Autistic-like behaviour in Scn1a\(^{+/-}\) mice and rescue by enhanced GABA-mediated neurotransmission

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Haploinsufficiency of the SCN1A gene encoding voltage-gated sodium channel Na\(_V\)1.1 causes Dravet’s syndrome, a childhood neuropsychiatric disorder including recurrent intractable seizures, cognitive deficit and autism–spectrum behaviours. The neural mechanisms responsible for cognitive deficit and autism–spectrum behaviours in Dravet’s syndrome are poorly understood. Here we report that mice with Scn1a haploinsufficiency exhibit hyperactivity, stereotyped behaviours, social interaction deficits and impaired context–dependent spatial memory. Olfactory sensitivity is retained, but novel food odours and social odours are aversive to Scn1a\(^{+/-}\) mice. GABAergic neurotransmission is specifically impaired by this mutation, and selective deletion of Na\(_V\)1.1 channels in forebrain interneurons is sufficient to cause these behavioural and cognitive impairments. Remarkably, treatment with low-dose clonazepam, a positive allosteric modulator of GABA\(_A\) receptors, completely rescued the abnormal social behaviours and deficits in fear memory in the mouse model of Dravet’s syndrome, 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\(_V\)1.1 channels in neuropsychiatric functions and provide a potential therapeutic strategy for cognitive deficit and autism–spectrum behaviours in Dravet’s syndrome.

Dravet’s 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 neuropsychiatric comorbidities, including hyperactivity, attention deficit, delayed psychomotor development, sleep disorder, anxiety-like behaviours, 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 behaviours\(^7\). DS is caused by heterozygous loss-of-function mutations in the SCN1A gene\(^7\), which encodes the pore-forming \(\alpha\)-subunit of the brain voltage-gated sodium channel type-1 (Na\(_V\)1.1)\(^7\). 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\(^8\)–\(^13\). Na\(_V\)1.1 channels are expressed in cell bodies and axon initial segments of excitatory and inhibitory neurons in the brain\(^11\)–\(^13\), but deletion of Na\(_V\)1.1 impairs Na\(^{+}\) currents and action potential firing of GABAergic interneurons specifically because Na\(_V\)1.1 is the primary Na\(^{+}\) channel in those cells\(^8\)–\(^10\). Specific deletion of Na\(_V\)1.1 channels in forebrain interneurons using a Cre-LoxP strategy recapitulates the symptoms of DS in mice\(^15\), confirming that loss of Na\(_V\)1.1 in GABAergic interneurons causes this disease. Emerging genetic evidence implicates SCN1A in autism\(^18\)–\(^22\), and there is increasing evidence that dysfunction of GABAergic signalling 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 behaviours in Scn1a\(^{+/-}\) mice and shown that they are caused by impaired GABAergic neurotransmission that can be rescued by drug treatment.

Hyperactivity, anxiety and stereotypies in Scn1a\(^{+/-}\) mice

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 (ref. 9). Scn1a\(^{+/-}\) mice develop multiple behavioural phenotypes, which are phenocopies of comorbidities in DS. During a 10-min open-field test, adult Scn1a\(^{+/-}\) mice travelled significantly farther than wild type (Fig. 1a), but spent less time in the centre of the open field (Fig. 1b and Supplementary Fig. 1). Scn1a\(^{+/-}\) mice also spent more time self-grooming than wild type (Fig. 1c and Supplementary Fig. 3a) and showed increased circling behaviour (Fig. 1d and Supplementary Fig. 3b). In the elevated plus maze, Scn1a\(^{+/-}\) mice entered open arms less frequently compared with wild type (Fig. 1e), and spent less time in the open arms (Fig. 1f and Supplementary Fig. 2). These observations indicate that Scn1a\(^{+/-}\) mice exhibit hyperactivity, increased anxiety and increased stereotyped behaviours, which are phenocopies of autistic traits in DS. Scn1a\(^{-/-}\) mice also have decreased nest-building ability compared to wild type (Supplementary Fig. 4), which could indicate deficits in social behaviour\(^30\).

Scn1a\(^{+/-}\) mice have deficits in social interaction

We performed behavioural tests to assess deficits in social interaction, a prominent symptom of ASD\(^31\). A three-chamber experiment showed that Scn1a\(^{+/-}\) mice have profound deficits in social interaction. Both Scn1a\(^{+/-}\) and wild type had no preference for two empty cages, located in the right and the left chambers during a habituation period (Supplementary Figs 5, 6, 7a). However, when we put a stranger mouse in the cage in one chamber, wild-type mice spent more time in the mouse-containing chamber than in the empty cage-containing chamber (Fig. 1g and Supplementary Fig. 5), and...
interacted more extensively with peer mice than with the empty cage (Supplementary Fig. 7b). In contrast, Scn1a+/− mice showed no preference for the stranger mouse (Fig. 1g and Supplementary Figs 5 and 1f). Wild-type mice exhibited strong habituation and dishabitation to odours of banana, male urine and standard food, whereas Scn1a+/− mice gave a normal response to food but failed to show habituation/dishabitation to banana or male urine (Supplementary Fig. 12a). However, Scn1a+/− mice had greatly increased digging behaviour when banana and male urine odours were presented, indicating that they detect these odours (Supplementary Fig. 12b). Moreover, in a Y-maze olfactory choice test, Scn1a+/− mice strongly avoided banana and male urine, whereas wild-type mice had a strong preference for both (Supplementary Fig. 12c, d). These data indicate that Scn1a+/− mice perceive food odours and social olfactory cues, but they have no interest or avoid unfamiliar odours and social odours. These results further establish a deficit in social interaction in ASDs and avoidance of environmental change in Scn1a+/− mice, as in ASDs.

**Scn1a+/− mice have deficits in context-dependent spatial memory**

Both wild-type and Scn1a+/− mice had similar ability to recognize a new object 24 h after training (Fig. 2a, b). In the context-dependent fear-conditioning test, Scn1a+/− and wild-type mice showed no freezing behaviour during the habituation period in context, and both of them had similar freezing behaviour immediately after a mild foot shock (Fig. 2c). However, whereas wild-type mice showed sustained freezing behaviours when returned to the shock cage 30 min and 24 h later, Scn1a+/− mice had substantially reduced freezing behaviour (Fig. 2c). The loss of fear-associated freezing behaviour 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 had 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 exhibit autism-related behaviours**

To determine whether the autism-related phenotypes of Scn1a+/− mice emerge specifically from reduced NaV1.1 activity in forebrain GABAergic neurons, we generated forebrain GABAergic neuron-specific conditional Scn1a+/− mutant mice using the Dlx112b-Cre transgene. We observed that Scn1a+/− mice exhibited increased immobilization behaviour when they encountered the caged stranger mouse (Supplementary Fig. 8b). Compared to wild type, this immobilization decreased distance travelled (Fig. 1k) and increased immobilization time by 400% (Fig. 1l). Taken together, these results indicate that Scn1a+/− mice have profound deficits in social behaviour.

In nocturnal rodents, social interaction and olfactory perception are tightly associated, and impairment of olfactory perception leads to decreased social interaction. We assessed olfaction in modified three-chamber experiments in which a tightly sealed Petri dish containing food pellets and an identical one with holes were placed in the side chambers. Both Scn1a+/− and wild-type mice spent more time in the food-odour chamber, showed a shorter latency to enter it, and entered it more frequently than the odourless chamber (Supplementary Fig. 10a–d). Alternatively, we used bedding from male or female cages as a social odour. Wild-type mice had a strong preference for the chamber containing bedding, whereas Scn1a+/− mice had no preference for these social odours (Supplementary Fig. 11a, b, d, e). In close-interaction analysis, Scn1a+/− mice avoided interacting with male social cues (Supplementary Fig. 11c), and both wild-type and Scn1a+/− mice strongly avoided fox urine (Supplementary Fig. 11f). Wild-type mice exhibited strong habitation and dishabitation to odours of banana, male urine and standard food, whereas Scn1a+/− mice exhibited increased digging behaviour when banana and male urine odours were presented, indicating that they detect these odours (Supplementary Fig. 12b). Moreover, in a Y-maze olfactory choice test, Scn1a+/− mice strongly avoided banana and male urine, whereas wild-type mice had a strong preference for both (Supplementary Fig. 12c, d). These data indicate that Scn1a+/− mice perceive food odours and social olfactory cues, but they have no interest or avoid unfamiliar odours and social odours. These results further establish a deficit in social interaction and avoidance of environmental change in Scn1a+/− mice, as in ASDs.
Cre-recombinase mouse line (Dlx1/2-Cre^{17,37,38}). These mice have a specific reduction of Na\textsubscript{v}1.1 channels in forebrain GABAergic neurons and have similar epilepsy and premature death as Scn1a\textsuperscript{-/-} mice\textsuperscript{15}. Dlx1/2 Scn1a heterozygous mutant mice (Dlx1/2-Scn1a\textsuperscript{+/-}) recapitulated the autism-related phenotypes and spatial learning deficit of Scn1a\textsuperscript{-/-} mice (Fig. 3), whereas control Cre-positive Scn1a\textsuperscript{+/-} mice did not (Supplementary Fig. 14). In the open field test, Dlx1/2-Scn1a\textsuperscript{+/-} mice moved farther compared to Cre-negative Scn1a\textsuperscript{+/loxp} littermates. In the elevated plus maze, Dlx1/2-Scn1a\textsuperscript{+/-} mice entered less frequently into open arms (d), and spent significantly less time in open arms (e). f. In the open field social interaction test, Dlx1/2-Scn1a\textsuperscript{+/-} mice showed decreased interaction with social cues compared to Scn1a\textsuperscript{+/loxp} littermates. g. In the 3-chamber test, Dlx1/2-Scn1a\textsuperscript{+/-} mice had no preference for the stranger mouse. h. In the contextual fear conditioning test, Dlx1/2-Scn1a\textsuperscript{+/-} mice had a normal fear response immediately after the foot shock during training but showed a profound deficit in short-term (30 min) and long-term (24 h) memory for the spatial context associated with a 2-s mild foot shock (0.5 mA) when compared to wild-type mice. Dlx1/2-Scn1a\textsuperscript{+/-} mice. i. Flox, Cre-negative Scn1a\textsuperscript{+/loxp} 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.

Deficit of Na\textsubscript{v}1.1 channels impairs GABAergic neurotransmission

To test our hypothesis that the autism-related phenotypes and spatial learning deficits in Scn1a\textsuperscript{-/-} mice are caused by decreased Na\textsubscript{v}1.1 activity in GABAergic interneurons in the forebrain, we compared the properties of cortical and hippocampal GABAergic interneurons in wild-type and Scn1a\textsuperscript{-/-} mice. Na\textsubscript{v}1.1 protein is expressed in adult hippocampal and neocortical interneurons, as assessed by co-immunolabelling of Na\textsubscript{v}1.1 channels and GABA in the hippocampal CA1 region (Fig. 4a) and prefrontal cortex (Supplementary Fig. 15). The proportion of GABAergic interneurons expressing a detectable level of Na\textsubscript{v}1.1 in Scn1a\textsuperscript{-/-} 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\textsubscript{v}1.1 in GABAergic cells with detectable staining was reduced by 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\textsuperscript{26–29}. Scn1a\textsuperscript{-/-} mice have reduced Na\textsuperscript{+} currents and impaired action potential firing in both hippocampal interneurons and cerebellar Purkinje neurons\textsuperscript{9,10}, which are GABAergic neurons. When action control and testing sessions, but significantly less freezing behaviour in the 30 min and 24 h after the training compared with Scn1a\textsuperscript{+/loxp} mice (Fig. 3h). These results show that Dlx1/2-Scn1a\textsuperscript{+/-} mice reproduce hyperactive and anxiety-like behaviours, deficits in social interactions, and impaired context-dependent fear conditioning of global Scn1a\textsuperscript{-/-} mice. This evidence indicates that the autism-related phenotype emerges from reduced Na\textsubscript{v}1.1 activity specifically within forebrain GABAergic interneurons.
consequence of decreased inhibition. Whereas excitatory synaptic activity was increased as an indirect result of the loss of their 30-min and 24-h contextual fear memory (Fig. 5e). Scn1a+/– mice show both cognitive deficits and autistic traits, including hyperactivity, sleep dysfunction, and thermally induced seizures. Ataxia also occurs in these mice, which have a specific deficit in NaV1.1 activity in hippocampal slices (Fig. 5a and Supplementary Fig. 21), 20-fold lower than typical anxiolytic doses. To test the effect of clonazepam on social behaviour, we performed three sets of identical trials at one-week intervals with the 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 behavioural tests were performed 30 min after intraperitoneal injection of 0.0625 mg kg−1 clonazepam. In the last trial, the tests were performed 30 min after intraperitoneal injection of vehicle. The data were analysed 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 behaviours of the Scn1a+/– mice, and this effect was reversed after the one-week clearing period (Fig. 5b, c and Supplementary Figs 22 and 23). In contrast, low-dose clonazepam had no effect on the social behaviour of wild-type mice. Treatment with low-dose clonazepam 30 min before testing also rescued impaired context-dependent fear conditioning. Whereas wild-type mice were unaffected by clonazepam (Fig. 5d), Scn1a+/– mice showed 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 and Supplementary Fig. 24a). The increased amplitude of spontaneous IPSCs after treatment with 10 μM clonazepam leads to a decrease in frequency of spontaneous IPSCs, without change in amplitude in Scn1a+/– hippocampal slices (Fig. 5g and Supplementary Fig. 24b). These results support our hypothesis that behavioural 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 NaV1.1 channel in these deficits in brain functions was unknown. Our results show that mice with heterozygous loss-of-function mutation in NaV1.1 channels show both cognitive deficits and autistic traits, including hyperactivity, anxiety, excessive stereotyped behaviours and social interaction deficits. Together with previously reported phenotypes of epilepsy, premature death, thermally induced seizures, ataxia, and sleep dysfunction, these studies demonstrate that Scn1a+/– mice phenocopy all the major symptoms of DS.

These cognitive and behavioural deficits in Scn1a+/– mice are caused by decreased action potential firing in forebrain GABAergic interneurons. Our previous studies indicated that deletion of NaV1.1 channels causes selective reduction in Na+ currents and action potential firing of GABAergic interneurons in hippocampus and cerebellum. 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−/− mice, which have a specific deficit in NaV1.1 strength of GABAergic transmission. To test this idea, we treated Scn1a+/– and wild-type mice with the benzodiazepine clonazepam, a positive allosteric modulator of the GABA<sub>A</sub> receptor. Benzodiazepines do not open the GABA<sub>A</sub> receptor chloride channel in the absence of GABA, but instead boost GABA signalling only when presynaptically released GABA binds to the receptor. First, we examined the effects of clonazepam in the open-field and elevated plus-maze tests to avoid potential sedative and anxiolytic effects in our behavioural 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−1 for Scn1a+/– mice (Fig. 5a and Supplementary Fig. 21), 20-fold lower than typical anxiolytic doses. To test the effect of clonazepam on social behaviour, we performed three sets of identical trials at one-week intervals with the 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 behavioural tests were performed 30 min after intraperitoneal injection of 0.0625 mg kg−1 clonazepam. In the last trial, the tests were performed 30 min after intraperitoneal injection of vehicle. The data were analysed 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 behaviours of the Scn1a+/– mice, and this effect was reversed after the one-week clearing period (Fig. 5b, c and Supplementary Figs 22 and 23). In contrast, low-dose clonazepam had no effect on the social behaviour of wild-type mice. Treatment with low-dose clonazepam 30 min before testing also rescued impaired context-dependent fear conditioning. Whereas wild-type mice were unaffected by clonazepam (Fig. 5d), Scn1a+/– mice showed 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.

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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. Sequencing of the genomes of autistic patients identified mutations of SCN1A gene in familial autism. Exome sequencing revealed that de novo mutations in the SCN1A gene cause autism. 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. With a prevalence of 1:20,000 births for DS and related SCN1A channelopathies, DS is less frequent than fragile-X syndrome (1:5,000) 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 inter-neurons. Thus, autistic traits in DS and in a broad range of ASDs may be caused by a reduction of GABAergic signalling. 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 behavioural 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 behavioural 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 analysed using Student’s t-test, one-way analysis of variance (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 and full statistical tests and values for behavioural data are presented in Supplementary Table 1.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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