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CaV2.1 ablation in cortical interneurons selectively impairs fast-spiking basket cells and causes generalized seizures

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Abstract

Background—Both the neuronal populations and mechanisms responsible for generalized spike-wave absence seizures are poorly understood. In mutant mice carrying loss-of-function mutations in *Cacna1a*, which encodes the $\alpha 1$ pore-forming subunit of Ca_v2.1 (P/Q-type) voltage-gated Ca²⁺ channels, generalized spike-wave seizures have been suggested to result from excessive bursting of thalamocortical cells. However, other cellular populations including cortical inhibitory interneurons may contribute to this phenotype.

Objective—We investigated how different cortical interneuron subtypes are affected by the loss of Ca_v2.1 channel function and how this contributes to the onset of generalized epilepsy.

Methods—We designed genetic strategies to induce a selective *Cacna1a* LOF mutation in different cortical GABAergic and/or glutamatergic neuronal populations in mice. We assessed the cellular and network consequences of these mutations by combining immunohistochemical assays, *in vitro* physiology, optogenetics and *in vivo* video-EEG recordings.

Results—We demonstrate that selective *Cacna1a* LOF from a subset of cortical interneurons, including parvalbumin (PV)- and somatostatin (SST)-positive interneurons, results in severe generalized epilepsy. Loss of Ca_v2.1 channel function compromises GABA release from PV-, but not SST-positive interneurons. Moreover, thalamocortical projection neurons do not show enhanced bursting in these mutants, suggesting that this feature is not essential for the development of generalized spike-wave seizures. Notably, the concurrent removal of Ca_v2.1 channels in cortical pyramidal cells and interneurons considerably lessens seizure severity by decreasing cortical excitability.

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Interpretation—Our findings demonstrate that conditional ablation of Ca_v2.1 channel function from cortical PV interneurons alters GABA release from these cells, impairs their ability to constrain cortical pyramidal cell excitability and is sufficient to cause generalized seizures.

Introduction

Epilepsy is a common human disorder affecting 0.5-1% of the general population.¹ It is a heterogeneous condition with a broad range of presentations, underlying etiologies and therapeutic outcomes. The non-lesional epileptic disorders are characterized by the onset of specific combinations of seizure subtypes at predictable developmental time-points.² For example, idiopathic generalized epilepsies (IGE) present during childhood with different combinations of generalized seizures that include absences, generalized tonic-clonic seizures and/or myoclonus.³ The precise anatomical and cellular origin of generalized seizures, however, remains unclear, limiting the development of more specific and efficient therapeutics.

Genetic studies of patients diagnosed with IGE syndromes have identified mutations in several genes suggesting a variety of underlying mechanisms.⁴⁻⁶ For instance, mutations leading to loss of Ca_v2.1 Ca²⁺ channel function have been shown to result in generalized seizures in mice and humans.⁷⁻¹² However, the site of origin of generalized absence seizures and the impact of such mutations on specific brain structures and cell types remains a matter of debate. Data from *Cacna1a* LOF mutant mice (*Cacna1a*^{tg/tg} tottering and *Cacna1a*^{-/-} knockout mice)¹³⁻¹⁷ suggest that their generalized spike-wave seizures (SWS) are due to excessive bursting of thalamocortical projection (TC) neurons, resulting from increased Ca_v3.1 (T-type) Ca²⁺ channel current or enhanced tonic thalamic inhibition. In addition, PLCβ4 mutations have been shown to enhance bursting of thalamocortical projection neurons in a T-type Ca²⁺ channel-dependant fashion and result in spike-wave absence seizures in mice.¹⁸ However, an increase in thalamic T-type Ca²⁺ currents and/or enhanced thalamocortical bursting is not always required for SWS generation.¹⁹ Therefore, other neuronal populations might contribute to the epileptic phenotype. The discovery of mutations in GABA_A receptor subunits in patients with IGE suggests a role for GABAergic deficits in generalized seizure disorders.²⁰⁻²⁵ In addition, experiments in anesthetized cats using focal cortical lesions or pharmacological disruption of inhibitory synaptic transmission suggest a cortical origin of generalized SWS.²⁶⁻²⁸ Mutant mice with aberrant development or positioning of telencephalic interneurons are known to develop severe seizures, also suggesting a critical role for GABAergic interneurons in controlling runaway excitation.²⁹⁻³³ Therefore, it may be that *Cacna1a* LOF mutations disrupting Ca_v2.1 channels contribute to the development of epilepsy through their impact on cortical interneuron function. While cortical inhibitory systems have been shown to be affected in *Cacna1a* mutants³⁴, the specific consequences of *Cacna1a* LOF on different cortical interneuron populations are unknown and the contribution of these defects to epilepsy onset has not been studied. Cortical interneuron populations are diverse and they play distinct functions in controlling excitation by targeting spatially and temporally divergent domains of principal neurons.³⁵ Hence, one could easily imagine that the disruption of a particular cortical interneuron subtype by the loss of *Cacna1a* gene function could be the cause of seizures in these mutants.

Here, we investigate how different cortical GABAergic populations are affected by the loss of Ca_v2.1 channel function, whether this leads to specific cortical GABAergic deficits, and whether dysfunction in these populations contributes to the genesis of generalized seizures. Our results suggest that compromised GABA release from cortical parvalbumin-positive, but not somatostatin-positive, interneurons is sufficient to induce severe generalized epilepsy, and is likely an important contributor to the genesis of generalized seizures in *Cacna1a* mutants.

Methods

See supplementary data for detailed methodology. Briefly, we generated mutant mice carrying a targeted *Cacna1a* exon 4 deletion, resulting in the loss of Ca_v2.1 Ca²⁺ channel function, in defined structures and neuronal populations. This was achieved by crossing mice carrying a *Cacna1a*^{c/c} conditional allele³⁶, with different *Cre* driver lines. We generated three mutant mouse lines in which *Cacna1a* gene function was ablated in: (i) cortical PV- and SST-positive GABAergic interneurons using the *Nkx2.1-BAC*^{Cre} driver^{37, 38}; (ii) SST-positive GABAergic interneurons in cortex and reticular thalamic using the *SST*^{Cre} driver³⁹; (iii) cortical pyramidal cells using the *Emx1*^{Cre} allele⁴⁰, which in conjunction with the *Nkx2.1-BAC*^{Cre} removes Ca_v2.1 channel function in cortical excitatory neurons and PV- and SST-positive interneurons, while sparing the thalamus. In all crosses, “cortical” refers to neocortical and limbic populations.

We compared the epileptic phenotypes between these different mutants by performing video-EEG recordings with implanted cortical and hippocampal electrodes as described in Batista-Brito et al²⁹. We investigated how the targeted loss of Ca_v2.1 channel function affects the maturation of cortical interneurons by quantifying immunohistochemical labeling for specific cell-type markers (i.e., GAD, PV, SST) and by assessing the intrinsic membrane properties of these interneurons using whole-cell electrophysiology *in vitro*. We also investigated the impact of the loss of Ca_v2.1 channel function on GABAergic transmission by performing paired electrophysiological recordings between cortical interneurons and pyramidal cells, or by using optogenetic approach (expressing ChR-2 in cortical interneurons through cellular infection with *Cre*-dependent AAV^{ChR-2}) to compare light-evoked GABAergic transmission between mutants and controls. Finally, we investigated the impact of the targeted loss of Ca_v2.1 channels in cortical interneurons on the physiological properties and bursting behaviors of thalamocortical cells using patch-clamp recordings *in vitro*.

Results

***Cacna1a* is expressed in the majority of cortical interneurons**

Cacna1a is widely expressed in the telencephalon from early embryonic stages into adulthood.⁴¹ We found *Cacna1a* expression in the majority of cortical GABAergic interneurons by P21, as demonstrated by double fluorescent *in situ* hybridization and co-labeling for *Cacna1a* together with GAD, and parvalbumin (PV) or somatostatin (SST) markers (60 ± 6% of GAD67-positive cells, 88 ± 8% of PV-positive and 65 ± 8 % of SST-positive express *Cacna1a* in wild-type (WT) somatosensory cortex at P21, n=3,

Supplementary Fig 1). PV and SST label the two major types of cortical GABAergic interneurons that originate from the medial ganglionic eminence (MGE), and our results suggest that these two populations might be particularly sensitive to the loss of *Cacna1a*. In addition, most hippocampal, striatal, thalamic, reticular-thalamic and hypothalamic GABAergic neurons express *Cacna1a* at P21.

Ablation of Ca_v2.1 Ca²⁺ channels in MGE-derived cortical interneurons is sufficient to cause generalized seizures

To study the role of Ca_v2.1 channels in interneurons, we selectively ablated *Cacna1a* gene function from the PV- and SST- positive MGE-derived populations (~70% of cortical inhibitory interneurons). This was achieved by crossing the *Nkx2.1BAC^{Cre}* transgenic mice³⁷ (Supplementary Fig 2 A-D) onto a homozygous conditional floxed *Cacna1a* background (*Cacna1a^{fl/c}*)³⁶, to generate *Nkx2.1BAC^{Cre}; Cacna1a^{fl/c}* mutants. Importantly, this approach spares *Cacna1a* gene function in all other *Cacna1a* expressing neuronal populations, including excitatory cortical and thalamic neurons, as well as in the GABAergic neurons of the reticular thalamus and in cortical interneurons that are CGE-derived (~30%), which include the VIP-positive multipolar and bipolar interneurons and neurogliaform cells.

Nkx2.1BAC^{Cre};Cacna1a^{fl/c} mutant mice appear normal until P17, when they develop a progressive and severe epileptic phenotype, as documented by video-EEG recordings (n=11), leading to death before P30. Notably, due to the sparing of cerebellar and subcortical structures, these mutants do not exhibit motor anomalies such as ataxia or dystonia seen in *Cacna1a^{tg/tg}* and *Cacna1a^{-/-}* mice.^{7, 42} On video-EEG recordings during the pre-symptomatic phase (P16-17), background cortical rhythms appear qualitatively normal in mutant animals; however, at around P17, sub-clinical interictal generalized epileptic discharges are recorded independently in the cortex and hippocampi and are most pronounced during sleep (Fig 1A). From this time-point onwards, the mutant mice develop a mixture of generalized seizures as documented through video monitoring including myoclonus (brief muscle jerks), absences (behavioral arrest with automatisms and myoclonus), tonic (diffuse contraction) and generalized tonic-clonic seizures (grand-mal seizures). In addition, absences and tonic seizures (when prolonged) occasionally evolve into generalized tonic-clonic seizures.

At the EEG level, myoclonus is accompanied by generalized bursts of polyspikes (Fig 1B). Tonic seizures present as fast runs of beta activity and polyspikes in the cortex and hippocampi (Fig 1E). Both the absences and the tonic-clonic seizures are characterized by generalized 5-7Hz spike-wave discharges in the hippocampi (Fig 1C, D). However, in the cortex, the absence seizures present as spike-wave activity interspersed with fast runs of beta activity (Fig 1D), whereas the tonic-clonic seizures display a combination of fast beta rhythms, polyspikes and spike-wave activity (Fig 1C). During absences and generalized tonic-clonic seizures, epileptic activity is recorded either synchronously or independently both in the cortex and hippocampi (Supplementary Fig 3). Therefore, a targeted *Cacna1a* LOF mutation in cortical MGE-derived interneurons, sparing the thalamus (including reticular nucleus GABAergic populations), is sufficient to trigger generalized seizures.

The maturation of MGE-derived cortical interneurons is unaffected in *Nkx2.1BAC^{Cre};Cacna1a^{c/c};RCE^{EGFP/+}* mutants

Immunohistochemical labeling for EGFP, PV, SST and somatostatin-calretinin (SST-CR) revealed both a similar number and laminar distribution of MGE-derived interneurons in cortical slices of *Nkx2.1BAC^{Cre};Cacna1a^{c/c};RCE^{EGFP/+}* mutant mice compared with control littermates, where the reporter allele *RCE^{EGFP/+}* labels all Cre-expressing cell lineages (i.e., somatosensory cortex: EGFP: 189 ± 17 vs. 181 ± 9 cells/field; PV: 132 ± 8 vs 138 ± 18 cells/field; SST: 99 ± 5 vs 98 ± 9 cells/field, including SST-CR double positive cells: 16 ± 2 vs 16 ± 3 cells/field, and SST (CR neg): 82 ± 4 vs 81 ± 7 cells/field in mutants vs controls respectively, $n=4$ mutants for EGFP, $n=6$ PV and $n=5$ for SST/CR, $n=3$ controls, unpaired Student's t-test, $p>0.5$, Fig. 2 A-C). Similar results were also observed in other cortical areas (cingulate and motor cortex) and in the hippocampal CA1 region of control versus mutant animals ($n=4$, Supplementary Fig 4). In addition, both wild-type and mutant EGFP-labeled cortical fast-spiking interneurons exhibited intact and mature intrinsic electrophysiological properties in terms of resting membrane potential, input resistance, action potential half-width and maximum firing rate as assessed in layer V of the somatosensory cortex at P17-19 (Fig 2 D-F). Together, these findings suggest that despite the developmental loss of *Cacna1a* gene function, the migration and maturation of MGE-derived cortical interneurons is preserved.

GABA release from fast-spiking PV- and SST-positive MGE interneurons is differentially affected by the loss of $\text{Ca}_v2.1 \text{ Ca}^{2+}$ channel function

MGE-derived cortical interneurons comprise two major classes of cells, the fast-spiking (FS, PV-expressing⁴³) and the SST-expressing neuronal populations. To assess the physiological consequences of *Cacna1a* LOF in these cell types and to parse out their respective contribution to the epileptic phenotype observed in *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutant mice, we studied the GABA release properties in these two interneuron subtypes. To test whether GABA release from FS interneurons is impaired, we performed paired electrophysiological recordings on genetically labeled INs and pyramidal cells (PCs) in *Nkx2.1BAC^{Cre};Cacna1a^{c/c};RCE^{EGFP/+}* mutants. For this purpose, we relied on the stereotypical fast-spiking firing pattern of PV interneurons⁴³ to target PV expressing interneurons within the *Nkx2.1* cell lineage. Previous results from our group indicated that FS interneurons normally rely exclusively on $\text{Ca}_v2.1 \text{ Ca}^{2+}$ channels for GABA release at P21⁴⁴ (also Supplementary Fig 5). Therefore, the loss of $\text{Ca}_v2.1$ channel function would be expected to eliminate inhibitory transmission from FS interneurons. However, we found that GABA release from FS cells in the *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutants, although notably impaired, was not entirely eliminated. This was *not* due to a failure of the *Nkx2.1^{cre}* driver to remove the *Cacna1a* expression in FS cells, as in mutants, the selective $\text{Ca}_v2.1$ (P/Q-type) Ca^{2+} channel antagonist ω -agatoxin IVa had no effect on unitary inhibitory postsynaptic currents (uIPSC) (Fig 3A, $105 \pm 8\%$ of control, $p>0.6$, $n=5$). In contrast, the selective $\text{Ca}_v2.2$ (N-type) Ca^{2+} channel antagonist ω -conotoxin GVIA, which is ineffective in blocking transmission in WT mice ($n=5$, Supplementary Fig 5), completely abolished uIPSCs in paired recordings between FS and PCs (Fig 3B, $2 \pm 1\%$ of control, $p<0.01$, $n=5$).

This indicates that, as in other cells^{42, 45, 46}, the loss of P/Q-type Ca^{2+} channels in FS cortical interneurons results in a compensatory upregulation of N-type Ca^{2+} channels.

However, despite compensation by N-type Ca^{2+} channels, the inhibitory transmission from FS cells was severely impaired in *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutant mice (Fig 3 C, D, E). First, the probability of detecting connected pairs, although unchanged in supragranular layers, was significantly lower in deep cortical layers (V-VI) of mutants compared to WT animals (24% vs. 38%, $p < 0.05$, z-test, Fig 3E). Second, the amplitude of uIPSCs recorded in PCs following stimulation of connected FS cells was significantly smaller in both deep and superficial cortical layers (by $82 \pm 16\%$ and $62 \pm 12\%$ respectively, Fig 3C). Third, we observed a dramatically increased failure rate of FS terminals, which while close to zero in wild-type animals ($0.5 \pm 1.4\%$) was $22 \pm 9.2\%$ and $30 \pm 8.4\%$ (in superficial and deep cortical layers, respectively) in mutant FS cells (Fig 3E). Fourth, IPSCs produced by FS interneurons were poorly time-locked to the action potential, with a $100 \pm 12\%$ ($n=12$, $p < 0.05$, Fig 3E) increase in jitter in the onset of the uIPSC (Fig 3E).

SST cells are much less abundant than FS cells and unitary inhibitory transmission events from cortical SST cells are difficult to assess by paired recordings due to their low connection probability to PCs, smaller postsynaptic responses and susceptibility to slicing artifacts.^{47, 48} To bypass these pitfalls, we used an alternative approach to measure GABA release from SST interneurons. We injected channelrhodopsin-2 (ChR-2) packaged into a *Cre*-activated 2/9AAV virus⁴⁹ in the somatosensory cortex of *SST^{Cre}* animals, thus ensuring the targeted expression of ChR-2 in cortical SST interneurons, which corresponds to the non-FS MGE-derived population within the *Nkx2.1* cell lineage. With this strategy, we observed that 100% of ChR-2 expressing cells also co-labeled with SST, confirming the specificity of our approach (Fig 4A). We then compared the GABA release induced by the light-activation of ChR-2 in cortical slices from *SST^{Cre};Cacna1a^{c/c}* mutants carrying a targeted *Cacna1a* LOF mutation in all SST populations, versus *SST^{Cre}* littermate controls.

First, we found that $\text{Ca}_v2.1$ (P/Q-type) Ca^{2+} channels mediate GABA release from SST cells in WT animals. Indeed, bath-application of the selective P/Q-type Ca^{2+} channel antagonist, ω -agatoxin IVa, completely abolished IPSCs in L5 PCs evoked by light-activation of ChR-2 in WT animals (Fig 4C, $3 \pm 3\%$ of control, $p < 0.01$, $n=5$). Second, we demonstrated that P/Q-type Ca^{2+} channels were successfully removed from SST interneurons in the *SST^{Cre};Cacna1a^{c/c}* mutant mice. In contrast to WT animals, ω -agatoxin IVa had no effect on the amplitude of the light-evoked GABA release (Fig 4D, $95 \pm 6\%$ of control, $p > 0.5$, $n=5$). However, the selective N-type Ca^{2+} channel antagonist ω -conotoxin GVIA largely ($24 \pm 8\%$ of control, $p < 0.01$, $n=6$, Fig 4D) or completely ($1 \pm 2\%$ of control, $p < 0.01$, $n=4$) blocked the release of GABA. This indicates that, as in FS interneurons, there is a compensation of P/Q-type Ca^{2+} channel loss by N-type Ca^{2+} channels at SST interneurons terminals.

Next we investigated the impact of $\text{Ca}_v2.1$ ablation on SST interneurons by comparing the amplitudes of light-evoked GABA release. To reduce variability in responses between slices, we compared light-evoked IPSCs in PCs from slices containing approximately similar number of ChR2-expressing SST interneurons (*SST^{Cre}* controls: 9 slices, 71 ± 11

infected cells per slice; *SST^{Cre};Cacna1a^{c/c}* mutant: 10 slices 65 ± 15 cells per slice; Fig 4B). To ensure maximal GABA release, we used saturating light intensity and duration of light stimulation. We found that despite the removal of P/Q-type Ca^{2+} channels from SST interneurons synapses, amplitudes of light-evoked IPSCs were insignificantly increased in *SST^{Cre};Cacna1a^{c/c}* mutant animals (391 ± 74 pA vs. 548 ± 66 pA; $p > 0.3$, $n = 13$, Fig 4E). In line with these findings, *SST^{Cre};Cacna1a^{c/c}* mutants did not display any gross neurobehavioral anomaly and do not develop spontaneous seizures as evaluated by daily video-EEG monitoring from P16 to P20 ($n = 3$) and from P28 to P30 ($n = 7$). Interestingly, *SST^{Cre}* also targets thalamic reticular neurons, indicating that a probable *Cacna1a* ablation in these cells (inferred from recombination of the *RCE^{EGFP}* reporter allele in these *SST^{Cre};Cacna1a^{c/c};RCE^{EGFP/+}* mutants) is insufficient to cause generalized epilepsy.

In summary, compensatory up-regulation of N-type Ca^{2+} channels upon P/Q-type Ca^{2+} channel loss of function in MGE-derived cortical interneurons is effective to sustain GABA release from terminals of SST-positive interneurons, but not of FS interneurons. Taken together, these findings indicate that the epileptic phenotype observed in *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutant mice is attributable to synaptic dysfunction within the cortical FS (presumed PV-positive) interneuron population.

Thalamocortical neurons do not exhibit enhanced bursting or low-threshold Ca^{2+} current up-regulation in *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutant mice

To assess whether the thalamus participates in seizures following targeted *Cacna1a* LOF mutations in cortical interneurons, EEG recordings with cortical (SS1), hippocampal (CA1) and thalamic (VB) implanted electrodes were obtained from *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutant mice at P16-P19 ($n = 3$, 12 seizures). All seizures recorded involved cortical and thalamic structures confirming that these indeed are corticothalamic seizures (Supplementary Fig 6A, A'). Seizures were recorded first in the cortex or hippocampus in 75% of events (9/12), with the remaining events observed simultaneously in all leads. The mean delay between the first epileptic spike recorded in the thalamus and the first spike recorded in any lead was 20 ms, suggesting that the thalamus participates in these seizures following activation from cortical and limbic structures (Supplementary Fig 6B).

Up-regulation of the low-threshold T-type Ca^{2+} current, which promotes bursting of thalamocortical (TC) cells, has been postulated to underlie generalized spike-wave discharges in *Cacna1a^{tg/tg}* tottering mutants.¹⁶ To investigate whether the loss of $\text{Ca}_v2.1$ channel function from cortical GABAergic interneurons produces similar thalamic changes, we first compared low-voltage activated (LVA) currents in thalamic relay cells in *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutant mice versus control animals at P19-21. We found no significant effect of the mutation on the density (Fig. 5A) or the voltage-dependence (Fig 5B) of the LVA current in TC neurons. Furthermore, we did not observe any effect on the burst-firing of these cells (Fig 5C). We conclude that in *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutants, thalamocortical projection neurons do not have an increased tendency to burst and that their LVA Ca^{2+} currents are comparable to those of WT animals. This suggests that increased intrinsic thalamocortical bursting due to up-regulation of LVA currents is not necessary for the development of generalized seizures in *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutant mice.

Seizure severity in *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutants is attenuated by the concomitant removal of *Cacna1a* gene function in cortical pyramidal neurons

Humans and mice with constitutive loss-of-function mutations in *Cacna1a* develop a generalized epileptic phenotype with absences and myoclonus substantially less severe than that observed in the conditional *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutant mice. We postulated that this might be due to a concomitant decrease in cortical excitability resulting from effects of the mutation on glutamate release. To test this hypothesis, we generated additional conditional mutants carrying both the *Emx1^{Cre}* and *Nkx2.1BAC^{Cre}* driver alleles (i.e., *Nkx2.1BAC^{Cre};Emx1^{Cre};Cacna1a^{c/-}*). These mutants undergo targeted deletion of *Cacna1a* embryonically within MGE-derived interneurons, as well as in most cortical excitatory neurons.^{40, 50}

We observed that the combined removal of *Cacna1a* in MGE-interneurons and cortical PCs significantly alleviated the epileptic phenotype observed in *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutant mice. The *Nkx2.1BAC^{Cre};Emx1^{Cre};Cacna1a^{c/-}* animals have a normal life expectancy and have no apparent gross neurological deficit. They develop, however, a generalized epileptic phenotype with myoclonus and brief absences, without motor seizures (tonic or tonic-clonic), resembling that seen in *Cacna1a^{tg/tg}* tottering mice.⁸ The absences displayed by the *Nkx2.1BAC^{Cre};Emx1^{Cre};Cacna1a^{c/-}* mutants are manifested on EEG recordings as generalized 5-7Hz spike-wave discharges (Fig 6A,B) similar to those observed in the *Nkx2.1^{Cre};Cacna1a^{c/c}* mutant mice and also appear around P17. However, they are much shorter and never lead to generalized tonic-clonic seizures (Fig 6C). After P23, the absences in *Nkx2.1BAC^{Cre};Emx1^{Cre};Cacna1a^{c/-}* animals become more frequent and prolonged (Fig 6D), particularly during somnolence and early slow-wave-sleep (Supplementary Fig 7). To confirm that the selective loss of Ca_v2.1 Ca²⁺ channel function in pyramidal cells is not sufficient to cause epilepsy, we further evaluated the *Emx1^{Cre};Cacna1a^{c/-}* mutants. These mutants did not display gross behavioural deficits or seizures, and their EEG recordings from P17-P19 (n=3) were exempt of any epileptic activity.

The relative mildness of the phenotype observed in the *Nkx2.1BAC^{Cre};Emx1^{Cre};Cacna1a^{c/-}* mutant mice seem likely due to a decreased excitatory drive from pyramidal cells. To test how cortical excitability changes following ablation of Ca_v2.1 Ca²⁺ channel function in PCs (*Emx1^{Cre};Cacna1a^{c/c}* mutants), we recorded PCs in thalamically projecting layers Vb/VI in cell-attached and whole-cell mode. First, the ability of electrical stimulation to evoke spikes in mutant PCs was significantly diminished compared to WT animals (Fig 7A). The response curves correlating the intensity of stimulation with the probability of spiking exhibited a significant rightward shift in mutant animals ($P_{0.5} = 38 \mu A$, n=11, $p < 0.01$, Fig 6B), indicating decreased excitability of cortical circuits. Second, we measured amplitudes and paired-pulse ratios of evoked EPSCs in PCs produced by a mild electrical stimulation (20 μA), to assess changes in cortical glutamatergic transmission. The amplitudes of evoked EPSCs, recorded at the reversal potential for Cl⁻, were significantly smaller in mutant PCs compared to those in WT ($58 \pm 8\%$, Fig 7C). Paired-pulse ratios were significantly higher in EPSC pairs from mutant animals (Fig. 7C), suggesting a presynaptic mechanism consistent with the removal of presynaptic P/Q-type Ca²⁺ channel from excitatory terminals.

In summary, loss of Cav2.1 Ca²⁺ channel function in telencephalic pyramidal cells decreases cortical excitability, and thereby in compound *Nkx2.1BAC^{Cre};Emx1^{Cre};Cacna1a^{c/-}* mutant mice shortens the duration of the absence seizures and prevents the motor seizures observed in the cortical interneuron-restricted *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutant mice (Supplementary Fig 8).

Discussion

In the present study, we demonstrate that the selective ablation of Cav2.1 P/Q-type Ca²⁺ channels in GABAergic MGE-derived cortical interneurons results in severe epilepsy manifested by multiple types of generalized seizures. Mechanistically, we show that, despite compensation by N-type Ca²⁺ channels, the loss of P/Q-type Ca²⁺ channel function results in unreliable, desynchronized and reduced neurotransmitter release from FS (PV) interneurons. By contrast, similar N-type channel compensation within the SST-positive interneuronal population is sufficient to maintain their physiological and synaptic properties. Moreover, unlike what was observed in tottering and constitutive *Cacna1a* knock-out mice¹⁶, the interneuron-specific ablation of Cav2.1 Ca²⁺ channels does not change the excitability of thalamocortical relay cells or the properties of their low-voltage activated (LVA) currents. Finally, we show that the compound removal of Cav2.1 Ca²⁺ channel function from interneurons and cortical pyramidal cells reduces cortical excitability and lessens seizure severity, resulting in absence seizures similar to those seen in the constitutive *Cacna1^{tg/tg}* mutant mice.⁷ Together, our findings suggest that the loss of Cav2.1 Ca²⁺ channel function leads to specific cortical GABAergic deficits by inducing synaptic dysfunction of cortical FS interneurons that may significantly contribute to the generalized seizures observed in *Cacna1a* constitutive mutants and underscores the importance of cortical dysfunction in generalized epilepsy.

Our data is consistent with a growing body of evidence suggesting that generalized seizures can originate in the cortex in both animals and humans.^{26, 51-55} The idea that cortical inhibitory deficits are sufficient to trigger generalized seizures has already been suggested by the ability of local cortical applications of GABA_A blockers^{26, 27} to induce generalized epileptic discharges even after hemi-thalamectomy.²⁶ However, these experiments could not distinguish the relative contribution of specific GABAergic cell types to the generation of seizures. The importance of GABAergic dysfunction in epilepsy has been highlighted recently through various reports of epilepsy following targeted mutations in transcription factors or ionic channels that impair specification, maturation or function of GABAergic interneurons.^{29-33, 56, 57} However, these experiments could not differentiate the contribution of specific cortical vs thalamic neuronal populations in the development of epilepsy. Our results demonstrate that a very specific impairment of synaptic GABA release from cortical PV interneurons is sufficient to produce generalized seizures, and that this likely contributes to the development of generalized seizures in *Cacna1a* loss-of-function mutants.

Implication of PV-positive interneuron impairment in the generation of epilepsy seems especially interesting given the role of these cells in cortical circuits. PV cells are believed to mediate feed-forward inhibition⁵⁸ and can be seen as a “stop-gate” that prevents runaway excitation.⁵⁹ In contrast, excitatory inputs onto SST cells are strongly facilitating, which

allows them to provide increased feedback inhibition to pyramidal neurons across layers and columns in proportion to the rate and duration of presynaptic activity.⁶⁰⁻⁶³ Such feedback inhibition can theoretically perform the role of a “circuit breaker”. However, according to our results, even in the presence of an intact GABAergic drive from SST interneurons, a synaptic deficit of GABA release from PV interneurons is sufficient to cause seizures. This likely reflects the preponderance and strength of the inhibition produced by PV interneurons, and adds to the body of evidence indicating that they are the main contributors to the maintenance of the overall excitation/inhibition balance in the cortex.^{64, 65}

Our results also reveal that the same *Cacna1a* mutation in different cellular populations leads to drastically different consequences in synaptic function despite similar compensation by other Ca^{2+} channel types. Indeed, the loss of P/Q-type Ca^{2+} channels from central synapses is usually compensated by other members of the Ca_v2 family, including N- or R-type Ca^{2+} channels.⁴² We show here that while compensation by N-type Ca^{2+} channels in *Cacna1a* mutant mice can sustain GABA release from SST cells, it is insufficient to compensate for the loss of P/Q-type Ca^{2+} channel function in FS interneurons, resulting in desynchronized and unreliable GABA release from these cells. P/Q-type Ca^{2+} channels in FS interneurons are known to be tightly coupled to the presynaptic release machinery and to establish Ca^{2+} nanodomains close to the release sites.⁶⁶ It is possible that N-type Ca^{2+} channels are unable to compensate for the loss of P/Q-type Ca^{2+} channel function in FS interneurons because they fail to form similar Ca^{2+} nanodomains. Alternatively, it has been suggested that different Ca^{2+} channels compete for specific presynaptic slots, which governs their contributions to neurotransmission.⁶⁷ The slots available to N-type Ca^{2+} channels in presynaptic terminals of FS cells may be located further away from these release sites, preventing the quick local build-up of Ca^{2+} that might be required for the faster, more efficient release of FS interneurons.

Finally, our results illustrate a surprising mechanistic relationship between different seizure types and various epileptic disorders that have been previously thought of as unrelated. Patients with severe developmental childhood epilepsies typically develop a wide variety of seizures that often lead to significant neurological impairment and high mortality rates.⁶⁸⁻⁷¹ In contrast, patients with IGE epileptic disorders generally have milder seizures with later onset.⁷² Here, we observe that a synaptic dysfunction in cortical GABAergic interneurons in mice can lead to generalized seizures resembling both severe developmental epileptic and mild IGE disorders depending on the additional involvement of cortical principal cells. In particular, decreased synaptic output from cortical pyramidal cells as a result of the loss of *Cacna1a* gene function prevents the occurrence of the motor seizures (tonic and tonic-clonic seizures) that are observed when the loss of this gene is confined to MGE-derived cortical interneurons. Assuming that these results can be generalized, this suggests that mutations that selectively impair cortical interneuron function may underlie severe epileptic syndromes, whereas mutations that impair both inhibitory and excitatory synaptic transmission will likely result in milder IGE phenotypes. These findings illustrate the importance of studying the cell-type specific consequences of given genetic mutations for elucidating the cellular impairments that result in disease states.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. The selective removal of *Cacna1a* within a subset of cortical interneurons results in a severe epileptic phenotype

A) Between P16 and P18, *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutants develop frequent interictal epileptic activity appearing independently in the cortex and hippocampi and occurred more frequently during sleep (SWS: slow-wave sleep and REM: rapid eye-movement sleep). In addition, *Nkx2.1BAC^{Cre};Cacna1a^{c/c}* mutants develop a variety of generalized seizures including: B) myoclonus, characterized at the EEG level by a burst of polyspikes crowning a slow wave, C) tonic-clonic seizures with mixed fast beta activity, polyspikes and spike-wave complexes in the cortex and 5-7 Hz spike-wave complexes in the hippocampus on EEG, D) myoclonic absences with generalized 5-7 Hz spike-wave activity and E) tonic seizures with predominant fast beta and polyspikes activity on EEG (see also Supplementary Fig. 3) (n=11)

Figure 2. The loss of *Cacna1a* in MGE-derived interneurons does not grossly alter their maturation or laminar distribution

In *Nkx2.1BAC^{Cre}; Cacna1a^{c/c}* mice carrying the *RCE^{EGFP/+}* reporter allele, genetically modified interneurons lacking *Cacna1a* gene function appear to mature normally. A) In these mutants, the normal expression of histochemical markers is observed as illustrated here by the PV expression of mutants (top) versus WT controls (bottom). This is quantified in B) though the demonstration that the total number of cells expressing EGFP, PV (parvalbumin), SST (somatostatin), subdivided as SST alone versus SST-CR (somatostatin-calretinin) double expression, and CR/SST- (calretinin alone) is unaltered in mutant animals. (n=3 wt controls, n= 7,5,6 for mutants EGFP, PV, SST/CR stains, unpaired Student's t-test with $p>0.05$, error bars= s.e.m.). In addition, the laminar distribution of cortical interneurons is normal as quantified in C) illustrating the number of EGFP cells per layer (n=3 controls, n=7 mutants). D) Paired recordings were performed as illustrated: (top) transmitted light image of mouse S1 cortex, with two recording electrodes in L5; (bottom) a fluorescent image of the dotted area in the top panel showing EGFP expression as driven by the *Nkx2.1* promoter. E) Representative examples of PC and PV interneuron firing patterns in wild-type and mutant animals. A summary table F) showing basic intrinsic properties of PV interneurons from wild-type and mutant animals (n=10, means $\pm$ s.d., FF – firing frequency).

Figure 3. The loss of *Cacna1a* in parvalbumin-positive interneurons leads to impaired synaptic transmission

Defects of inhibitory synaptic transmission in *Nkx2.1-BAC^{Cre};Cacna1a^{c/c}* animals were evident in paired recordings of PV interneurons (INs) and PCs. A) A representative example of a connected L5 pair of PV IN and PC recorded in a whole cell patch-clamp mode (insets – firing patterns of both cells, scale bars: 20 mV, 50 ms). Action potentials (APs) in a presynaptic PV IN produced a unitary inhibitory PSCs (uIPSCs) in postsynaptic PC, 20 of which are overlaid, average of all successful events is shown in blue (top); note the absence of failures and synchronous onset of uIPSCs. B) Similar analysis in mutant animals showed a marked increase in variance of responses to PV IN stimulation and a high failure rate. C) Statistical comparison of connection probabilities (connected/all tested), uIPSC amplitude and failure rates. Each dot represents the corresponding value for a recorded pair, whereas bars represent means. Frequency histogram of a synaptic delay distribution of uIPSCs measured relative to the peak of presynaptic action potential. The data was pooled from all connected pairs and fitted with a Gaussian; jitter is calculated as a half-width of a Gaussian. #*p*<0.05, z-test; **p*<0.01, ***p*<0.001, ****p*<0.0001 – Mann-Whitney u-test.

Figure 4. GABAergic transmission from SST-positive interneurons is preserved following *Cacna1a* removal

Viral infection of SST-positive interneurons with ChR-2. A) Expression of EGFP in slices from an adult *SST^{Cre};RCE^{EGFP/+}* animal (left); expression of ChR-2 linked to mCherry (center); an overlay of both fluorophores showing a 100% confinement of mCherry signal to a subset of SST-positive interneurons (right). B) An overlay of ChR2-mCherry expression and a transmitted light image of a 300 μ m coronal brain slice showing cell bodies of SST-positive cells. C) Inhibitory currents produced by a paired-pulse light stimulation of ChR-2 (700 mW, 5 ms, 20Hz) recorded in PCs of cortical layer 5 in slices from wild-type animals. Numbers i, ii and iii (top) correspond to marked boxes at the time-course of changes in le-IPSCs amplitude (bottom) and represent effects of N- and P/Q-type Ca^{2+} channel antagonists on the amplitude of first and second le-IPSC. D) Same as in C) but in cells from *SST^{Cre};Cacna1a^{c/c}* animals. E) Pooled data of le-IPSC amplitudes recorded from 11 wild-type and 13 mutant slices. N.S. – no significant difference, $p > 0.3$ Mann-Whitney u-test.

Figure 5. LVA calcium channel availability and burst firing of relay neurons in ventrobasal thalamus of *Nkx2.1^{Cre};Cacna1a^{c/c}* mutants

A) Representative current traces for steady-state inactivation of LVA Ca^{2+} currents in cells from WT (left) and mutant (right) animals. A standard double-pulse protocol for steady-state inactivation was given from the holding potential of -70 mV. A 1-sec pre-pulse at potentials ranging from -100 to -40 mV preceded each depolarization, followed by a voltage step to -40 mV. The interpulse interval was 10 sec. B) Pooled maximal current densities (cyan trace in A) divided by cell capacitance) in relay neurons from WT and mutant animals. C) Pooled current densities, calculated from the inactivation protocol and normalized to the maximum are plotted as a function of prepulse membrane potentials and fitted with a Boltzmann function. D) A burst of action potentials in response to a 600 ms, 20 mV hyperpolarization step recorded in thalamic relay neurons from WT (left) and mutant (right) animals. Insets show cell positions in the thalamus. E) Pooled averages of number of spikes in the burst (20 trials per cell, n=10 and 11 cells respectively). N.S. – no statistical difference as to unpaired Student's *t*-test.

Figure 6. Compound removal of *Cacna1a* in cortical pyramidal cells and interneurons results in a mild IGE-like epileptic phenotype

Nkx2.1BAC^{Cre};Emx1^{Cre};Cacna1a^{c/-} mutants develop an IGE-like epileptic disorder combining A) absences characterized on EEG by 5-7Hz generalised spike-wave discharges and B) myoclonus with a generalized polyspikes and slow-wave complex (arrow). Seizure duration (C) and frequency (D) are compared between mutants and controls across genotypes at early (P16-18, black bars) and late (P28-30, grey bars) time-points. Note that seizures are more prolonged in *Nkx2.1BAC^{Cre};Cacna1a^{c/-}* mutants than in *Nkx2.1BAC^{Cre};Emx1^{Cre};Cacna1a^{c/-}* mutants at P16-18. In addition, absences become more frequent and more prolonged in *Nkx2.1BAC^{Cre};Emx1^{Cre};Cacna1a^{c/-}* mutants with age. (Pooled data at P16-18: n=9 *Nkx2.1BAC^{Cre};Cacna1a^{c/-}* mutants, n=3 for *Nkx2.1BAC^{Cre};Emx1^{Cre};Cacna1a^{c/-}* mutants, at P28-30, n= 3 for *Nkx2.1BAC^{Cre};Emx1^{Cre};Cacna1a^{c/-}* mutants, Student's unpaired t-test: *p<0.05)

Figure 7. The loss of *Cacna1a* gene function from cortical pyramidal cells resulted in reduced cortical excitability

A) Representative cell-attached recordings from infragranular PCs during extracellular stimulation by a laterally positioned bipolar concentric electrode (see inset in C). The same stimulation intensities produced markedly reduced number of spikes in mutant animals compared to wild-type controls. B) Summary data representing the dependence of spiking probability during a stimulus train (5 stimuli at 20 Hz) on the stimulus intensity. A response curve for each recorded cell was calculated as a logistic function fit of experimental data; note the right shift of response curves from mutant animals (red) compared to wild-type controls (black). C) Representative whole-cell recordings of evoked EPSCs in PCs from mutant animals (red) and from wild-type controls (black) in response to the same intensity stimulation. Inset shows location of recording (1) and stimulating (2) electrodes. Bottom, pooled data on evoked EPSCs amplitudes and paired-pulse ratios from mutant and control animals. *p<0.05; **p<0.01 Student's unpaired t-test.