20]. Exome sequencing revealed that *de novo* mutations in the *SCN1A* gene cause autism[21]. Our results suggest the hypothesis that DS should be included in the category of ASD-related syndromes, such as Fragile-X Syndrome, Rett Syndrome, and Timothy Syndrome[41]. With a prevalence of 1:20,000 births for DS and related *SCN1A* channelopathies[42], DS is less frequent than Fragile-X Syndrome (1:5000) or Rett Syndrome (1:10,000) but much more common than Timothy Syndrome (<1:1,000,000). Interestingly, mutations in many ASD susceptibility genes also exhibit cytogenetic dysfunctions in GABAergic interneurons[24–29,43,44]. Thus, autistic traits in DS and in a broad range of ASDs are likely caused by a reduction of GABAergic signaling. Our results suggest that low-dose benzodiazepine treatment may be effective in alleviating these autistic traits and cognitive impairment in DS and possibly in ASDs more broadly.

METHODS SUMMARY

Animals

The mice used for all behavioral analyses were 6 – 8 month-old adult male mice except DLX1/2 conditional mutant mice which were 3 – 5 months old. Adult mice 10 months old were used for Immunohistochemical staining, and young mice 3 – 4 weeks old were used for

electrophysiological recording. All behavioral tests were done blind to genotypes with age-matched littermate pairs of male mice. All experiments with animals were performed according to the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the University of Washington Institutional Animal Care and Use Committee.

Statistical analysis

All data are shown as mean ± s.e.m. and analyzed using Student's t-test, one-way ANOVA with Tukey's post hoc comparison, and two-way ANOVA with Bonferroni's post hoc comparison. All the statistical analyses were done using Prism 4 (GraphPad). Details of particular tests in each experiment are described in the supplementary methods section, and full statistical tests and values for behavioral data are presented in Supplementary Table 1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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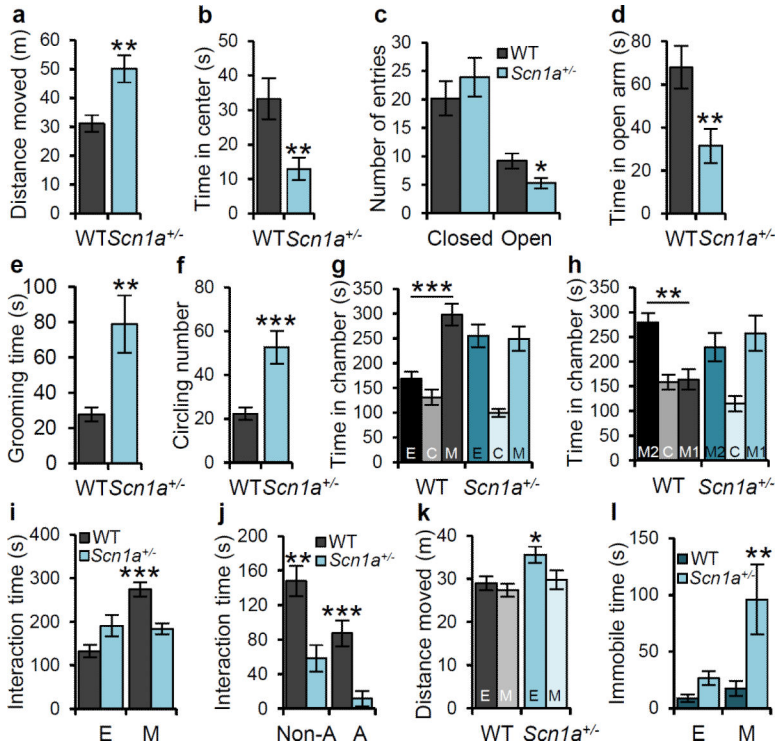

Figure 1. *Scn1a*$^{+/-}$ mice display hyperactivity, anxiety-like behavior, increased stereotypies, poor nest-building, and impaired social behavior

In the open field test, *Scn1a*$^{+/-}$ mice travel longer distances compared with WT mice **(a)**, spend less time in the center **(b)**, spend more time grooming **(e)** and circling **(f)** than WT mice. In the elevated plus maze, *Scn1a*$^{+/-}$ mice enter less frequently in the open arms **(c)** and spend less time in the open arms (**d**). In (**f**) one complete turn is counted as one circle, regardless of direction. **g**, **h**, Three-chamber experiment. **g**, Whereas WT mice spend more time in the chamber housing a stranger mouse (M) than the chamber housing an empty cage (E), *Scn1a*$^{+/-}$ mice have no preference for either chamber. **h**, Whereas WT mice spend more time in the chamber housing a novel mouse (M2) than in a chamber housing a familiar mouse (M1), *Scn1a*$^{+/-}$ mice have no preference for either chamber. **i - l, Social interaction test. i,** *Scn1a*$^{+/-}$ mice show decreased interaction with a caged stranger mouse when compared with WT mice. **j**, In a 10-min reciprocal interaction test, pairs of WT and *Scn1a*$^{+/-}$ unfamiliar mice had significantly less non-aggressive (Non-A) and aggressive (A) interactions than pairs of WT and WT unfamiliar mice. Aggressive behaviors included attacking, wrestling, and biting the dorsal surface, and non-aggressive behaviors include nose-to-nose sniffing, anogenital sniffing, and grooming. All data shown are means ± s.e.m. from 10 – 12 mice per genotype. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. **k**, *Scn1a*$^{+/-}$ mice move significantly less when they encountered the stranger mouse compared to an empty cage, whereas there is no difference in movement for WT. **l**, *Scn1a*$^{+/-}$ mice, but not WT mice, show increased immobilization behavior in the presence of the caged stranger mouse than in the presence of an empty cage.

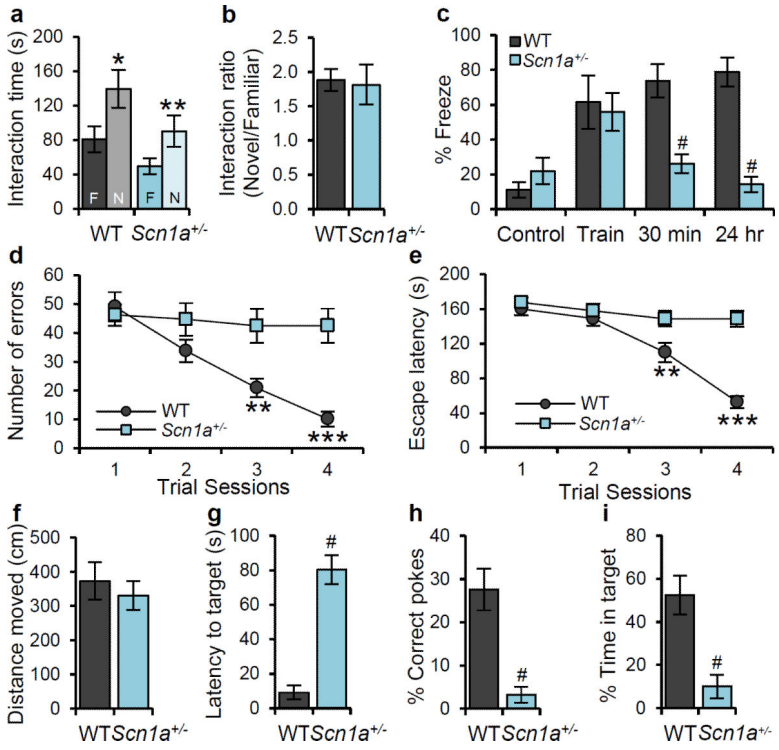

Figure 2. Profound deficits in context-dependent spatial learning and memory formation in *Scn1a*$^{+/-}$ mice

a, In the novel object recognition test, *Scn1a*$^{+/-}$ mice show normal recognition memory for preconditioned object (F: Familiar), which was presented 24 hr before the test, so that they spend more time with novel object (N: Novel). **b**, Discrimination index, the normalized ratio of time spent with the familiar object and time spent with the novel object, shows that there is no difference between WT and *Scn1a*$^{+/-}$ mice for novel object recognition ability. **c**, In the contextual fear conditioning test, *Scn1a*$^{+/-}$ mice display a profound deficits in short-term (30 min) and long-term (24 hr) memory of 2 s mild foot shock (0.5 mA)-associated context, but normal fear response immediately after the training (Train) when compared to WT mice. **d**, **e**, In the Barnes circular maze test, *Scn1a*$^{+/-}$ mice display profound deficit in spatial learning. WT mice make less errors to find the target hole (d), and display decreased latency to escape the maze (e) during the 4-day repeated training trials, but *Scn1a*$^{+/-}$ mice display no improved performance for both the number of errors made to find the target hole (**d**), and the time to escape the maze (**e**). **f - i**, During the probe trial at 5th day of trials, *Scn1a*$^{+/-}$ mice display profound deficit in spatial memory. They spend significantly more time to find the target hole (**g**), poke target hole with significantly lower correct choice (**h**), and stay significantly less time in the target area (**i**) when compared with WT mice, although total moved distance is not significantly different with that of WT mice (**f**). All data shown are means ± s.e.m. from 6 – 10 mice per genotype. **P* < 0.05, #, ****P* < 0.001.

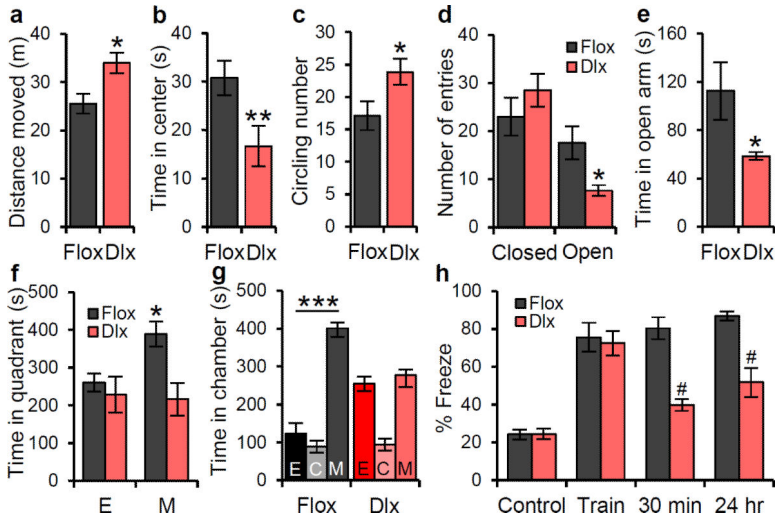

Figure 3. *Dlx1/2-Scn1a*$^{+/-}$ mice have the impaired spatial learning and autism-related phenotypes observed in *Scn1a*$^{+/-}$ mice

a, In the open field test, *Dlx1/2-Scn1a*$^{+/-}$ mice run longer compared with Cre-negative floxed littermate mice. **b**, In the open field test, *Dlx1/2-Scn1a*$^{+/-}$ mice spend less time in the center during the open field test. **c**, *Dlx1/2-Scn1a*$^{+/-}$ mice show increased circling behavior. One complete turn, regardless of direction is counted as one circling. **d**, **e**, In the elevated plus maze, *Dlx1/2-Scn1a*$^{+/-}$ mice enter less frequently in open arms (**d**), and spend significantly less time in open arms (**e**). **f**, In the social interaction test, *Dlx1/2-Scn1a*$^{+/-}$ mice display decreased interaction with social cue when compared to negative floxed littermate mice. **g** , In the 3-chamber test, *Dlx1/2-Scn1a*$^{+/-}$ mice have no preference for the stranger mouse. **h**, In the contextual fear conditioning test, *Dlx1/2-Scn1a*$^{+/-}$ mice display profound deficit in short-term (30 min) and long-term (24 hr) memory of 2 s mild foot shock (0.5 mA)-associated context, but normal fear response immediately after the foot shock during training (Train) when compared to WT mice. Dlx, *Dlx1/2-Scn1a*$^{+/-}$ mice. Flox, Cre-negative floxed *Scn1a*$^{+/+}$ mice. E, Empty cage. C, Center. M, Mouse. All data shown are means ± s.e.m. from 7 – 9 mice per genotype. *$P < 0.05$; **$P < 0.01$; $^{\#}$, ***$P < 0.001$.

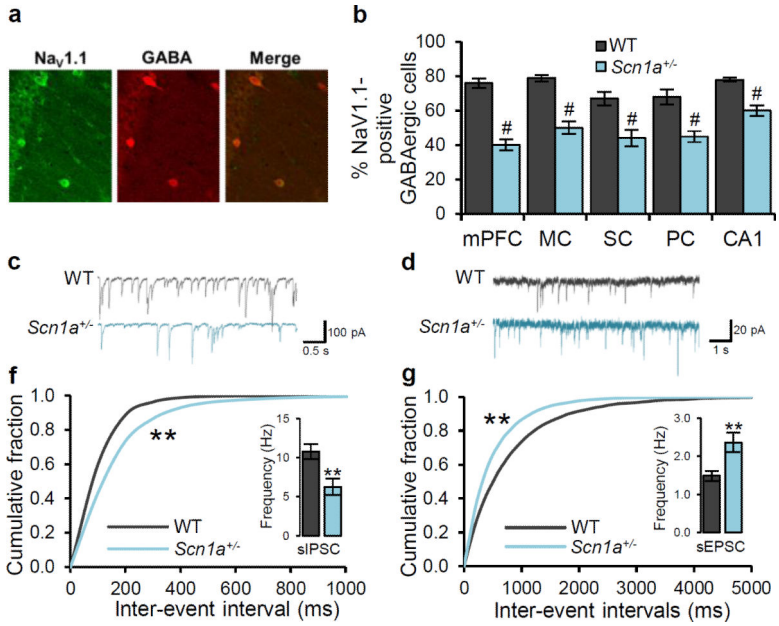

Figure 4. Deficit of Na$_V$1.1 channels and GABAergic neurotransmission in *Scn1a*$^{+/-}$ hippocampal GABAergic interneurons

Immunocytochemical staining of forebrain neurons from 10-month old mice for Na$_V$1.1 channels. **a,** Co-immunolabeling of Na$_V$1.1 and GABA reveals co-expression of Na$_V$1.1 and GABA in the hippocampal CA1 region in WT mice. **b**, Co-immunolabeling of Na$_V$1.1 and GABA reveals a decreased expression of Na$_V$1.1 channels in GABAergic interneurons in various forebrain regions in *Scn1a*$^{+/-}$ mice. **c**, Example traces of sIPSC from WT and *Scn1a*$^{+/-}$ mice hippocampal CA1 region. **d**, Example traces of sEPSC from WT and *Scn1a*$^{+/-}$ mice hippocampal CA1 region. **f**, Cumulative plot and average values (inset) of sIPSC frequency. The frequency of sIPSC is decreased, but the amplitude of sIPSC is unchanged in *Scn1a*$^{+/-}$ hippocampal CA1 slices when compared to WT slices (Suppl. Fig. 17a). **g**, Cumulative plot and average values (inset) of sEPSC frequency. The frequency of sEPSC is increased, but the amplitude of sEPSC is unchanged in *Scn1a*$^{+/-}$ hippocampal CA1 slices when compared to WT slices (Suppl. Fig. 17b). mPFC, medial prefrontal cortex. MC, motor cortex. SC, sensory cortex. PC, parietal cortex. CA1, hippocampal CA1 region. All data shown are means ± s.e.m. from 15 – 19 recordings per genotype. $^{\#}$, $^{**}P < 0.01$.

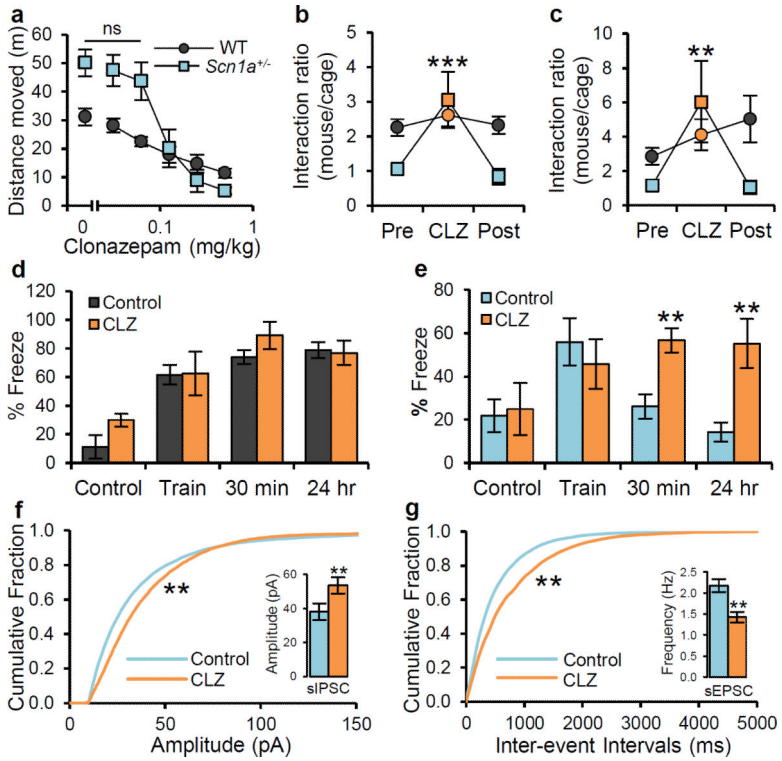

Figure 5. Complete rescue of impaired social behavior and fear-associated memory deficits by low-dose clonazepam treatment

a, Both WT and *Scn1a*$^{+/-}$ mice show dose-dependent sedation by CLZ. Maximal concentration of CLZ without sedative effect is 0.0625 mg/kg. **b**, **c**, In the social interaction test (**b**) and 3-chamber test (**c**), decreased social interaction in *Scn1a*$^{+/-}$ mice is completely recovered by a single i.p. injection of 0.0625 mg/kg CLZ, 30 min prior to the test; this CLZ effect on social interaction completely disappears after 1 week of clearance period in the same *Scn1a*$^{+/-}$ mice. CLZ effects on social interaction are absent in WT mice. **d**, **e**, In the contextual fear conditioning test, a single i.p. injection of 0.0625 mg/kg CLZ, 30 min prior to the training, leads to a complete rescue of short-term (30 min) and long-term (24 h) fear-associated contextual memory in *Scn1a*$^{+/-}$ mice (**e**), but no significant change of fear-associated contextual memory by CLZ is observed in WT mice (**d**). Pre, Pre-clonazepam treated. CLZ, Clonazepam. Post, Post-Clonazepam treated. All data shown are means ± s.e.m. from 6 – 12 mice per genotype. ns, not significant. **f**, Cumulative plot and average value (inset) of sIPSC amplitude. The treatment of 10 μM CLZ increases the amplitude of sIPSC, but the frequency of sIPSC is unchanged by 10 μM CLZ in *Scn1a*$^{+/-}$ hippocampal CA1 slices (Suppl. Fig. 24a). **g**, Cumulative plot and average value (inset) of sEPSC frequency. The treatment of 10 μM CLZ decreases the frequency of sEPSC, but the amplitude of sEPSC is unchanged by 10 μM CLZ in *Scn1a*$^{+/-}$ hippocampal CA1 slices (Suppl. Fig. 24b). All data shown are means ± s.e.m. from 15 – 20 recordings per treatment group. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.