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Dravet Syndrome: A Sodium Channel Interneuronopathy

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Abstract

Dravet Syndrome is a devastating childhood epilepsy disorder with high incidence of premature death plus comorbidities of ataxia, circadian rhythm disorder, impaired sleep quality, autistic-like social-interaction deficits and severe cognitive impairment. It is primarily caused by heterozygous loss-of-function mutations in the *SCN1A* gene that encodes brain voltage-gated sodium channel type-1, termed $Na_V1.1$. Here I review experiments on mouse genetic models that implicate specific loss of sodium currents and action potential firing in GABAergic inhibitory interneurons as the fundamental cause of Dravet Syndrome. The resulting imbalance of excitatory to inhibitory neurotransmission in neural circuits causes both epilepsy and co-morbidities. Promising therapeutic approaches involving atypical sodium channel blockers, novel drug combinations, and cannabidiol give hope for improved outcomes for Dravet Syndrome patients.

Keywords

Epilepsy; autism; cognitive deficit; sodium channels; interneurons

Introduction

Voltage-gated sodium (Na_V) channels initiate action potentials in neurons and other excitable cells [1]. They are composed of a large central pore-forming α subunit in complex with one or two auxiliary β subunits [2]. In response to depolarizing stimuli, brain Na_V channels rapidly activate, open, and then inactivate with 1–2 msec [2,3]. A further slow inactivation process is engaged by long trains of stimuli or prolonged depolarizations in the range of 100 msec [4]. The kinetics and voltage dependence of sodium channel activation and inactivation strongly influence the threshold for action potential firing and the initiation, firing frequency, and durations of trains of action potentials [4]. Because information is encoded in the frequency and pattern of trains of action potentials, sodium channels play critical roles in information processing in neural circuits as well as in information transmission throughout the brain.

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Dravet Syndrome

Dravet Syndrome is a devastating childhood epilepsy disorder with a high incidence of premature death plus co-morbidities of developmental delay, severe cognitive impairment, ataxia, circadian rhythm disorder, impaired sleep quality, and autistic-like social interaction deficits [5]. It is primarily caused by heterozygous loss-of-function mutations in the *SCN1A* gene that encodes the brain voltage-gated sodium channel type-1, termed $Na_V1.1$ [5]. Approximately 80% of patients with a clinical diagnosis of Dravet Syndrome have identified mutations in exons encoding $Na_V1.1$ [5]. As the exons of *SCN1A* only comprise 6 kb of the gene, which is over 100 kb in size, many of the remaining patients may have loss-of-function mutations in the non-coding portion of the gene that prevent $Na_V1.1$ protein expression.

Dravet Syndrome typically begins at 6 to 9 months of age with seizures induced by elevation in core body temperature due to a fever, a hot day, or a hot bath [5]. Spontaneous seizures follow within weeks, and they become progressively more frequent and severe throughout childhood. After six years of age, the frequency and severity of seizures are reduced, but seizures continue into adulthood [5]. Seizures in Dravet Syndrome are resistant to standard pharmacotherapy, even with mixtures of multiple antiepileptic drugs [6]. Historically, up to 30% of children with Dravet Syndrome died before their teenage years. Even with modern diagnosis and care, up to 15% of affected children die prematurely.

Children with Dravet Syndrome also have debilitating co-morbidities. As their seizures reach peak frequency and severity during years 1–5, developmental milestones are lost, and cognitive achievements regress, resulting in a typical IQ of 50 in teenage years [5]. This severe cognitive deficit is as problematic as seizures for older patients because they require constant care. Most Dravet Syndrome patients also have autistic-like social interaction deficits, and ataxia, circadian rhythm defect, and sleep impairment are common [5]. In this article, I review research on mouse genetic models that has provided important insights into the pathophysiology and therapy of this devastating disease

Mouse Genetic Models of Epilepsy and Premature Death in Dravet Syndrome

It is a paradox that loss-of-function mutations in a Na_V channel cause epilepsy. Two mouse genetic models of Dravet Syndrome based on different disease mutations both showed spontaneous seizures (Fig. 1a). Surprisingly, these loss-of-function mutations have a specific effect to reduce the sodium currents and electrical excitability of GABAergic interneurons [7,8], which would imbalance the ratio of excitation and inhibition in neural circuits throughout the brain and lead to general hyperexcitability [9]. By postnatal day (P) 21, mice with heterozygous mutations in $Na_V1.1$ become susceptible to thermal induction of seizures by slowly raising their core body temperature to 38.5°C or higher (Fig. 1c) [10]. One day later, at P22, they begin to have spontaneous seizures, which increase to peak frequency and severity at P24-P27 [7,11]. The frequency of spontaneous seizure declines with increasing age, but these mice continue to have seizures through adulthood [11–13]. The temporal evolution of Dravet Syndrome is similar in humans and mice, when compared to the

developmental time courses of the two species, with seizures beginning near the time of weaning (P20 vs. 6–9 months), reaching peak during early development (P24–27 vs. 2–5 years), and declining to a steady state at puberty (P60 vs.13 years) [14]. The onset of Dravet Syndrome in humans and mice is approximately correlated with the normal developmental loss of embryonic $Na_V1.3$ channels paired with the failure of normal development of $Na_V1.1$ channels to replace them [14].

As expected from the deficit in action potential firing in GABAergic interneurons, inhibitory GABAergic neurotransmission is reduced. Recordings of spontaneous, action potential-driven synaptic activity in hippocampal slices show an increase in the time interval between inhibitory postsynaptic currents (IPSCs) and a reduction in the frequency of these synaptic events (Fig. 2a). In response to the reduced inhibitory neurotransmission, excitatory neurotransmission is increased (Fig. 2b). These results reveal an increase in the ratio of excitatory to inhibitory neurotransmission in neural circuits in Dravet Syndrome.

The key role of sodium channel mutations in GABAergic interneurons as the fundamental genetic cause of Dravet Syndrome was further supported by specific Cre-Lox deletion of $Na_V1.1$ in forebrain GABAergic interneurons, which precisely recapitulates the thermally induced seizures, spontaneous seizures, and premature death of the disease [15]. Thermal induction of seizures is half-maximal at 38.5°C, just as for mice with global mutation of $Na_V1.1$ (Fig. 1b, d), suggesting that mutation in forebrain GABAergic interneurons quantitatively recapitulates the epilepsy syndrome [15]. In contrast, specific deletion in excitatory neurons ameliorates the effects of Dravet Syndrome in mice [16]. Remarkably, these mouse genetic models also recapitulate each of the co-morbidities of Dravet Syndrome, and failure of firing of GABAergic inhibitory neurons is implicated in each case.

Co-Morbidities in Mouse Genetic Models of Dravet Syndrome

Ataxia.

The first co-morbidity to be analyzed in a mouse model of Dravet Syndrome was ataxia. A mild ataxia phenotype was observed in digital video recordings [17]. Electrophysiological studies revealed a defect in action potential firing in cerebellar Purkinje neurons [17]. This defect is sufficient to cause ataxia, because deficits of similar magnitude in Purkinje cell function cause ataxia in other contexts (Table 1).

Circadian rhythm.

Dravet Syndrome children have a circadian rhythm defect, which prevents them from establishing a normal sleep-wake cycle [18, 19]. Dravet Syndrome mice have an abnormally long circadian cycle length, defects in phase shift after change of their light-dark cycle, analogous to exaggerated jet lag in humans, and impaired light-induced shifts of their sleep-wake cycle [19]. GABAergic neurotransmission is impaired in the suprachiasmatic nucleus of the hypothalamus, the primary site of the circadian clock [19]. These deficits were substantially reversed by treatment with the benzodiazepine clonazepam, a positive allosteric modulator of the postsynaptic response of $GABA_A$ receptors to synaptically released GABA [19]. These results add further support for the conclusion that co-morbidities of Dravet

Syndrome are caused by failure of action potential firing of GABAergic interneurons (Table 1).

Sleep quality.

Children with Dravet Syndrome wake frequently, independent of their pattern of seizures during sleep [18,19]. Dravet Syndrome mice also have a defect in sleep quality [21], including excess brief waking episodes. This sleep defect is observed in mice with specific deletion of $Na_V1.1$ in GABAergic interneurons in the forebrain, confirming that it is independent of the impairment of circadian rhythms in the suprachiasmatic nucleus [21]. Amplitudes of delta waves and sleep spindles in NREM sleep are decreased more than 60%. Sleep spindles and behavioral aspects of sleep are driven by a tri-synaptic pathway of excitatory neurons in the basal nucleus of the thalamus, pyramidal cells in the cerebral cortex, and GABAergic inhibitory neurons in the reticular nucleus of the thalamus, which control this circuit by firing rapid trains of action potentials upon release from a hyperpolarizing stimulus [22]. This rebound firing is reduced by 60% in Dravet Syndrome mice [21], consistent with the conclusion that impaired action potential firing of these GABAergic interneurons is responsible for the defect in sleep quality (Table 1).

Autistic-like behaviors.

Children with Dravet Syndrome also exhibit autistic-like behaviors [23–25]. Dravet Syndrome mice have a substantial deficit in social interactions (Fig. 3) [26,27]. In the three-chamber test, Dravet Syndrome mice (Fig. 3a) show no preference for interaction with a stranger mouse in preference to a novel object. In the open field, they interact less with a stranger mouse, and they exhibit rapid defensive escapes from social interactions [26,27]. These deficits in social interaction are also present in mice with specific deletion of $Na_V1.1$ channels in forebrain inhibitory neurons (Dlx, Fig. 3c) [26], indicating that this autistic-like behavior is caused by reduced action potential firing in inhibitory neurons (Table 1). Consistent with this mechanism, autistic-like behaviors are rescued by treatment of Dravet Syndrome mice with low doses of the benzodiazepine clonazepam [26].

Spatial learning and memory.

According to parental reports, children with Dravet Syndrome have exceptional difficulty learning and remembering the location of dangerous situations, and therefore have an unusual number of accidents from falling on stairs, jumping into swimming pools, running into the street, etc. [19]. Dravet Syndrome mice also have a major deficit in spatial learning and memory [26]. In the context-dependent fear conditioning test, Dravet Syndrome mice exhibit a normal acute ‘freezing’ response to a fearful foot shock, but they do not remember the spatial context of that fearful event when later returned to the same location (Fig. 3b) [26]. They also are impaired in the Barnes maze test, in which mice learn to find a dark refuge hole on the periphery of a brightly lighted field [26]. These impairments in spatial learning and memory are also observed when the $Na_V1.1$ channel is specifically deleted in forebrain inhibitory neurons (Fig. 3d), and they are rescued by a single low dose of clonazepam [26]. Thus, this defect in fear-associated spatial learning is characteristic of mice and humans with Dravet Syndrome, and it is caused by loss of electrical excitability in forebrain GABAergic interneurons (Table 1).

Interneuron Types

In the cerebral cortex, interneurons can be divided into three non-overlapping classes, recognizable by their expression of the marker proteins parvalbumin (PV), somatostatin (SST), and serotonin receptor 3a (5-HT3aR) [28]. PV interneurons make synapses on the cell bodies and axon initial segments of pyramidal neurons, where their fast-spiking discharges exert potent inhibition of action potential firing by their postsynaptic target [28]. SST interneurons make synapses on distal synapses of pyramidal neurons. Their activity opposes incoming EPSCs from synapses on the distal dendrites and exerts disynaptic inhibition of the activity of neighboring excitatory neurons [28]. 5-HT3aR neurons form synapses on both inhibitory neurons and excitatory neurons, which may provide opposing influences on pyramidal neuron excitability [28]. In Dravet Syndrome mice, action potential firing is impaired in some subtypes of 5HT3aR inhibitory neurons in layers M/MI [29] and in both PV and SST interneurons in Layer V (Fig. 4) [30]. Disynaptic inhibition between pairs of pyramidal neurons, which is mediated by SST interneurons [31], was also markedly impaired [30]. No impairment of action potential firing was observed in pyramidal neurons in these cortical brain areas [29,30].

We used the Cre-Lox method to delete the $Na_V1.1$ channel in each of these classes of interneurons [32]. Action potential firing was impaired in all thee classes of interneurons [29,32]. Deletion in PV interneurons gave autistic-like social interaction deficits and pro-epileptic effects (Table 1) [32]. Deletion in SST interneurons gave hyperactivity and pro-epileptic effects that were not as strong as deletion in PV interneurons (Table 1) [32]. Combined deletion in PV and SST interneurons gave synergistic pro-epileptic effects, cognitive impairments, and premature death (Table 1) [32]. In contrast, deletion in 5-HT3aR neurons caused comparatively mild effects, with no evident pro-epileptic effects or premature death, no cognitive deficit, and relatively mild social interaction impairment [29]. Evidently, Dravet Syndrome is caused primarily by defects in action potential firing in PV and SST interneurons. These deficits create an imbalance of excitatory glutamatergic neurotransmission over inhibitory GABAergic neurotransmission, which is a key piece in the puzzle of Dravet Syndrome (Fig. 5). This imbalance of electrical signaling in brain circuits leads to the pleiotropic effects of this disease on ataxia, circadian defect, sleep impairment, autistic-like behaviors, and cognitive deficit (Table 1).

Genetic Background Effects

Children with apparently complete loss-of-function mutations in $Na_V1.1$ have different time course and severity of Dravet Syndrome symptoms, implicating strong effects of genetic background in determining disease severity [23]. All of our studies cited above were carried out with $Na_V1.1$ mutations expressed in homozygous C57BL/6J mice, which recapitulate all of the phenotypes of human Dravet Syndrome [12,26]. With this genetic background, all of the effects of these mutations are caused by failure of action potential firing by GABAergic interneurons [12,15,26,33]. However, in our initial studies, we found that the effect of deletion of $Na_V1.1$ channels was much more severe in the C57BL/6J genetic background than in the 129SvJ genetic background, because the ~70% of C57BL/6 mice died by P80 whereas 129SvJ mice died no more frequently than WT [7].

Mistry et al. [34] compared the Dravet Syndrome $Na_V1.1^{+/-}$ genotype in 50:50 C57BL/6:J:129S6/SvEvTac genetic background to homozygous 129S6/SvEvTac genetic background. As in our previous work [7], they found that mice in a 50:50 C57BL/6J genetic background had spontaneous seizures and premature death, whereas mice in the homozygous 129S6/SvEvTac genetic background had both spontaneous seizures and premature death [34]. Electrophysiological studies revealed reduced sodium currents in hippocampal GABAergic interneurons in 50:50 C57BL/6:J:129S6/SvEvTac but not in homozygous 129S6/SvEvTac genetic background [34], consistent with a causal role for impaired action potential generation in GABAergic interneurons in Dravet Syndrome. They also observed increased sodium currents in pyramidal neurons of P21 mice that may contribute to disease phenotypes in these genetic backgrounds.

Further studies of genetic background effects in mice with homozygous genetic backgrounds showed that $Na_V1.1^{+/-}$ mice in the 129SvJ genetic background have a less severe phenotype, with no evident spontaneous seizures, premature death, or cognitive deficit [35]. This less severe phenotype resembles Generalized Seizures with Febrile Seizures Plus (GEFS+) [12], which is a milder $Na_V1.1$ genetic epilepsy syndrome. The mild phenotype of Dravet Syndrome in 129SvJ mice was correlated with less reduction of action potential firing in GABAergic interneurons and less impairment of postsynaptic boosting of synaptic inputs in pyramidal neurons [35]. No changes in pyramidal cell excitability were observed in these genetic backgrounds [35]. Overall, these results indicate that genetic background effects on the phenotypes of $Na_V1.1$ mutations are potentially strong enough to change the clinical diagnosis from Dravet Syndrome to GEFS+, consistent with the model that mutations with a spectrum of severity and genetic background can account for the spectrum of clinical diagnoses from familial febrile seizures to GEFS+ to Dravet Syndrome [12].

Gene mapping studies are in progress to identify potential modifier genes for Dravet Syndrome. The initial phase of this work revealed sites of significant genetic background effects on chromosomes 5, 7, 8, and 11 [36]. Follow-up studies of chromosome 5 identified a 9 Mb region containing candidate genes encoding $GABA_A$ receptor subunits [37]. Genetic background effects of polymorphisms in $GABA_A$ receptor subunits would be expected in a disease caused by failure of action potential firing in GABAergic interneurons. It will be of great interest to discover the specific polymorphisms in these genes and analyze their effects on GABA receptor function.

Novel Therapeutic Approaches

Current therapy.

Current treatment of Dravet Syndrome is not sufficient to prevent the storm of seizures and debilitating co-morbidities that are characteristic of this disease, even though combinations of antiepileptic drugs are used [6]. One standard treatment includes four antiepileptic drugs: valproate, clobazam, topiramate, and stiripentol [6]. The nontraditional antiepileptic drug leviteracetam is also frequently used as an add-on medication [38]. Unfortunately, even with these complex drug cocktails, control of seizures and prevention of co-morbidities are usually not achieved.

Sodium channel-blocking antiepileptic drugs.

Drugs that block sodium channels are a mainstay of antiepileptic therapy, including phenytoin and lamotrigine. However, as would be expected in a disease caused by loss-of-function mutations in a sodium channel, these traditional sodium channel blockers actually exacerbate seizures in Dravet Syndrome in both children and mice [39,40]. Although traditional sodium channel blocking antiepileptic drugs are not effective, the atypical sodium channel blocker GS967 has beneficial effects on seizure frequency and premature death in Dravet Syndrome mice [41]. This atypical compound preferentially inhibits sustained sodium currents compared to peak sodium currents [41]. This mechanism of action may allow GS967 to prevent the synchronous repetitive action potential firing characteristic of epileptic seizures while leaving normal action potential generation and propagation in inhibitory neurons relatively unaffected.

Enhancing GABAergic neurotransmission.

Because the primary effect of mutation of $Na_V1.1$ is failure of action potential generation in inhibitory interneurons, the most direct approach to restoring normal nervous system function would be to enhance GABAergic neurotransmission. Our evidence that epilepsy, premature death, and all co-morbidities of Dravet Syndrome are caused by failure of action potential firing in inhibitory neurons suggests that both epilepsy and co-morbidities could potentially be prevented by appropriate enhancement of phasic GABAergic neurotransmission. An ideal therapeutic approach would be to both increase the level of GABA in the synaptic cleft and enhance its postsynaptic effects. Tiagabine and related drugs inhibit the uptake of GABA from the synaptic cleft by inhibiting the GAT1 transporter [42,43]. Benzodiazepines like clonazepam enhance the postsynaptic response to GABA by serving as positive allosteric modulators [44]. We found synergistic effects of tiagabine and clonazepam on thermally induced seizures in Dravet Syndrome mice and reduced side effects for equi-effective combined doses compared to the single drugs [45]. Unfortunately, sedative side effects of clonazepam and increased incidence of myoclonic seizures caused by tiagabine were still noticeable with the optimum drug combination [45].

Cannabidiol.

Parental reports indicate that the nonpsychotropic cannabis component cannabidiol is effective in reducing the frequency and severity of seizures in Dravet Syndrome [46]. We found that treatment with a single dose of cannabidiol decreases the duration and severity of thermally induced seizures (Fig. 6), reduces the frequency of spontaneous seizures, and ameliorates the social interaction deficits in Dravet Syndrome mice [47], and a small clinical trial reported efficacy in reducing the frequency of spontaneous seizures in Dravet Syndrome children [48]. The beneficial effects of cannabidiol in mice were correlated with increased GABAergic inhibitory neurotransmission, reduced ratio of excitatory to inhibitory neurotransmission, enhanced action potential firing in GABAergic interneurons in the dentate gyrus, and, at high stimulus intensity, inhibition of action potential firing of excitatory dentate granule neurons [47]. These electrophysiological effects were mimicked and occluded by inhibition of the lipid-activated G protein-coupled receptor GPR55 [47],

suggesting a key role for this signaling pathway in mediating the effects of cannabidiol. Drugs that act directly on the GPR55 pathway may also be efficacious in Dravet Syndrome.

Conclusion

Studies of multiple mouse genetic models of Dravet Syndrome all lead to the conclusion that the primary pathogenic event is loss of action firing in GABAergic interneurons. This loss of electrical excitability in GABAergic interneurons leads to an imbalance of excitation over inhibition in many neural circuits. This imbalance leads directly to the severe epilepsy, premature death, and many co-morbidities of Dravet Syndrome. Genetic dissection of the phenotypes of Dravet Syndrome indicates different roles for specific classes of interneurons in the disease. Secondary up-regulation of sodium channel expression and function may also contribute to the disease phenotypes, and genetic background effects have a major impact on disease severity. Recent pharmacological studies in mice and humans give a more optimistic outlook for future effective therapies for this devastating disease.

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Highlights

- Dravet Syndrome results from heterozygous loss-of-function mutation of Nav1.1 channel
- Mouse genetic models recapitulate all aspects of the disease
- Epilepsy is caused by loss of action potential firing in GABAergic inhibitory neurons
- Co-morbidities also correlate with loss of excitability of inhibitory neurons
- Novel therapeutic approaches show promise for improved treatment

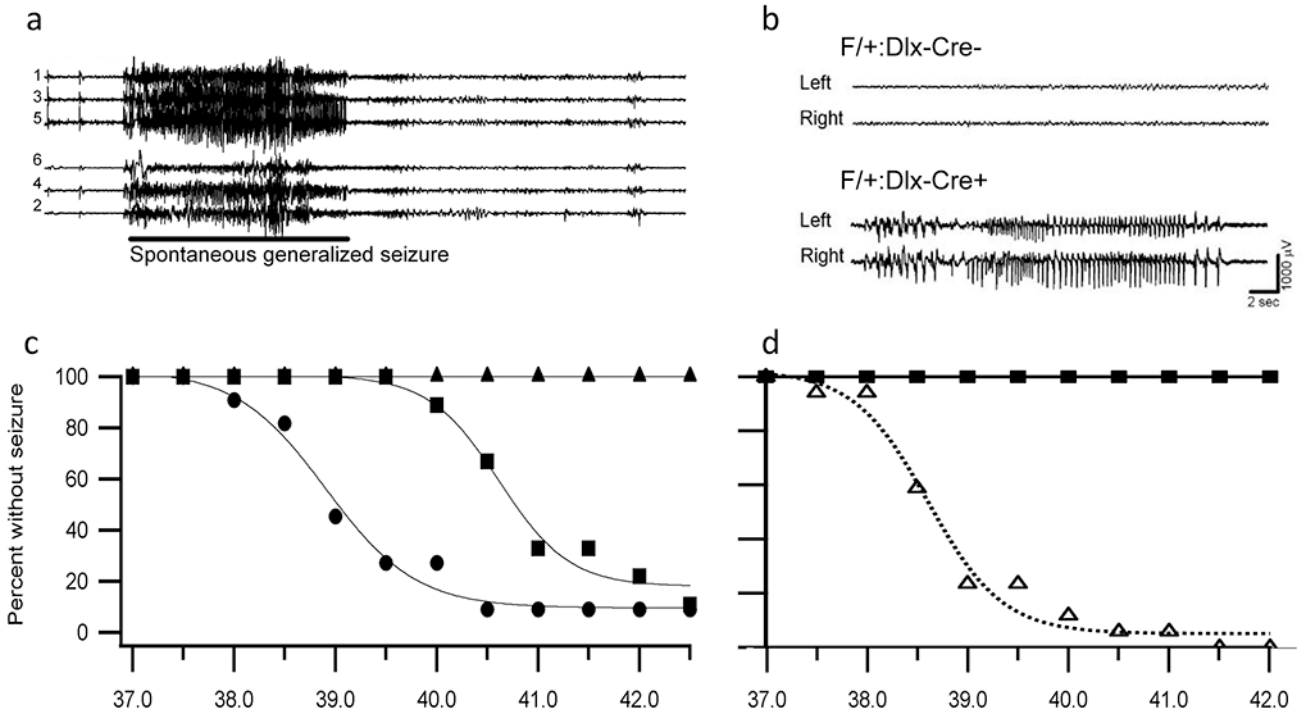

Figure 1. Spontaneous and thermally induced seizures in DS mice.

The body core temperature of mice was raised to the indicated levels by a thermal feedback circuit and heat lamp in steps of 0.5°C per two minutes, and seizures were recorded by video camera and implanted EEG electrodes. **(a)** Representative EEG traces during a spontaneous seizure in a DS mouse. (**b**) Representative EEG traces of F/+:Dlx-Cre^{-} and F/+:Dlx-Cre^{+} mice during thermal induction of seizures at P24. *Top*, F/+:Dlx-Cre^{-} mouse at 39.5°C. *Bottom*, F/+:Dlx-Cre^{+} mouse during a seizure at 39.5°C. (**c**) Temperature dependence of thermal induction of seizures in DS mice. Thermally-induced seizures were evoked in DS mice at P30–46 (circles, mean temperature of 39°C) and P20–22 (squares, mean temperature of induction 40.2°C), but no seizures were induced in P17–18 mice (triangles). (**d**) Temperature dependence of thermal induction of seizures in Dlx-Cre^{+} mice. Thermally-induced seizures were evoked in all F/+:Dlx-Cre^{+} animals with a mean temperature of 39°C. No F/+:Dlx-Cre^{-} mice had a seizure.

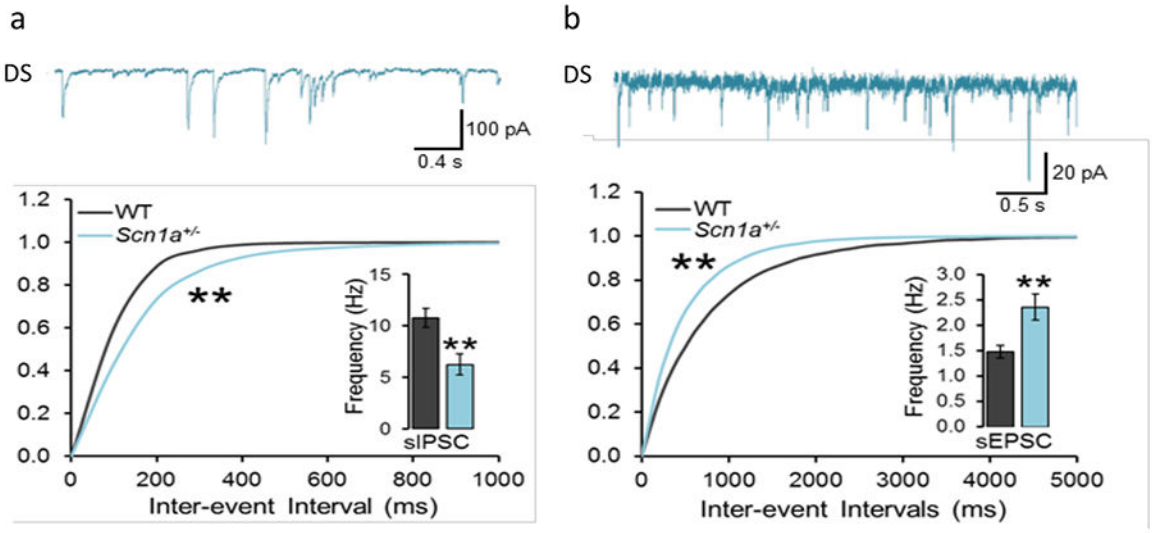

Figure 2. Spontaneous inhibitory and excitatory synaptic activity in WT and DS mice.

Hippocampal slices were prepared from WT and DS mice, and spontaneous, AP-dependent synaptic events were recorded by whole-cell voltage clamp of CA1 neurons. (**a**) Top. Representative recording of inhibitory postsynaptic currents (IPSCs) from a CA1 neuron from a DS mouse. Bottom. Histogram of inter-event intervals between IPSCs. *Inset.* Mean frequency of IPSCs. (**b**) Top. Representative recording of excitatory postsynaptic currents (EPSCs) from a CA1 neuron from a DS mouse. Bottom. Histogram of inter-event intervals between EPSCs. *Inset.* Mean frequency of EPSCs.

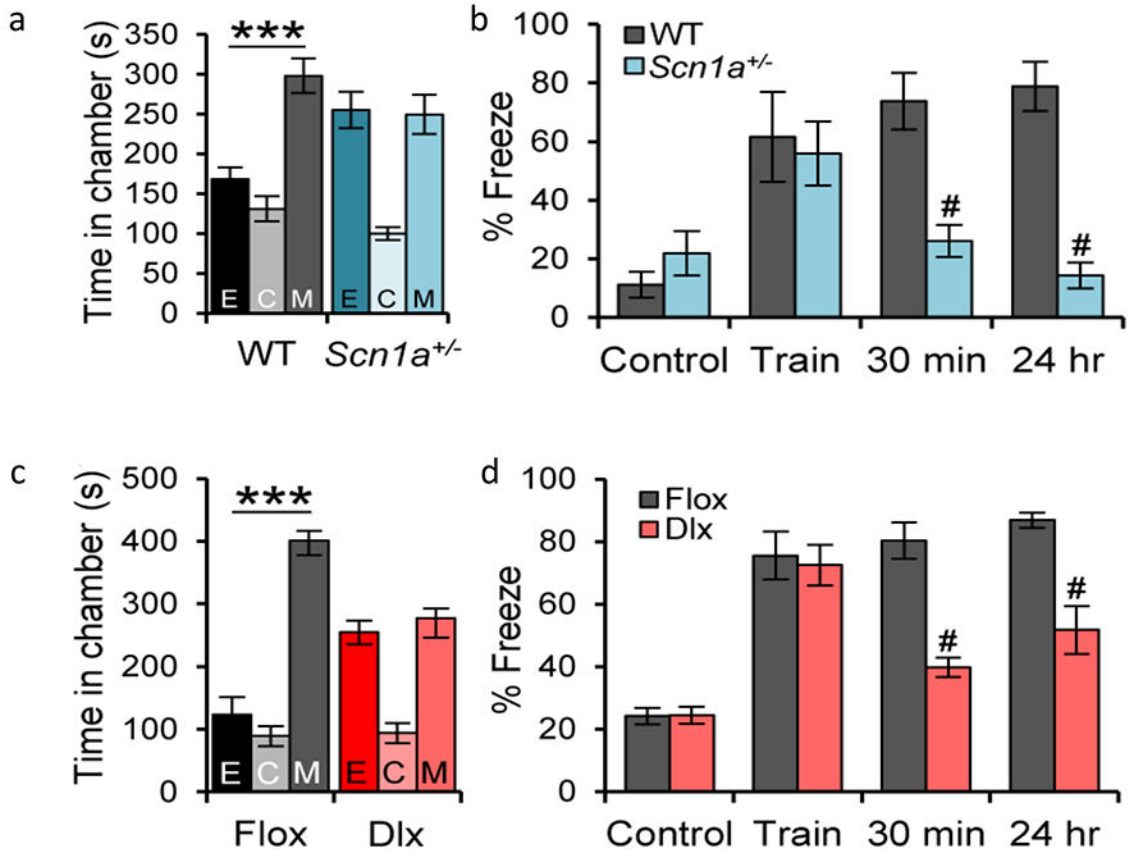

Figure 3. Autism and cognitive deficit in DS mice.

(a) A test mouse was placed in the center chamber (C) of a three-chamber apparatus with connecting passageways, and its movement among the three chambers was recorded and digitally analyzed. WT mice spend more time in the side chamber containing a stranger mouse in a small inverted wire cup (M) compared to the side chamber containing an empty wire cup (E). In contrast, DS mice spend equal time in the E and M chambers. **(b)** In the contextual fear-conditioning test, a mouse was placed in a well-marked cage with an electric grid as its floor, and the movement of the mouse was recorded and digitally analyzed. The % of time that the mouse displays fear-induced freezing behavior was quantified. WT and DS test mice freeze infrequently while exploring the cage (Control), but they freeze in fear during and immediately after a mild foot shock (Train, 2s, 0.5 mA). Test mice were transported to their home cage. Upon return to the context of the fearful shock after 30 min and 24 h, WT mice freeze in anticipation of a shock, even though none is administered. In contrast, DS mice display a profound deficit in short-term (30 min) and long term (24 hr) memory of the spatial context of the foot shock (0.5 mA), (**c**) In the 3-chamber test, Flox control mice prefer to interact with the stranger mouse (M) compared to the empty wire cup, whereas *Dlx1/2-Scn1a*$^{+/-}$ mice have no preference for the stranger mouse. (**d**) In the contextual fear-conditioning test, *Dlx1/2-Scn1a*$^{+/-}$ mice have a normal fear response during and immediately after the foot shock (Train) but display a profound deficit in short-term (30 min) and long-term (24 hr) memory of the spatial context of the foot shock compared to Flox control mice.

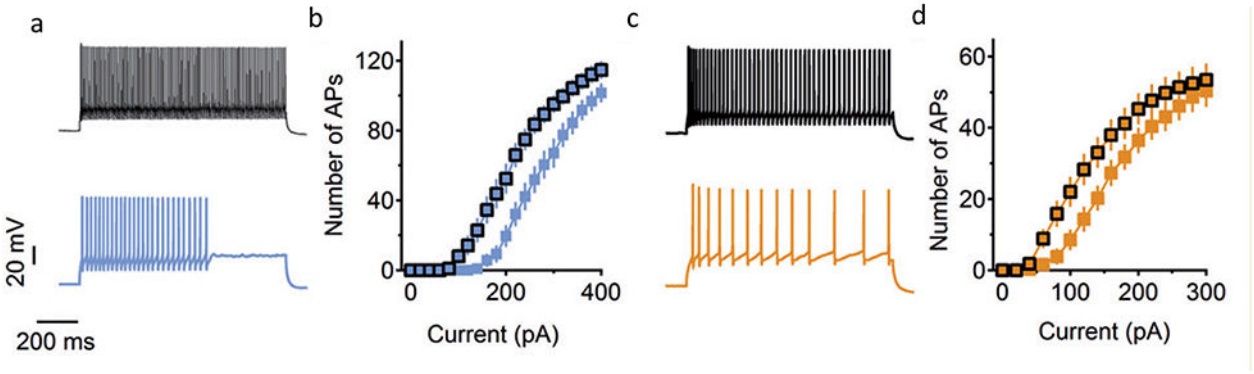

Figure 4. Impaired action potential generation in PV and SST interneurons from from Cre^{+} mice.

DS Flox mice were bred with mice expressing PV-Cre (PV mice) or with mice expressing SST-Cre (SST mice). Cerebral cortical slices were prepared, PV and SST interneurons were identified by specific labeling, and APs were recorded in whole-cell current clamp mode in response to injection of the indicated amounts of depolarizing current. (**a**) Representative trains of action potentials recorded in whole-cell current clamp during injection of 240 pA of depolarizing current. WT, black; PV, blue. (**b**) Action potential number plotted vs. the level of depolarizing current. (**c**) Representative trains of action potentials recorded in whole-cell current clamp during injection of 160 pA of depolarizing current. WT, black; SST, yellow. (**d**) Action potential number plotted vs. the level of depolarizing current.

Figure 5. Imbalance of excitatory vs. inhibitory neurotransmission in DS.

Failure of AP firing in GABAergic interneurons in DS causes a reduction in GABA release and an imbalance of excitatory vs. inhibitory neurotransmission in circuits in the brain. This imbalance leads to epilepsy, cognitive deficit, and autistic-like behaviors plus other co-morbidities in DS.

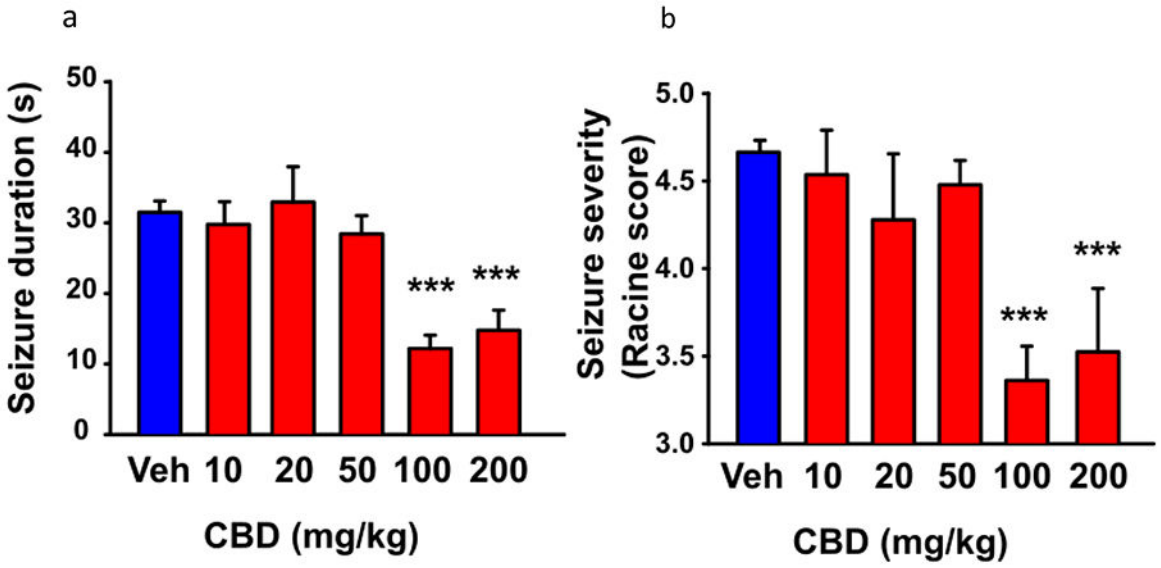

Figure 6. CBD reduced seizure duration and severity in DS mice.

The core body temperature of mice was warmed to 38.5°C as in Fig. 1, and the duration and severity of the resulting seizures were video recorded. **(a)** Dose dependence of CBD reduction in duration of thermally induced seizures. **(b)** Dose dependence of CBD reduction in seizure severity based on Racine Score.

Table 1

Co-Morbidity	Symptoms in DS Mice	Physiological Correlates	Causal Evidence for Mechanism
Ataxia	Abnormal foot placement in walking	Failure of AP firing in GABAergic cerebellar Purkinje neurons	
Circadian Rhythm	Long circadian cycle; weak light-induced phase shift; increased negative masking	Failure of AP firing in GABAergic neurons in the suprachiasmatic nucleus	Not observed in forebrain-specific knockout mouse; reversed by clonazepam
Sleep Impairment	Reduced non-REM sleep, delta wave power, sleep spindles	Failure of rebound AP firing in GABAergic neurons in the RNT	Observed in forebrain-specific knockout mouse
Cognitive Deficit	Failure of spatial learning and memory	Failure of AP firing by GABAergicneurons in hippocampus and cerebral cortex	Observed in forebrain-specific knockout mouse and PV+SST-specific knockout; reversed by clonazepam
Autistic-like Behavior	Impaired social interaction; repetitive behaviors	Failure of AP firing by PV-expressing GABAergicneurons in hippocampus and cerebral cortex	Observed in forebrain-specific and PV-specific knockout mouse; reversed by clonazepam

AP, action potential; SCN, suprachiasmatic nucleus of the hypothalamus; RNT, reticular nucleus of the thalamus; REM, rapid-eye-movement.