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► To cite this version:

Nathalie Leresche, Vincenzo Crunelli. Corticothalamic network abnormalities underlying absence seizures. The Cerebral Cortex and Thalamus, Oxford University Press, In press. hal-04229142

HAL Id: hal-04229142

<https://hal.science/hal-04229142>

Submitted on 5 Oct 2023

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Corticothalamic network abnormalities underlying absence seizures

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Key words: Ca²⁺ channels; channelopathy; genetic generalized epilepsy; HCN channels; Na⁺
channels; neuronal synchronization; spike-wave discharges; somatosensory cortex; thalamic
reticular nucleus; ventrobasal thalamic nucleus.

Word count: Abstract 202, Main Text: 4903. Number of references: 67

Acknowledgments

Work in our labs is supported by the CNRS, Sorbonne Université and Inserm (grants to NL)
and the Ester Floridia Neuroscience Research Foundation (grant 1502 to VC).

Conflict of interest

The authors declare no conflict of interest.

Abstract

Absence seizures (ASs) are genetic generalized seizures and one of the critical neurological phenotypes that originates from abnormalities in corticothalamic (CT) networks. Genetic analyses of cohorts with only ASs have identified 2p16.1 and 2q22.3 as significant loci and a few genes related to intrinsic and synaptic membrane channels. In normal animals, the genetic mutation of a single voltage- or ligand-gated channel in a single cortical or thalamic neuronal population can lead to spontaneous ASs. The electrographic activity of ASs invariably initiates from a localized cortical area but the full expression of these seizures' clinical symptoms requires interactions between cortical and thalamic networks. In contrast to focal and other genetic generalized seizures, the ictal activity of cortical and thalamic neurons during ASs is predominantly characterized by a decreased, but highly synchronized, firing with individual neurons often showing a different firing pattern from one seizure to the next.. The high remittance rate of children with ASs indicates that CT networks, and/or their modulatory inputs from the basal ganglia and brain stem, have the inherent capacity to compensate for the developmental abnormalities that lead to ASs, but the mechanisms of remittance have not been investigated since all available models lack this feature of human ASs.

1. Introduction

As early as the first half of the 20th century, the German neurologist Hans Berger hypothesized that the 3Hz oscillations that he observed in the EEG of a patient during an absence seizure (AS) resulted from an abrupt withdrawal of tonic inhibitory influences exerted by the thalamus on the cortex (see Avoli, 2012). Thus, although at that time the mechanism of these electroencephalographic (EEG) oscillations (now known as spike-and-wave discharges, SWDs) was completely unknown, the cortex and the thalamus were already considered major players in the generation of this paroxysmal activity. Nowadays, there is a general consensus that ASs are a disease of corticothalamic (CT) networks with a critical modulation by the basal ganglia and brain stem (Crunelli et al., 2020).

Following a short summary of the clinical presentation, therapy and comorbidities of ASs, we first discuss their genetics and the mechanisms involved in their initiation and maintenance in humans and in models of ASs. The main body of this chapter is a focused review of the current state of knowledge of the molecular and cellular abnormalities in different neuronal populations of CT networks that can bring about ASs, using evidence both from investigations that have used selective genetic manipulations in normal non-epileptic animals and from studies in five genetic models with spontaneous ASs that have been validated for their strong face-, construct- and content-validity: the polygenic GAERS and WAG/Rij rats, and the monogenic stargazer (STG), tottering (TG) and lethargic (LH) mice (Pitkänen et al., 2017). Due to space limitations, studies using only pharmacological manipulations are not discussed.

2. Clinical presentation of ASs

An AS is a sudden and relatively brief lapse of consciousness associated with lack of voluntary movements and distinctive SWDs at 2.5-4Hz in the EEG (Crunelli et al., 2020). ASs are present, together with other seizure types, in many genetic generalised epilepsies (GGEs), here we focus on ASs observed in childhood absence epilepsy (CAE) since in this epilepsy type ASs are the only clinical symptom present and not compromised by other epileptic phenotypes. In CAE, ASs begin between 3 and 8 years of age and can occur up to ~200 per day. Approximately 70% of CAE patients go into spontaneous remission around adolescence (Crunelli and Leresche, 2002), though the mechanisms underlying remission have not been elucidated, since currently researched ASs models lack spontaneous remission. Pharmacological treatment of ASs relies primarily on ethosuximide (ETX), valproic acid,

lamotrigine or their combinations, but ~30% of children are pharmaco-resistant and ~60% show neuropsychiatric comorbidities (see Crunelli et al., 2020).

3. Genetics of ASs

Strong evidence exists for a genetic component in CAE, possibly of a polygenic nature (Perucca et al., 2020) and concordances of 70%–85% and 33% have been reported in monozygotic twins and first-degree relatives, respectively (Gruenbaum et al., 2021). However, most CAE patients do not have family history of epilepsy and siblings of affected individuals have an 8% risk of ASs, which is lower than the expected risk for a recessive (25%) or dominant (50%) inherited trait (Perucca et al., 2020).

Most AS genetic studies have used cohorts with a complex phenotype that included various epilepsies and other neurological deficits, rendering it difficult to demonstrate an association between gene abnormalities and ASs. Further, these studies focused on a few families and reported diverse mutations mainly in γ -aminobutyric acid receptor (GABA_AR) subunits and voltage-gated Ca²⁺ channels (reviewed in Crunelli and Leresche, 2002). More recently, in a large study of GGEs (that included CAE as well as juvenile absence epilepsy), no single gene reached exome-wide significance, though lead associations were found for *CACNA1G*, *EEF1A2* and *GABRG2*, and, notably, GABA_AR genes, but not excitatory receptor genes, were enriched for missense variants (Feng et al., 2019).

The first genome-wide association study of a large, pure CAE cohort (778 Caucasian, 15 African) has identified two significant loci: 2p16.1 and 2q22.3 (International League Against Epilepsy Consortium on Complex Epilepsies, 2018). Though the precise genes in these loci that contribute to the CAE phenotype are still unknown, prioritized genes include *FANCL*, *BCL11A* and *ZEB2*, i.e., genes not linked to membrane channels or transmitter pathways that are generally considered key proteins for AS expression. Notwithstanding, mutations in *SCN1A*, *SCN8A*, *CACNA1H* and *CLCN2* have been identified in different Chinese CAE cohorts (Xie et al., 2019).

A large number of spontaneous single gene mutations bring about spontaneous ASs in mice (Frankel, 2009), some of which have been validated as ASs models, including *CACNG2* (in STG mice), *CACNA1A* (in TG mice) and *CACNB4*, (in LH mice). Whole-genome sequencing of the polygenic GAERS rats has identified *CACNA1H* (R1584P) as the only gene-variant that selectively co-segregate with high-seizuring F2 progenies (Casillas-Espinosa et al., 2017). Notably, a combined proteomic and metabolomic analysis of GAERS rats has found

an increase in pathways involved in synaptic transmission and metabolism of neurotransmitters and the down-regulation of the lysine degradation pathway in both cortex and thalamus (Harutyunyan et al., 2022). However, adult rats were used in this study, raising the issue of whether the observed changes in proteins and metabolites are the cause or a consequence of ASs.

In summary, single gene variants are causally linked to the expression of spontaneous ASs in mice models. However, the human homologs of these genes as well as the mutant genes found in the Chinese CAE cohorts are not located in the significant loci (2p16.1 and 2q22) identified in a large CAE cohort.

4. Cortical initiation networks of ASs

Although ASs are classified as generalized seizures, many studies in humans report that SWDs originate from cortical regions before spreading to the entire cortical surface and other brain regions, though the cohorts of these studies had a mixed phenotype including different GGEs (reviewed in Crunelli et al., 2020). The first study on a pure CAE cohort reported cortical-selective increases in the amplitude of blood-oxygen-level-dependent (BOLD) signals occurring as early as 14s before the clinical and electrographic signatures of an AS could be observed (Bai et al., 2010). The cortical area(s) showing these pre-ictal increases in BOLD amplitude are mostly in frontal regions and can differ from child to child, but are generally consistent across seizures for a given child (Bai et al., 2010). Importantly, the same cortical areas show a post-ictal decrease in BOLD amplitude that can persist for up to 10s after seizure-offset (Bai et al., 2010).

Changes in BOLD signals also occur in the thalamus during ASs (particularly, in the anterior, centromedian, parafascicular and reticular nuclei), supporting its critical involvement in the expression of these seizures (Bai et al., 2010; Guo et al., 2016).

The lack of generalization at the onset of an AS, i.e., the existence of a cortical initiation network (CIN), had been originally reported in two rat models of ASs. It was found that the peri-oral region of the primary somatosensory cortex (S1) was the cortical initiation network in GAERS and WAG/Rij rats (Figure 1A) and that S1 Layer 5/6 excitatory pyramidal neurons were the first to fire at the start of SWDs (Figure 1B) (Meeren et al., 2002; Polack et al., 2007). Moreover, BOLD signals in awake GAERS rats recapitulate human BOLD results with an AS-linked decrease and increase in signal in S1 and ventrobasal (VB) thalamus, respectively (McCafferty et al., 2023).

All these pre-seizure changes observed with non-invasive imaging and EEG in humans and animal models may be the substrate for impaired consciousness, including loss of responses to external stimuli and a pause in ongoing interactive behaviours, which occur not only during, but even before the development of, SWDs (Guo et al., 2016). Notably, the magnitude of BOLD and EEG changes in the cortex and thalamus that precede an AS are positively linked to the severity of altered behavioural responses and consciousness during a seizure and may thus be pre-determined by the state of CT networks before seizure-onset (Bai et al., 2010; Guo et al., 2016). Notably, in GAERS rats, the expression of the AS-related behavioural phenotype requires the mutual interactions of cortical and thalamic networks, as shown by the lack of any overt behavioural sign when short (1-2sec) small amplitude SWDs are present in the cortex but not in the thalamus (Figure 1C) (Polack et al., 2007), indicating that the recruitment of the thalamus is a necessary step for the expression and maintenance of ASs (see Part 6 below for intralaminar thalamic nuclei).

In summary, it is now well established that 1) SWDs are not generalized from the start but originate from selective CINs, with S1 Layer 5/6 pyramidal neurons being the first to fire in animal models; 2) the cortex and thalamus are critical elements for the full expression of ASs; and 3) the severity of the clinical symptoms of ASs may be predicted by the degree of pre-seizure EEG and BOLD changes and predetermined by persistently abnormal CT networks.

5. Mechanisms of altered excitability in deep layers of the CIN

The presence of a CIN in animal models has allowed to study the mechanism(s) underlying the AS initiation. Strong evidence obtained *in vivo* and *in vitro* in four AS models (GAERS and WAG/Rij rats, STG and TG mice) has shown enhanced excitability of Layer 5/6 pyramidal neurons (Kole et al., 2007; Polack et al., 2007) that is evident from the presence of spontaneous giant depolarizing potentials, an increased number of neurons with intrinsic strong bursting and N-methyl-D-aspartate (NMDA)-mediated EPSPs as well as an enhanced firing rate, response to weak stimuli and gain of input-output function of Layer 5/6 neurons (D'Antuono et al., 2005; Polack et al., 2007). Moreover, in WAG/Rij rats, it has elegantly been shown that a selective decrease in HCN1, but not HCN2 channels in the distal apical dendrites of Layer 5 pyramidal neurons leads to a decreased dendritic I_h current, larger and faster backpropagating action potentials and increased somato-dendritic coupling, so that the summation of synaptic potentials is enhanced (Figure 1D) (Kole et al., 2007). These intrinsic HCN channel-dependent changes in excitability are strengthened by a decreased peak

conductance and paired-pulse depression (PPD) of evoked GABA_A inhibitory post-synaptic potentials (eIPSPs) in S1 Layer 5 (and Layer 2/3) pyramidal neurons and evoked excitatory post-synaptic potentials (eEPSPs) with a reduced PPD and sensitivity to baclofen, a GABA_BR agonist. The net result of all these changes in synaptic activity in S1 Layer 5/6 pyramidal neurons of WAG/Rij rats is an increased EPSP/IPSP ratio since i) the PPD of both EPSPs and IPSP is decreased, ii) the number of NMDA EPSPs is increased, and iii) the peak conductance of IPSCs is decreased.

Notably, a similar decrease in cortical inhibition is present in S1 of the TG mice, where a comprehensive study of EPSPs and IPSPs evoked either by thalamic stimulation or locally (either in Layer 4 or 5), has compellingly shown that the feed-forward inhibition from the thalamus to Layer 4 is markedly compromised and correlated to the developmental onset of ASs (Sasaki et al., 2006).

Notably, there is a lower expression of PV+ interneurons in all S1 layers of WAG/Rij rats (Papp et al., 2018). Functionally, chemogenetic silencing of S1 PV+ interneurons in normal mice is sufficient to generate ETX-sensitive ASs (Panthi and Leitch, 2019), whereas DREADD-mediated activation of S1 PV+ interneurons, i.e., activation of local feed-forward inhibition, protects against pentylenetetrazol-induced SWDs (Panthi and Leitch, 2019) (see Part 7, below).

In summary, in WAG/Rij rats, we have a clear mechanistic explanation of the aberrant behaviour of S1 Layer 5/6 pyramidal neurons where SWDs originate: intrinsic HCN1 loss-of-function (leading to enhanced synaptic potential summation) combines with an increased EPSPs/IPSPs ratio to bring about the enhanced excitability observed in this neuronal population. A decreased cortical inhibition is also present in TG mice, but similar detailed mechanistic studies of S1 are not available for GAERS rats and STG and LH mice. PV+ interneurons have an anti-absence role since their selective silencing in normal non-epileptic animals leads to AS expression.

6. Cellular activity in the neocortex and thalamus during ASs

Using electrophysiological and imaging approaches in non-anesthetized AS models, the ictal neuronal activities that occur in the cortex and thalamus have recently been studied with single-cell resolution. Using Ca²⁺-sensitive dyes in the primary visual cortex in STG mice, Meyer et al. (2018) reported that ~80% of neurons in all cortical layers reduce their activity before a SWD is detected in the EEG, while smaller neuronal pools show an increased, or no

change in, activity (Figure 2A). Interestingly, the ictal involvement of each neuron is not fixed but fluctuates from seizure to seizure. An overall decrease in neuronal activity during ASs has been confirmed in S1 cortical neurons of GAERS rats (McCafferty et al., 2023). However, on closer examination, four patterns of ictal activity could be identified. While 37% of neurons experienced a sustained ictal decrease in firing, 28% showed a transient firing peak at seizure-onset. Importantly, regardless of the type of firing, all cortical neurons show a marked increase in rhythmicity during SWDs compared to interictal periods and all changes precede seizure-onset by up to 80 seconds.

Simultaneous ensemble recordings in cortical neurons and VB thalamocortical (TC) and nucleus reticularis thalami (NRT) neurons during spontaneous ASs in GAERS rats and γ -hydroxybutyric acid (GHB)-induced ASs in Wistar rats under freely moving conditions show that cortical afferent activity drives thalamic firing and temporally frames thalamic output via feed-forward inhibition of TC neurons by NRT neurons (Figure 2D) (McCafferty et al., 2018). This results in a marked decrease in the overall firing of TC neurons (with only rare T-type Ca^{2+} channel-dependent burst firing being present ictally) (Figure 2B), which is causally linked to the constitutively enhanced tonic GABA_A current of TC neurons that is present in this AS model as well as in STG and LH mice (Cope et al., 2009). Nevertheless, the total firing of TC (and NRT) neurons is strictly in phase with each SWD cycle (Figure 2C), indicating that the synchrony and strength of the ictal output of TC neurons to the neocortex is not a feature of single, rhythmically bursting TC neurons, as previously suggested (Sorokin et al., 2017), but an emergent property of this neuronal population. Notably, an altered firing that precedes the start of an AS is also present in WAG/Rij rats and STG mice (Sorokin et al., 2017).

A clear limitation in our understanding of thalamic activity during ASs is that the vast majority of available data have been obtained in first-order somatosensory thalamic nuclei (see Part 7, below), whereas higher-order and intralaminar thalamic nuclei have been relatively neglected. Notwithstanding, the rodent thalamic posterior nucleus may be one of the key thalamic hubs in view of its critical involvement in pre- and post-ictal activity (Lüttjohann and Pape, 2019). Notably, TC neurons in intralaminar thalamic nuclei show a similar decrease in total firing as that of first-order thalamic nuclei during ASs but present two patterns of ictal burst firing: one with a transient increase in bursts at seizure-onset and one with a sustained increase in burst firing that lasts for the duration of the seizure (Atherton et al., 2023). Moreover, it is interesting to note that extracellular alkalosis elicits ASs by

activating intralaminar thalamic, but not cortical and first-order thalamic neurons of WAG/Rij rats (Salvati et al., 2022b), a finding of key clinical significance since hyperventilation is a trigger of ASs in many CAE patients.

Finally, although ASs are generated by CT network, both the direct and indirect basal ganglia pathways provide a fine control of the expression of these seizures (Deransart and Depaulis, 2002). Ictal changes in MRI BOLD signals in the basal ganglia of CAE cohorts have been consistently reported (Bai et al., 2010). Moreover, genetic deletion of the Munc-18-1 protein in cortico-striatal Layer 5 neurons elicits ETX-sensitive ASs, and activation of DREADD-transfected striatal fast-spiking interneurons blocks the spontaneous ASs of mice with whole-brain KO of Munc-18-1 (Miyamoto et al., 2019).

In summary, most cortical neurons decrease their firing during ASs but there are significant neuronal pools that either increase or do not change their firing rate. In the thalamus, the total and burst firing rate of TC neurons are reduced but that of the GABAergic NRT neurons is increased. Each cortical and thalamic neuron may show different activity changes from one seizure to the next. Intrathalamic thalamic nuclei are critical for AS synchronization and maintenance.

7. Induced genetic alterations in CT networks that lead to ASs

To understand how alterations in different neuronal populations of CT networks lead to the expression of ASs, i.e., to an abnormal function of these networks, many studies have used genetic tools to manipulate voltage- and ligand-gated membrane channels in normal animals. In the following, we provide a detailed summary of all the studies that genetically manipulated voltage- and ligand-gated membrane channels in normal non-epileptic animals and compare these findings with studies that have investigated abnormalities in voltage- and ligand-gated membrane channels of five well-validated AS models: GAERS and WAG/Rij rats, and STG, TG and LH mice. Due to space limitations, we report changes in the expression levels of intrinsic and synaptic membrane channels only if their functional consequences have been investigated. Moreover, the abnormalities are described in comparison with the respective non-epileptic control animals of each model.

7.1 Ligand-gated channels

GABA_AR $\alpha 3$ subunit knock-out (KO) mice do not express spontaneous ASs but show a decrease in GHB-induced SWDs (Winsky-Sommerer et al., 2008), whereas GABA_AR $\beta 3$ subunit KO mice show a complex epileptic phenotype that only at times includes ASs

(DeLorey et al., 1998). Spontaneous ASs are observed in mice knock-in (KI) for the human $\gamma 2$ subunit R43Q mutation (Tan et al., 2007) (which had been identified in probands with febrile seizures and ASs (see Crunelli and Leresche, 2002), whereas whole-brain KO of GABA_BR1 or GABA_BR2 lead only to rare or no spontaneous SWDs, respectively (Gassmann et al., 2004; Schuler et al., 2001).

Paradoxically, whole brain ablation of one of the subunits of the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (*GRIA4* KO mice) results in spontaneous ASs with decreased EPSCs in NRT but not TC neurons (Paz et al., 2011). Notably, decreased eEPSCs are present in TC neurons of TG mice and reduced NMDA and non-NMDA eEPSCs in LH mice (Caddick et al., 1999).

A common finding in GAERS rats and STG and LH mice is the constitutively enhanced tonic GABA_A current of TC neurons that results from a non-genetic loss-of-function of GAT1 (one of the GABA transporters) that is necessary and sufficient for AS expression (Cope et al., 2009). In TC neurons of LH mice, GABA_B eIPSCs are decreased but there is no change in GABA_A eIPSCs and baclofen-evoked currents (Caddick et al., 1999). The lack of changes in GABA_A IPSCs is shared by other models since no difference was found in GABA_A miniature IPSCs (mIPSCs) and eIPSCs in TC neurons of GAERS rats (Bessaïh et al., 2006; Cope et al., 2009) and TG mice (Caddick et al., 1999), respectively. An increased amplitude, and decreased frequency and duration of mIPSCs but no change in PPD was reported in GAERS NRT neurons (Bessaïh et al., 2006).

As far as excitatory transmitter function is concerned, a detailed analysis of synaptic potentials in NRT neurons has only been carried out in STG mice. Using a combination of electrical stimulation and photolysis of caged-glutamate, it was elegantly shown that a trafficking defect of AMPA receptors (i.e., decreased synaptic and increased perisynaptic receptors) leads to a reduction of AMPA EPSCs and a compensatory increase of NMDA EPSCs efficacy, leading to an increased NMDA/AMPA charge of EPSCs of both thalamic and cortical origin (Lacey et al., 2012).

7.2 Voltage-gated Ca^{+2} channels

Selective knock-down (KD) of Cav2.1 channels in Layer 6 pyramidal neurons of normal mice leads to spontaneous ETX-sensitive ASs accompanied by increased T-type Ca^{2+} currents in the TC and NRT neurons and increased burst firing in the latter but not the former thalamic neural population (Bomben et al., 2016) (Figure 3). Since no changes in cortical Cav2.1

channels expression were observed with light microscopy, T-type Ca^{2+} currents and associated burst firing was not investigated in cortical excitatory and inhibitory neurons in these Layer 6 pyramidal neuron-selective KD mice, making it difficult to accept the authors' conclusion that changes in thalamic neuron excitability were the only ones causally linked to AS expression. Indeed, ablation of $\text{Cav}2.1$ channels in adult mice brings about ASs that are not associated with changes in T-type Ca^{2+} currents or burst firing in TC neurons (Miao et al., 2019). Similarly, selective KO of $\text{Cav}2.1$ channels in PV+ and somatostatin-positive (SST+) cortical interneurons leads to ASs with no change in T-type Ca^{2+} currents or burst firing of TC neurons (Figure 3) (Rossignol et al., 2013), demonstrating that a constitutive or induced increase in these TC neuron parameters, as observed in TG mice and mice with selective Layer 6 pyramidal neurons KD of $\text{Cav}2.1$ channels, is not necessary for the expression of ASs.

$\text{Cav}2.3$ channels are expressed in cortical and NRT, but not TC neurons, and their whole-brain KO leads to a decrease in GHB-induced ASs (Zaman et al., 2011). However, whether this pathological phenotype is due to NRT or cortical $\text{Cav}2.3$ channels remains to be investigated.

Whole-brain overexpression of $\text{Cav}3.1$ channels lead to spontaneous ETX-sensitive ASs (Ernst et al., 2009), whereas mice with KO of these channels show a decrease in baclofen and GHB-induced SWDs (Kim et al., 2001). Notably, the spontaneous ASs observed in $\text{Cav}2.1$ KO mice (Bomben et al., 2016; Miao et al., 2019; Song et al., 2004) are abolished by the simultaneous KO of $\text{Cav}3.1$ channels. However, despite the substantial expression of $\text{Cav}3.1$ channels in cortical territories (Talley et al., 1999), the analysis of the cellular/network abnormalities in these single and double KO mice was only carried out in the thalamus. So, though these studies strongly point to a key role of $\text{Cav}3.1$ channels in the genesis of ASs, they do not clarify the brain area(s) and neuronal population(s) where these channels are critical for AS expression (see below).

Notably, elevated T-type Ca^{2+} currents (mainly due to $\text{Cav}3.1$ channels) are present in TC neurons of different thalamic nuclei of four AS models (WAG/Rij rat, and STG, TG and LG mice) (Broicher et al., 2008; Zhang et al., 2002). The block of ASs by whole-brain genetic ablation of $\text{Cav}3.1$ channels in STG, TG and LH mice has been causally linked to the loss of function of these channels in TC neurons (Song et al., 2004). However, such conclusion is flawed since the block of ASs in these mice could also be due to the simultaneous ablation of $\text{Cav}3.1$ channels in the cortex where they are highly expressed (Talley et al., 1999). Indeed,

ASs in GAERS rats are not affected by selectively blocking Cav3.1 channels and related burst firing of TC neurons with a potent T-type Ca^{2+} channels antagonist (McCafferty et al., 2018). Notwithstanding, TC neuron firing is necessary for AS expression since brief optogenetic manipulation of TC neurons abort ASs in WAG/Rij rats and STG mice (Sorokin et al., 2017).

Mice with whole-brain KO or selective KD of Cav3.3 channels in the NRT do not show spontaneous ASs, but an unexpected increase in GHB-induced ASs (Lee et al., 2014) (Figure 3). The same phenotype is observed in double KO mice of Cav3.3 and Cav3.2 channels (Lee et al., 2014) where the T-type Ca^{2+} current of NRT neurons is completely abolished. Thus, notwithstanding the key role of the NRT neurons T-type Ca^{2+} current (and the burst firing that it generates) in physiological activities expressed by CT networks (Crunelli et al., 2018), full ablation of these NRT channels leads to potentiation of pharmacologically induced ASs. Whether this is due to the increased tonic firing of NRT neurons that is present in these single and double KO mice (Lee et al., 2014) or results from alterations in the activity of cortical neuronal populations remains to be elucidated since the analysis of the cellular and network abnormalities of these mice was only carried out in the thalamus.

Elevated T-type Ca^{2+} currents are also present in the NRT neurons of GAERS and WAG rats (Broicher et al., 2008; Cain et al., 2018) but have not been studied in the NRT of STG, TG and LH mice. In GAERS rats, this increased current is due to the R158P missense variant of *CACNA1H* (the Cav3.2 channel gene) and selective KD of these channels in NRT neurons markedly reduces ASs and normalizes their elevated burst firing (Cain et al., 2018). Moreover, KI of the human *CACNA1H* variant C456S in S1 of normal non-epileptic rats elicits spontaneous ASs in >60% of the injected animals (Wang et al., 2015).

7.3 Voltage-gated Na^+ and Na^+/K^+ channels

Ablation of Nav1.2 channels in the whole-brain or selectively in telencephalic and diencephalic neurons leads to spontaneous ETX-sensitive ASs with smaller SWDs being present in the thalamus compared to those in S1, medial prefrontal cortex, and hippocampus (Ogiwara et al., 2018). Ablation of Nav1.6 channels either in the whole-brain, only in inhibitory neurons or in the NRT (Figure 3) brings about ETX-sensitive ASs, whereas no seizures are observed when these channels are ablated in either all cortical neurons, in glutamatergic excitatory neurons, or only in inhibitory neurons of the cortex, hippocampus, and striatum (Makinson et al., 2017).

Mice with whole-brain KO of hyperpolarization-activated, cyclic nucleotide-gated Na^+/K^+ channels subtype 2 (HCN2) show spontaneous ASs (Ludwig et al., 2003) but HCN4 channel

KO mice do not (Zobeiri et al., 2019). However, extensive analysis of thalamic neuron abnormalities but no data on cortical neurons were provided in these two studies, leaving a knowledge gap on the relative contribution of HCN2 and HCN4 channels in the cortical and thalamic sectors of the CT network. In TC neurons of different first-order thalamic nuclei of both GAERS and WAG/Rij rats, there is an increased I_h (Budde et al., 2005; Cain et al., 2015; Kanyshkova et al., 2012), that most likely results from an increased HCN1 channel density with no change in HCN2 and HCN4 expression (Cain et al., 2015). Notably, the increased density of HCN1 channels in thalamic nuclei of WAG/Rij rats (Kuisle et al., 2006) is in contrast to their decreased expression in S1 (Kole et al., 2007) (see Part 5, above), indicating that in a given AS model, and potentially in humans with ASs, the same channels can be differentially expressed in diverse sectors of the CT network. Notably, pharmacological block of all HCN subtypes systemically, or locally in S1 or VB, blocks ASs (Iacone et al., 2021).

In summary, the following significant conclusions arise from these studies:

1. genetic ablations of diverse single channel abnormalities in single neuronal populations of the CT network lead to spontaneous ETX-sensitive ASs (Figure 3); Cav2.1 channels in cortical layer 6 neurons (Bomben et al., 2016), Cav2.1 channels in cortical PV+ and SST+ interneurons (Rossignol et al., 2013), Nav1.6 channels in NRT neurons (Makinson et al., 2017), phospholipase C β 1 subunit in NRT neurons (Chang et al., 2022) and phospholipase C β 4 subunit in TC neurons (Cheong et al., 2009), whereas selective KD of Cav3.3 channels in NRT neurons does not lead to spontaneous ASs but increases GHB-induced SWDs (Lee et al., 2014);
2. secondary (compensatory) changes in a CT sector other than the one where the primary genetic manipulation had been made may be the critical element for ASs expression indicated by the increased T-type Ca^{2+} currents in TC neurons of mice with selective KD of Cav2.1 channels in cortical Layer 6 neurons (Bomben et al., 2016);
3. different changes of membrane proteins expression may occur in diverse areas of CT network, as indicated by the decreased and increased expression of HCN1 channels in the cortex and thalamus, respectively, of WAG/Rij rats (Cain et al., 2015; Kole et al., 2007);
4. in view of the cortical location of SWDs initiation in humans and animal models (see Parts 4 and 5, above), it is unfortunate that almost all investigations that used whole-brain genetic manipulations have studied abnormalities only in thalamic but not cortical neurons, even when the manipulated channels are (highly) expressed in both the cortical and thalamic sectors of CT networks.

8. Conclusions

Abnormal genes found either in small Chinese CAE cohorts or in mice AS models are not located in the significant loci (2p16.1 and 2q22) identified in the only large genome-wide CAE study, though *CACNA1H* variants are present in Chinese probands and the GAERS rat model of ASs. Selective ablation of Cav2.1, Cav3.3, and Nav1.6 channels as well as phospholipase C β 1 and β 4 subunits in either Layer 6 pyramidal neurons, cortical PV+ and SST+ interneurons, TC or NRT neurons of normal non-epileptic animals leads to ASs. In contrast to focal seizures and other GGEs, the ictal activity of cortical and thalamic neurons is predominantly characterized by a decreased, but highly synchronized, firing with all component members of a neuronal population often changing firing pattern from one seizure to the next. Selective chemogenetic and optogenetic manipulation of S1 PV+ interneurons and TC neurons, respectively, conclusively demonstrates that these neuronal populations are necessary for AS expression. The evidence in normal non-epileptic animals that a single manipulation of different voltage- and ligand-gated membrane channels does lead to ASs indicates the liability of CT networks towards the generation of these seizures. On the other hand, the high remittance rate of children with CAE indicates that CT networks, and/or their modulatory inputs from the basal ganglia and brain stem, have the inherent capacity to compensate for the developmental abnormalities that lead to ASs. However, the mechanism of remittance has not been investigated since all available AS models lack this feature of the human disease.

References

- Atherton Z, Nagy O, Barcsai L, Sere P, Zsigri N, Földi T, Berényi A, Crunelli V, Lőrincz ML. 2023. Higher-order thalamic nuclei facilitate the generalization and maintenance of spike-and-wave discharges of absence seizures. *Neurobiol Dis* 178: 106025.
- Avoli M. 2012. A brief history on the oscillating roles of thalamus and cortex in absence seizures. *Epilepsia* 53:779–789.
- Bai X, Vestal M, Berman R, Negishi M, Spann M, Vega C, Desalvo M, Novotny EJ, Constable RT, Blumenfeld H. 2010. Dynamic Time Course of Typical Childhood Absence Seizures: EEG, Behavior, and Functional Magnetic Resonance Imaging. *J Neurosci* 30:5884–5893.
- Bessaïh T, Bourgeois L, Badiu CI, Carter DA, Toth TI, Ruano D, Lambolez B, Crunelli V, Leresche N. 2006. Nucleus-specific abnormalities of GABAergic synaptic transmission in a genetic model of absence seizures. *J Neurophysiol* 96:3074–3081.
- Bomben VC, Aiba I, Qian J, Mark MD, Herlitze S, Noebels JL. 2016. Isolated P/Q Calcium Channel Deletion in Layer VI Corticothalamic Neurons Generates Absence Epilepsy. *J Neurosci* 36:405–418.
- Broicher T, Kanyshkova T, Meuth P, Pape H-C, Budde T. 2008. Correlation of T-channel coding gene expression, IT, and the low threshold Ca²⁺ spike in the thalamus of a rat model of absence epilepsy. *Molecular and Cellular Neuroscience* 39:384–399.
- Budde T, Caputi L, Kanyshkova T, Staak R, Abrahamczik C, Munsch T, Pape H-C. 2005. Impaired Regulation of Thalamic Pacemaker Channels through an Imbalance of Subunit Expression in Absence Epilepsy. *J Neurosci* 25:9871–9882.
- Caddick SJ, Wang C, Fletcher CF, Jenkins NA, Copeland NG, Hosford DA. 1999. Excitatory But Not Inhibitory Synaptic Transmission Is Reduced in Lethargic (*Cacnb4* lh) and Tottering (*Cacna1a* tg) Mouse Thalami. *Journal of Neurophysiology* 81:2066–2074.
- Cain SM, Tyson JR, Choi H, Ko R, Lin PJC, LeDue JM, Powell KL, Bernier L, Rungta RL, Yang Y, Cullis PR, O'Brien TJ, MacVicar BA, Snutch TP. 2018. CaV3.2 drives sustained burst-firing, which is critical for absence seizure propagation in reticular thalamic neurons. *Epilepsia* 59:778–791.
- Cain SM, Tyson JR, Jones KL, Snutch TP. 2015. Thalamocortical neurons display suppressed burst-firing due to an enhanced Ih current in a genetic model of absence epilepsy. *Pflugers Arch - Eur J Physiol* 467:1367–1382.
- Casillas-Espinosa PM, Powell KL, Zhu M, Campbell CR, Maia JM, Ren Z, Jones NC, O'Brien TJ, Petrovski S. 2017. Evaluating whole genome sequence data from the Genetic Absence Epilepsy Rat from Strasbourg and its related non-epileptic strain. *PLoS ONE* 12:e0179924.
- Chang B, Byun J, Kim KK, Lee SE, Lee B, Kim K-S, Ryu H, Shin H-S, Cheong E. 2022. Deletion of Phospholipase C β 1 in the Thalamic Reticular Nucleus Induces Absence Seizures. *Exp Neurobiol* 31:116–130.
- Cheong E, Zheng Y, Lee K, Lee J, Kim S, Sanati M, Lee S, Kim Y-S, Shin H-S. 2009. Deletion of phospholipase C β 4 in thalamocortical relay nucleus leads to absence seizures. *Proc Natl Acad Sci U S A* 106:21912–21917.
- Cope DW, Di Giovanni G, Fyson SJ, Orbán G, Errington AC, Lorincz ML, Gould TM, Carter DA, Crunelli V. 2009. Enhanced tonic GABAA inhibition in typical absence epilepsy. *Nat Med* 15:1392–1398.
- Crunelli V, Leresche N. 2002. Childhood absence epilepsy: genes, channels, neurons and networks. *Nat Rev Neurosci* 3:371–382.

- Crunelli V, Lőrincz ML, Connelly WM, David F, Hughes SW, Lambert RC, Leresche N, Errington AC. 2018. Dual function of thalamic low-vigilance state oscillations: rhythm-regulation and plasticity. *Nat Rev Neurosci* 19:107–118.
- Crunelli V, Lőrincz ML, McCafferty C, Lambert RC, Leresche N, Di Giovanni G, David F. 2020. Clinical and experimental insight into pathophysiology, comorbidity and therapy of absence seizures. *Brain* 143:2341–2368.
- D’Antuono M, Inaba Y, Biagini G, D’Arcangelo G, Tancredi V, Avoli M. 2005. Synaptic hyperexcitability of deep layer neocortical cells in a genetic model of absence seizures: Neocortical excitability and absence epilepsy. *Genes, Brain and Behavior* 5:73–84.
- DeLorey TM, Handforth A, Anagnostaras SG, Homanics GE, Minassian BA, Asatourian A, Fanselow MS, Delgado-Escueta A, Ellison GD, Olsen RW. 1998. Mice Lacking the $\beta 3$ Subunit of the GABAA Receptor Have the Epilepsy Phenotype and Many of the Behavioral Characteristics of Angelman Syndrome. *J Neurosci* 18:8505–8514.
- Deransart C, Depaulis A. 2002. The control of seizures by the basal ganglia? A review of experimental data. *Epileptic Disord* 4 Suppl 3:S61-72.
- Ernst WL, Zhang Y, Yoo JW, Ernst SJ, Noebels JL. 2009. Genetic enhancement of thalamocortical network activity by elevating alpha 1g-mediated low-voltage-activated calcium current induces pure absence epilepsy. *J Neurosci* 29:1615–1625.
- Feng Y-CA, Howrigan DP, Abbott LE, Tashman K, Cerrato F, Singh T, Heyne H, Byrnes A, Churchhouse C, Watts N, Solomonson M, Lal D, Heinzen EL, Dhindsa RS, Stanley KE, Cavalleri GL, Hakonarson H, Helbig I, Krause R, May P, Weckhuysen S, Petrovski S, Kamalakaran S, Sisodiya SM, Cossette P, Cotsapas C, De Jonghe P, Dixon-Salazar T, Guerrini R, Kwan P, Marson AG, Stewart R, Depondt C, Dlugos DJ, Scheffer IE, Striano P, Freyer C, McKenna K, Regan BM, Bellows ST, Leu C, Bennett CA, Johns EMC, Macdonald A, Shilling H, Burgess R, Weckhuysen D, Bahlo M, O’Brien TJ, Todaro M, Stamberger H, Andrade DM, Sadoway TR, Mo K, Krestel H, Gallati S, Papacostas SS, Kousiappa I, Tanteles GA, Štěrbová K, Vlčková M, Sedláčková L, Laššuthová P, Klein KM, Rosenow F, Reif PS, Knake S, Kunz WS, Zsurka G, Elger CE, Bauer J, Rademacher M, Pendziwiat M, Muhle H, Rademacher A, van Baalen A, von Spiczak S, Stephani U, Afawi Z, Korczyn AD, Kanaan M, Canavati C, Kurleman G, Müller-Schlüter K, Kluger G, Häusler M, Blatt I, Lemke JR, Krey I, Weber YG, Wolking S, Becker F, Hengsbach C, Rau S, Maisch AF, Steinhoff BJ, Schulze-Bonhage A, Schubert-Bast S, Schreiber H, Borggräfe I, Schankin CJ, Mayer T, Korinthenberg R, Brockmann K, Kurleman G, Dennig D, Madeleyn R, Kälviäinen R, Auvinen P, Saarela A, Linnankivi T, Lehesjoki A-E, Rees MI, Chung S-K, Pickrell WO, Powell R, Schneider N, Balestrini S, Zagaglia S, Braatz V, Johnson MR, Auce P, Sills GJ, Baum LW, Sham PC, Cherny SS, Lui CHT, Barišić N, Delanty N, Doherty CP, Shukralla A, McCormack M, El-Naggar H, Canafoglia L, Franceschetti S, Castellotti B, Granata T, Zara F, Iacomino M, Madia F, Vari MS, Mancardi MM, Salpietro V, Bisulli F, Tinuper P, Licchetta L, Pippucci T, Stipa C, Minardi R, Gambardella A, Labate A, Annesi G, Manna L, Gagliardi M, Parrini E, Mei D, Vetro A, Bianchini C, Montomoli M, Doccini V, Marini C, Suzuki T, Inoue Y, Yamakawa K, Tumiene B, Sadleir LG, King C, Mountier E, Caglayan SH, Arslan M, Yapıcı Z, Yis U, Topaloglu P, Kara B, Turkdogan D, Gundogdu-Eken A, Bebek N, Uğur-İşeri S, Baykan B, Salman B, Haryanyan G, Yücesan E, Kesim Y, Özkara Ç, Poduri A, Shiedley BR, Shain C, Buono RJ, Ferraro TN, Sperling MR, Lo W, Privitera M, French JA, Schachter S, Kuzniecky RI, Devinsky O, Hegde M, Khankhanian P, Helbig KL, Ellis CA, Spalletta G, Piras Fabrizio, Piras Federica, Gili T, Ciullo V, Reif A, McQuillin A, Bass N, McIntosh A, Blackwood D, Johnstone M, Palotie A, Pato MT, Pato CN, Bromet EJ, Carvalho CB, Achtyes ED, Azevedo MH, Kotov R, Lehrer DS, Malaspina D, Marder

- SR, Medeiros H, Morley CP, Perkins DO, Sobell JL, Buckley PF, Macciardi F, Rapaport MH, Knowles JA, Fanous AH, McCarroll SA, Gupta N, Gabriel SB, Daly MJ, Lander ES, Lowenstein DH, Goldstein DB, Lerche H, Berkovic SF, Neale BM. 2019. Ultra-Rare Genetic Variation in the Epilepsies: A Whole-Exome Sequencing Study of 17,606 Individuals. *The American Journal of Human Genetics* 105:267–282.
- Frankel WN. 2009. Genetics of complex neurological disease: challenges and opportunities for modeling epilepsy in mice and rats. *Trends in Genetics* 25:361–367.
- Gassmann M, Shaban H, Vigot R, Sansig G, Haller C, Barbieri S, Humeau Y, Schuler V, Müller M, Kinzel B, Klebs K, Schmutz M, Froestl W, Heid J, Kelly PH, Gentry C, Jatón A-L, Van der Putten H, Mombereau C, Lecourtier L, Mosbacher J, Cryan JF, Fritschy J-M, Lüthi A, Kaupmann K, Bettler B. 2004. Redistribution of GABAB(1) Protein and Atypical GABAB Responses in GABAB(2)-Deficient Mice. *J Neurosci* 24:6086–6097.
- Gruenbaum BF, Sandhu MRS, Bertasi RAO, Bertasi TGO, Schonwald A, Kurup A, Gruenbaum SE, Freedman IG, Funaro MC, Blumenfeld H, Sanacora G. 2021. Absence seizures and their relationship to depression and anxiety: Evidence for bidirectionality. *Epilepsia* 62:1041–1056.
- Guo J, Kim R, Chen Y, Negishi M, Jhun S, Weiss S, Ryu JH, Bai X, Xiao W, Feeney E, Rodriguez-Fernandez J, Mistry H, Crunelli V, Crowley MJ, Mayes LC, Todd Constable R, Blumenfeld H. 2016. Mechanism of impaired consciousness in absence seizures: a cross-sectional study. *Lancet Neurol* 15:1336–1345.
- Harutyunyan A, Chong D, Li R, Shah AD, Ali Z, Huang C, Barlow CK, Perucca P, O'Brien TJ, Jones NC, Schittenhelm RB, Anderson A, Casillas-Espinosa PM. 2022. An Integrated Multi-Omic Network Analysis Identifies Seizure-Associated Dysregulated Pathways in the GAERS Model of Absence Epilepsy. *Int J Mol Sci* 23:6063.
- Iacone Y, Morais TP, David F, Delicata F, Sandle J, Raffai T, Parri HR, Weisser JJ, Bundgaard C, Klewe IV, Tamás G, Thomsen MS, Crunelli V, Lőrincz ML. 2021. Systemic administration of ivabradine, a hyperpolarization-activated cyclic nucleotide-gated channel inhibitor, blocks spontaneous absence seizures. *Epilepsia* 62:1729–1743.
- International League Against Epilepsy Consortium on Complex Epilepsies. 2018. Genome-wide mega-analysis identifies 16 loci and highlights diverse biological mechanisms in the common epilepsies. *Nat Commun* 9:5269.
- Kanyshkova T, Meuth P, Bista P, Liu Z, Ehling P, Caputi L, Doengi M, Chetkovich DM, Pape H-C, Budde T. 2012. Differential regulation of HCN channel isoform expression in thalamic neurons of epileptic and non-epileptic rat strains. *Neurobiol Dis* 45:450–461. doi:10.1016/j.nbd.2011.08.032
- Kim D, Song I, Keum S, Lee T, Jeong M-J, Kim S-S, McEnery MW, Shin H-S. 2001. Lack of the Burst Firing of Thalamocortical Relay Neurons and Resistance to Absence Seizures in Mice Lacking $\alpha 1G$ T-Type Ca^{2+} Channels. *Neuron* 31:35–45.
- Kole MH, Bräuer AU, Stuart GJ. 2007. Inherited cortical HCN1 channel loss amplifies dendritic calcium electrogenesis and burst firing in a rat absence epilepsy model. *J Physiol* 578:507–525.
- Kuisle M, Wanaverbecq N, Brewster AL, Frère SGA, Pinault D, Baram TZ, Lüthi A. 2006. Functional stabilization of weakened thalamic pacemaker channel regulation in rat absence epilepsy. *J Physiol* 575:83–100.
- Lacey CJ, Bryant A, Brill J, Huguenard JR. 2012. Enhanced NMDA Receptor-Dependent Thalamic Excitation and Network Oscillations in Stargazer Mice. *J Neurosci* 32:11067–11081.

- Lee SE, Lee J, Latchoumane C, Lee B, Oh SJ, Saud ZA, Park C, Sun N, Cheong E, Chen CC, Choi EJ, Lee CJ, Shin H-S. 2014. Rebound burst firing in the reticular thalamus is not essential for pharmacological absence seizures in mice. *Proc Natl Acad Sci USA* 111:11828–11833.
- Ludwig A, Budde T, Stieber J, Moosmang S, Wahl C, Holthoff K, Langebartels A, Wotjak C, Munsch T, Zong X, Feil S, Feil R, Lancel M, Chien KR, Konnerth A, Pape H-C, Biel M, Hofmann F. 2003. Absence epilepsy and sinus dysrhythmia in mice lacking the pacemaker channel HCN2. *EMBO J* 22:216–224.
- Lüttjohann A, Pape H-C. 2019. Regional specificity of cortico-thalamic coupling strength and directionality during waxing and waning of spike and wave discharges. *Sci Rep* 9:2100.
- Makinson CD, Tanaka BS, Sorokin JM, Wong JC, Christian CA, Goldin AL, Escayg A, Huguenard JR. 2017. Regulation of Thalamic and Cortical Network Synchrony by *Scn8a*. *Neuron* 1–22.
- McCafferty C, David F, Venzi M, Lőrincz ML, Delicata F, Atherton Z, Recchia G, Orban G, Lambert RC, Di Giovanni G, Leresche N, Crunelli V. 2018. Cortical drive and thalamic feed-forward inhibition control thalamic output synchrony during absence seizures. *Nat Neurosci* 21:744–756.
- McCafferty C, Gruenbaum B, Tung R, Li J-J, Zheng X, Salvino P, Vincent P, Kratochvil Z, Ryu JH, Khalaf A, Swift K, Akbar R, Islam W, Antwi P, Johnson EA, Vitkovskiy P, Sampognaro J, Freedman I, Kundishora A, Depaulis A, David F, Crunelli V, Sanganahalli BG, Herman P, Hyder F, Blumenfeld H. 2023. The neuronal basis of impaired consciousness in absence seizures. *Nat Commun*, in press.
- Meeren HKM, Pijn JPM, Luijtelaa ELJMV, Coenen AML, Silva FHL da. 2002. Cortical Focus Drives Widespread Corticothalamic Networks during Spontaneous Absence Seizures in Rats. *J Neurosci* 22:1480–1495.
- Meyer J, Maheshwari A, Noebels J, Smirnakis S. 2018. Asynchronous suppression of visual cortex during absence seizures in stargazer mice. *Nat Commun* 9:1938.
- Miao Q-L, Herlitze S, Mark MD, Noebels JL. 2019. Adult loss of *Cacna1a* in mice recapitulates childhood absence epilepsy by distinct thalamic bursting mechanisms. *Brain* 143:161–174.
- Miyamoto H, Tatsukawa T, Shimohata A, Yamagata T, Suzuki T, Amano K, Mazaki E, Raveau M, Ogiwara I, Oba-Asaka A, Hensch TK, Itohara S, Sakimura K, Kobayashi Kenta, Kobayashi Kazuto, Yamakawa K. 2019. Impaired cortico-striatal excitatory transmission triggers epilepsy. *Nat Commun* 10:1917.
- Ogiwara I, Miyamoto H, Tatsukawa T, Yamagata T, Nakayama T, Atapour N, Miura E, Mazaki E, Ernst SJ, Cao D, Ohtani H, Itohara S, Yanagawa Y, Montal M, Yuzaki M, Inoue Y, Hensch TK, Noebels JL, Yamakawa K. 2018. *Nav1.2* haplodeficiency in excitatory neurons causes absence-like seizures in mice. *Commun Biol* 1:96.
- Panthi S, Leitch B. 2019. The impact of silencing feed-forward parvalbumin-expressing inhibitory interneurons in the cortico-thalamocortical network on seizure generation and behaviour. *Neurobiology of Disease* 132:104610.
- Papp P, Kovács Z, Szocsics P, Juhász G, Maglóczy Z. 2018. Alterations in hippocampal and cortical densities of functionally different interneurons in rat models of absence epilepsy. *Epilepsy Research* 145:40–50.
- Paz JT, Bryant AS, Peng K, Fenno L, Yizhar O, Frankel WN, Deisseroth K, Huguenard JR. 2011. A new mode of corticothalamic transmission revealed in the *Gria4*(-/-) model of absence epilepsy. *Nat Neurosci* 14:1167–1173.

628 Perucca P, Bahlo M, Berkovic SF. 2020. The Genetics of Epilepsy. *Annual Review of*
629 *Genomics and Human Genetics* 21:205–230.

630 Pitkänen A, Buckmaster P, Galanopoulou AS, Moshé SL, editors. 2017. *Models of Seizures*
631 *and Epilepsy*, 2nd Edition. ed. Elsevier.

632 Polack PO, Guillemain I, Hu E, Deransart C, Depaulis A, Charpier S. 2007. Deep Layer
633 Somatosensory Cortical Neurons Initiate Spike-and-Wave Discharges in a Genetic Model
634 of Absence Seizures. *J Neurosci* 27:6590–6599.

635 Rossignol E, Kruglikov I, van den Maagdenberg AMJM, Rudy B, Fishell G. 2013. CaV2.1
636 ablation in cortical interneurons selectively impairs fast-spiking basket cells and causes
637 generalized seizures. *Ann Neurol* 74:209–222.

638 Salvati KA, Ritger ML, Davoudian PA, O'Dell F, Wyskiel DR, Souza GMPR, Lu AC, Perez-
639 Reyes E, Drake JC, Yan Z, Beenhakker MP. 2022a. AMPK-mediated potentiation of
640 GABAergic signalling drives hypoglycaemia-provoked spike-wave seizures. *Brain*
641 145:2332–2346.

642 Salvati KA, Souza GM, Lu AC, Ritger ML, Guyenet P, Abbott SB, Beenhakker MP. 2022b.
643 Respiratory alkalosis provokes spike-wave discharges in seizure-prone rats. *eLife*
644 11:e72898.

645 Schuler V, Lüscher C, Blanchet C, Klix N, Sansig G, Klebs K, Schmutz M, Heid J, Gentry C,
646 Urban L, Fox A, Spooren W, Jatón A-L, Vigouret J-M, Pozza M, Kelly PH, Mosbacher J,
647 Froestl W, Käslin E, Korn R, Bischoff S, Kaupmann K, van der Putten H, Bettler B. 2001.
648 Epilepsy, Hyperalgesia, Impaired Memory, and Loss of Pre- and Postsynaptic GABAB
649 Responses in Mice Lacking GABAB(1). *Neuron* 31:47–58.

650 Song I, Kim D, Choi S, Sun M, Kim Y, Shin H-S. 2004. Role of the $\alpha 1G$ T-Type Calcium
651 Channel in Spontaneous Absence Seizures in Mutant Mice. *J Neurosci* 24:5249–5257.

652 Sorokin JM, Davidson TJ, Frechette E, Abramian AM, Deisseroth K, Huguenard JR, Paz JT.
653 2017. Bidirectional control of generalized epilepsy networks via rapid real-time switching
654 of firing mode. *Neuron* 93:194–210.

655 Suls A, Mullen SA, Weber YG, Verhaert K, Ceulemans B, Guerrini R, Wuttke TV, Salvo-
656 Vargas A, Deprez L, Claes LRF, Jordanova A, Berkovic SF, Lerche H, De Jonghe P,
657 Scheffer IE. 2009. Early-onset absence epilepsy caused by mutations in the glucose
658 transporter GLUT1. *Annals of Neurology* 66:415–419.

659 Talley EM, Cribbs LL, Lee JH, Daud A, Perez-Reyes E, Bayliss DA. 1999. Differential
660 distribution of three members of a gene family encoding low voltage-activated (T-type)
661 calcium channels. *J Neurosci* 19:1895–1911.

662 Tan HO, Reid CA, Single FN, Davies PJ, Chiu C, Murphy S, Clarke AL, Dibbens L, Krestel
663 H, Mulley JC, Jones MV, Seeburg PH, Sakmann B, Berkovic SF, Sprengel R, Petrou S.
664 2007. Reduced cortical inhibition in a mouse model of familial childhood absence
665 epilepsy. *Proc Natl Acad Sci USA* 104:17536–17541.

666 Tangwiriyasakul C, Perani S, Centeno M, Yaakub SN, Abela E, Carmichael DW, Richardson
667 MP. 2018. Dynamic brain network states in human generalized spike-wave discharges.
668 *Brain* 141:2981–2994.

669 Wang G, Bochorishvili G, Chen Y, Salvati KA, Zhang P, Dubel SJ, Perez-Reyes E, Snutch
670 TP, Stornetta RL, Deisseroth K, Erisir A, Todorovic SM, Luo J-H, Kapur J, Beenhakker
671 MP, Zhu JJ. 2015. Ca V3.2 calcium channels control NMDA receptor-mediated
672 transmission: a new mechanism for absence epilepsy. *Genes Dev* 29:1535–1551.

673 Winsky-Sommerer R, Knapman A, Fedele DE, Schofield CM, Vyazovskiy VV, Rudolph U,
674 Huguenard JR, Fritschy JM, Tobler I. 2008. Normal sleep homeostasis and lack of

675 epilepsy phenotype in GABAA receptor $\alpha 3$ subunit-knockout mice. *Neuroscience*
676 154:595–605.

677 Xie H, Su W, Pei J, Zhang Yujia, Gao K, Li J, Ma X, Zhang Yuehua, Wu X, Jiang Y. 2019.
678 De novo SCN1A, SCN8A, and CLCN2 mutations in childhood absence epilepsy.
679 *Epilepsy Research* 154:55–61.

680 Zaman T, Lee K, Park C, Paydar A, Choi JH, Cheong E, Lee CJ, Shin H-S. 2011. CaV2.3
681 Channels Are Critical for Oscillatory Burst Discharges in the Reticular Thalamus and
682 Absence Epilepsy. *Neuron* 70:95–108.

683 Zhang Y, Mori M, Burgess DL, Noebels JL. 2002. Mutations in high-voltage-activated
684 calcium channel genes stimulate low-voltage-activated currents in mouse thalamic relay
685 neurons. *J Neurosci* 22:6362–6371.

686 Zobeiri M, Chaudhary R, Blaich A, Rottmann M, Herrmann S, Meuth P, Bista P, Kanyshkova
687 T, Lüttjohann A, Narayanan V, Hundehage P, Meuth SG, Romanelli MN, Urbano FJ,
688 Pape H-C, Budde T, Ludwig A. 2019. The Hyperpolarization-Activated HCN4 Channel is
689 Important for Proper Maintenance of Oscillatory Activity in the Thalamocortical System.
690 *Cereb Cortex* 29:2291–2304.

691

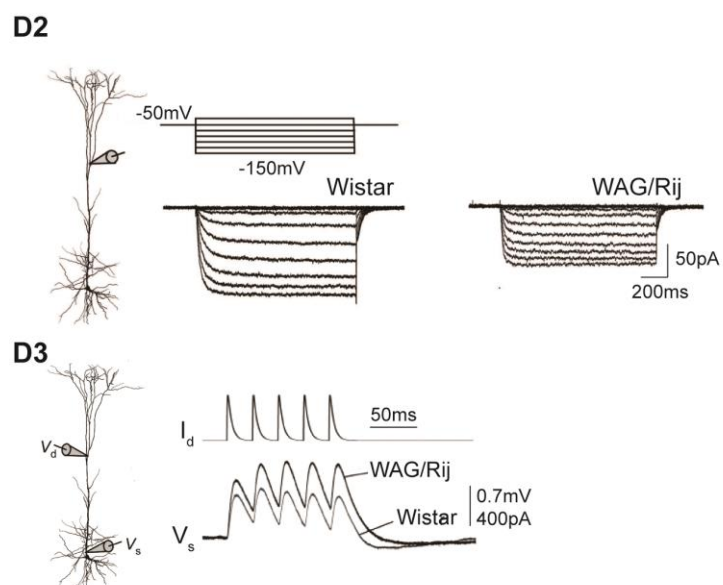
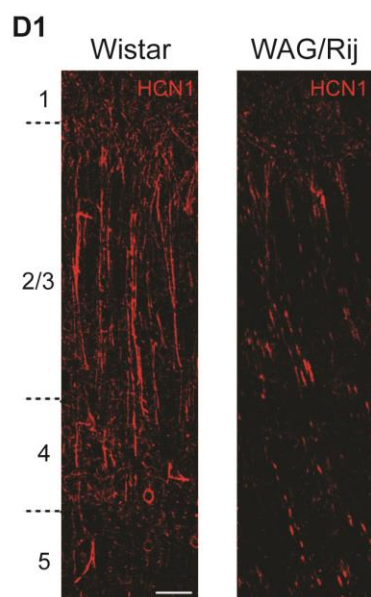
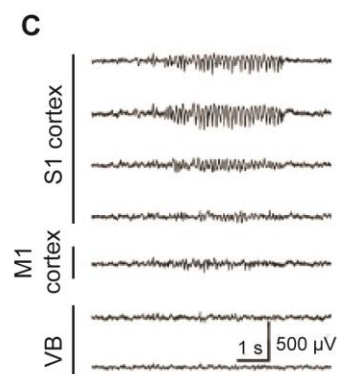
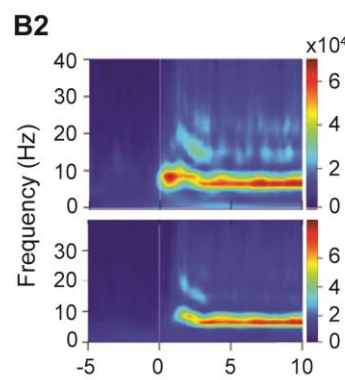
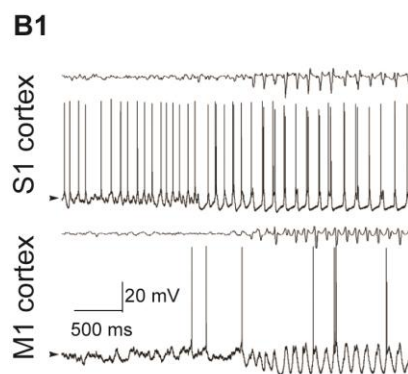
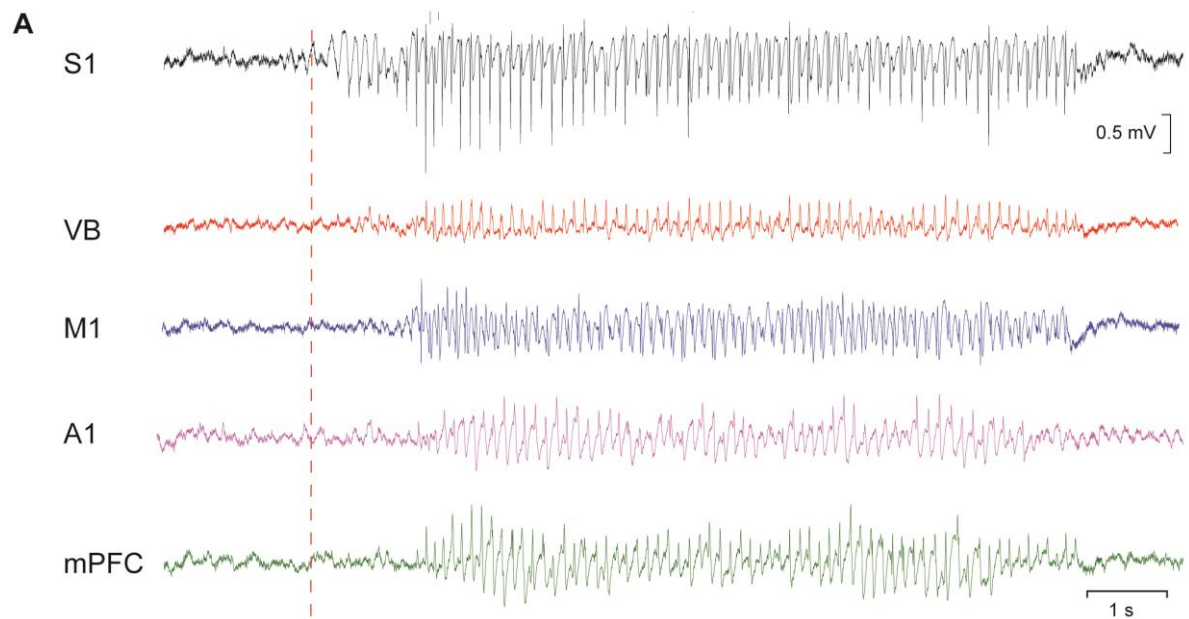


Figure 1. Cortical initiation of absence seizures (ASs) and cellular abnormalities in Layer 5 neurons of AS models.

(A) Electroencephalographic (EEG) traces from the indicated cortical and thalamic areas in a freely moving GAERS rat show the initiation of SWDs in the primary somatosensory cortex (S1); VB: ventrobasal thalamic nucleus, M1: primary motor cortex, A1: primary auditory cortex, mPFC: medial prefrontal cortex. **(B1)** Interictal and ictal intracellular activity (bottom trace in each pair) of Layer 5 neurons, recorded in the same GAERS rat simultaneously with the corresponding EEG (top trace in each pair). Note the hyperactivity of the neuron located in the primary somatosensory cortex (S1 cortex), the cortical initiation network in this animal model, compared to the neuron in the primary motor cortex (M1 cortex). **(B2)** Averaged time-frequency maps showing the occurrence of 6-8Hz SWDs in S1 before M1. **(C)** EEG traces from different sites in the CT network of a GAERS rat show large amplitude SWDs in S1 (and very small SWDs in M1) but not in the VB, the somatotopic thalamic nucleus of S1, which were not accompanied by any behavioural sign. **(D1)** HCN1 immunofluorescence in S1 shows weaker labeling in a WAG/Rij rat compared to a control Wistar rat (scale bar, 100 μ m). **(D2)** Dendritic cell-attached patch-clamp recordings in the apical dendrites of an S1 Layer 5 pyramidal neuron show significantly lower amplitude of I_h in a WAG/Rij rat compared to a control Wistar rat. **(D3)** Simultaneous dendritic and somatic whole-cell recordings in an S1 Layer 5 pyramidal neuron. EPSPs were simulated by current injection in the dendrite (I_d , top trace) and their summation at the soma (V_s , bottom traces) was increased in a WAG/Rij rat neuron compared to a control Wistar rat neuron. Adapted from McCafferty et al. (2018) (A), Polack et al. (2007) (B, C) and Kole et al. (2007) (D1-D3).

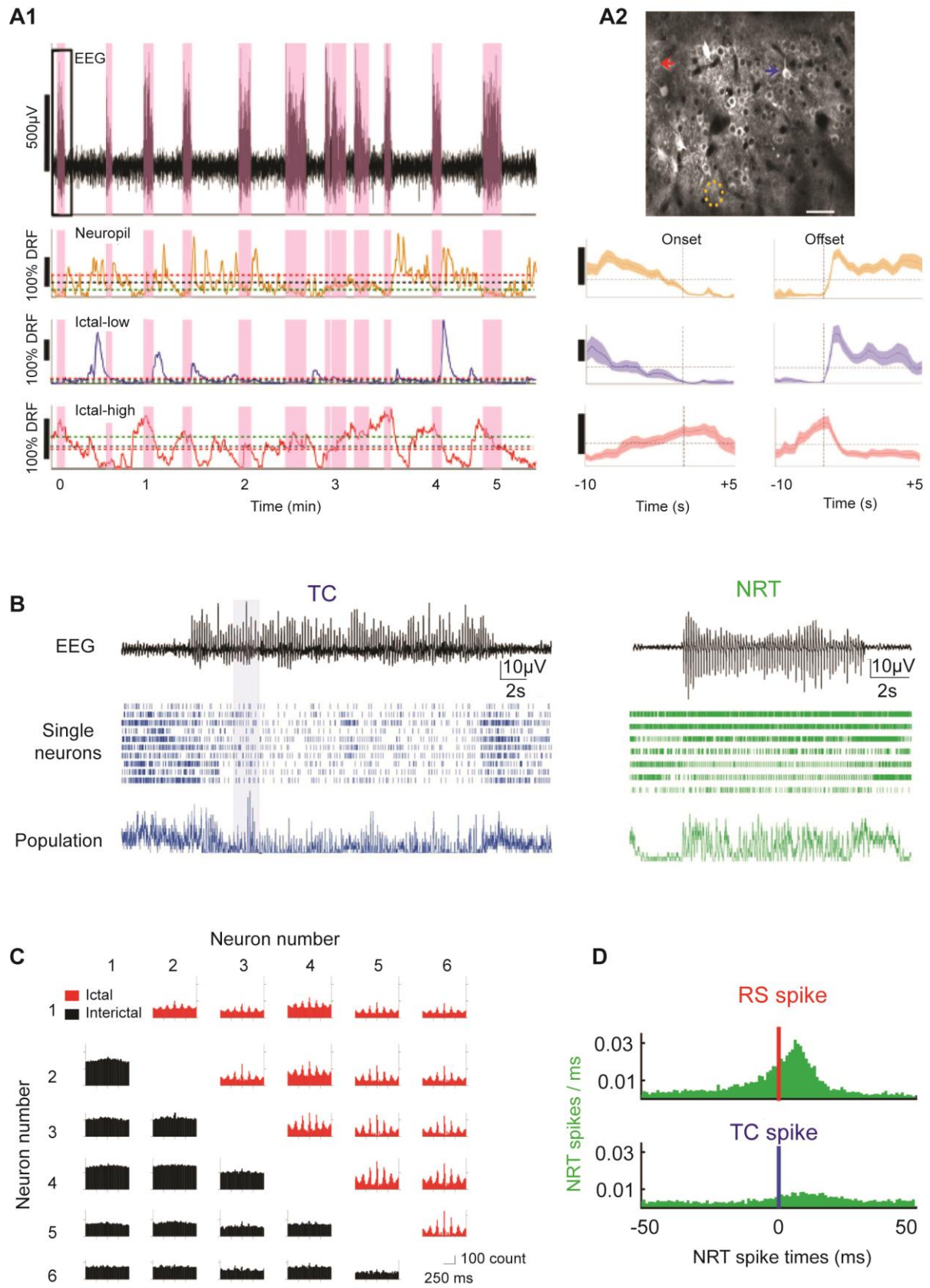


Figure 2. Activity of cortical and thalamic neurons during absence seizures (ASs).

(A1) Concomitant electroencephalographic (EEG) (top trace) and intracellular Ca^{2+} changes from Layer 2/3 GCaMP6-filled neurons in the visual cortex of a head-restrained STG mouse. The Ca^{2+} changes of each neuron/neuropil patch over time are shown with the seizure epochs highlighted in pink. Mean ictal activity (green dashed line), mean interictal activity (red dashed line), and overall mean activity (black dashed line). **(A2)** The average activity (vertical bar = 20% $\Delta F/F$) time-locked to the seizure onset and offset is shown for exemplary neuropil (top), a neuron with low activity during ASs (ictal-low, middle) and a neuron with high activity during ASs (ictal-high, bottom). The ictal-low and ictal-high neuron are marked by a blue and red arrow, respectively in the 2-photon microscopy image shown at the top, with the neuropil area highlighted by a yellow dotted circle. Scale bar = 50 μm . **(B)** EEG (top trace) and spike-time raster plots from 10 thalamocortical (TC) neurons in the ventrobasal thalamic nucleus and 7 nucleus reticularis thalami (NRT) neurons simultaneously recorded with a silicon probe in a freely moving GAERS rats. Most TC neurons show a decrease in firing rate during the ASs, which is also evident from the population activity (bottom trace). NRT neurons show two different types of ictal firing: one group increases their firing ictally (top three cells in raster-plot) and another group shows a decreased activity (bottom four cells). **(C)** Cross-correlograms of the total firing of 6 simultaneously recorded GAERS TC neurons during interictal periods (black plots) and ASs (red plots). Despite the overall decrease in firing during ASs (red plots), there is a marked increase in neuronal synchrony. **(D)** Histograms of the firing of an NRT neuron recorded simultaneously with a cortical regular spiking (RS) neuron (top) or a TC neuron (bottom) in a freely moving GAERS rat. Note that the ictal NRT neuron firing (green bars) is mainly driven by the cortical RS neuron spikes rather than the TC neuron spikes. Time zero marks the firing of the RS and TC neurons (red and blue lines, respectively). Adapted from Meyer et al. (2018) (A) and McCafferty et al. (2018) (B-D).

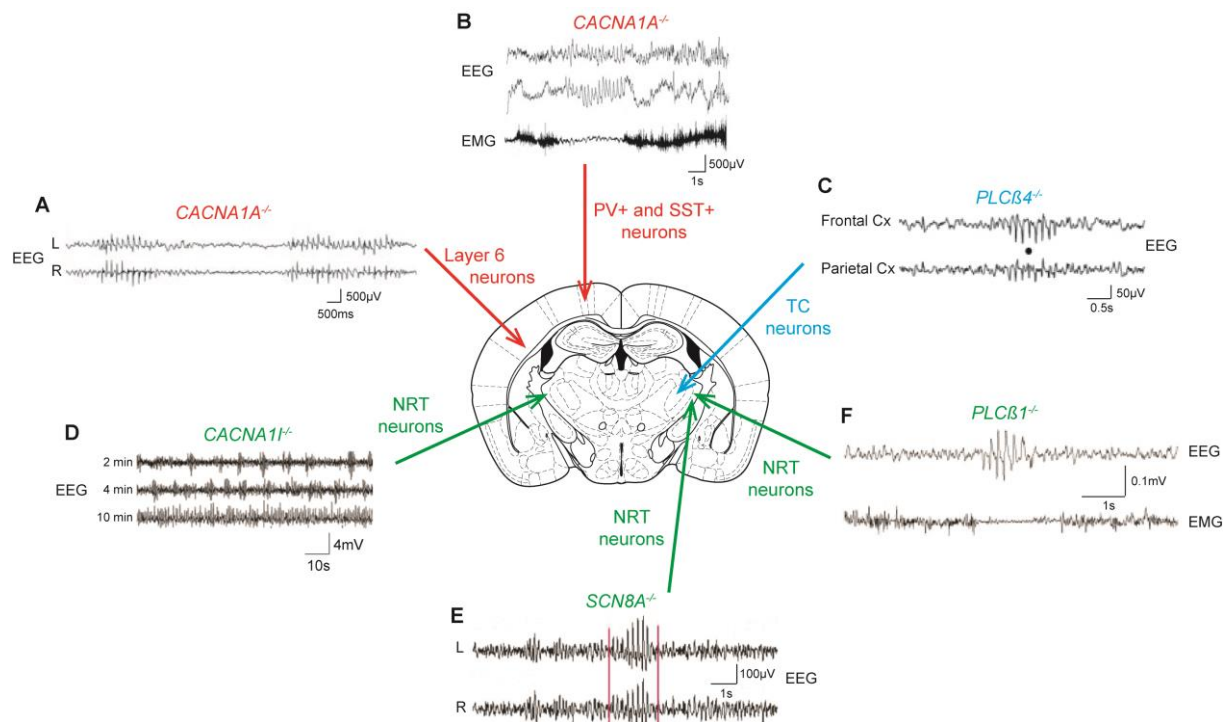


Figure 3. Single genetic ablations that lead to absence seizures (ASs).

Schematic brain drawing (center panel) showing the location of the different single mutations induced in either Layer 6 neurons, cortical parvalbumin- and somatostatin-positive (PV+ and SST+, respectively) interneurons, thalamocortical (TC) and nucleus reticularis thalami (NRT) neurons that have been shown to elicit spontaneous ASs (A, B, C, E, and F) or increase γ -hydroxybutyric acid-induced ASs (D). Panels show electroencephalographic (EEG) and electromyographic (EMG) activity in mice with the illustrated genetic ablation. Minutes on the left-hand side of the traces in D indicate the time from the γ -hydroxybutyric acid injection (Cx: cortex; L: left; R: right). Adapted from Bomben et al. (2016) (A), Rossignol et al. (2013) (B), Cheong et al. (2009) (C), Lee et al. (2014) (D), Makinson et al. (2017) (E), and Chang et al. (2022) (F).