



HAL
open science

Corticothalamic network abnormalities underlying absence seizures

Nathalie Leresche, Vincenzo Crunelli

► **To cite this version:**

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

HAL Id: hal-04229142

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

Submitted on 5 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Corticothalamic network abnormalities**
2 **underlying absence seizures**

3
4 Nathalie Leresche^{1*} and Vincenzo Crunelli^{2*}
5
6

7 ¹Sorbonne Université, CNRS, INSERM, Neurosciences Paris Seine - Institut de Biologie
8 Paris Seine, Paris, France; ²Neuroscience Division, School of Bioscience, Cardiff University,
9 Museum Avenue, Cardiff, UK.

10
11
12 *Correspondence: nathalie.leresche@sorbonne-universite.fr; crunelli@cardiff.ac.uk
13
14
15
16

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

20
21
22 Word count: Abstract 202, Main Text: 4903. Number of references: 67
23
24

25 **Acknowledgments**

26 Work in our labs is supported by the CNRS, Sorbonne Université and Inserm (grants to NL)
27 and the Ester Florida Neuroscience Research Foundation (grant 1502 to VC).
28
29

30 **Conflict of interest**

31 The authors declare no conflict of interest.
32
33

34 **Abstract**

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

51

52

53 **1. Introduction**

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

63 Following a short summary of the clinical presentation, therapy and comorbidities of ASs, we
64 first discuss their genetics and the mechanisms involved in their initiation and maintenance in
65 humans and in models of ASs. The main body of this chapter is a focused review of the
66 current state of knowledge of the molecular and cellular abnormalities in different neuronal
67 populations of CT networks that can bring about ASs, using evidence both from
68 investigations that have used selective genetic manipulations in normal non-epileptic animals
69 and from studies in five genetic models with spontaneous ASs that have been validated for
70 their strong face-, construct- and content-validity: the polygenic GAERS and WAG/Rij rats,
71 and the monogenic stargazer (STG), tottering (TG) and lethargic (LH) mice (Pitkänen et al.,
72 2017). Due to space limitations, studies using only pharmacological manipulations are not
73 discussed.

74

75 **2. Clinical presentation of ASs**

76 An AS is a sudden and relatively brief lapse of consciousness associated with lack of
77 voluntary movements and distinctive SWDs at 2.5-4Hz in the EEG (Crunelli et al., 2020).
78 ASs are present, together with other seizure types, in many genetic generalised epilepsies
79 (GGEs), here we focus on ASs observed in childhood absence epilepsy (CAE) since in this
80 epilepsy type ASs are the only clinical symptom present and not compromised by other
81 epileptic phenotypes. In CAE, ASs begin between 3 and 8 years of age and can occur up to
82 ~200 per day. Approximately 70% of CAE patients go into spontaneous remission around
83 adolescence (Crunelli and Leresche, 2002), though the mechanisms underlying remission
84 have not been elucidated, since currently researched ASs models lack spontaneous remission.
85 Pharmacological treatment of ASs relies primarily on ethosuximide (ETX), valproic acid,

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

88

89 **3. Genetics of ASs**

90 Strong evidence exists for a genetic component in CAE, possibly of a polygenic nature
91 (Perucca et al., 2020) and concordances of 70%–85% and 33% have been reported in
92 monozygotic twins and first-degree relatives, respectively (Gruenbaum et al., 2021).
93 However, most CAE patients do not have family history of epilepsy and siblings of affected
94 individuals have an 8% risk of ASs, which is lower than the expected risk for a recessive
95 (25%) or dominant (50%) inherited trait (Perucca et al., 2020).

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

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

113 A large number of spontaneous single gene mutations bring about spontaneous ASs in mice
114 (Frankel, 2009), some of which have been validated as ASs models, including *CACNG2* (in
115 STG mice), *CACNA1A* (in TG mice) and *CACNB4*, (in LH mice). Whole-genome sequencing
116 of the polygenic GAERS rats has identified *CACNA1H* (R1584P) as the only gene-variant
117 that selectively co-segregate with high-seizuring F2 progenies (Casillas-Espinosa et al.,
118 2017). Notably, a combined proteomic and metabolomic analysis of GAERS rats has found

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

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

128

129 **4. Cortical initiation networks of ASs**

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

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

144 The lack of generalization at the onset of an AS, i.e., the existence of a cortical initiation
145 network (CIN), had been originally reported in two rat models of ASs. It was found that the
146 peri-oral region of the primary somatosensory cortex (S1) was the cortical initiation network
147 in GAERS and WAG/Rij rats (Figure 1A) and that S1 Layer 5/6 excitatory pyramidal neurons
148 were the first to fire at the start of SWDs (Figure 1B) (Meeren et al., 2002; Polack et al.,
149 2007). Moreover, BOLD signals in awake GAERS rats recapitulate human BOLD results
150 with an AS-linked decrease and increase in signal in S1 and ventrobasal (VB) thalamus,
151 respectively (McCafferty et al., 2023).

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

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

170

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

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

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

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

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

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

211

212 **6. Cellular activity in the neocortex and thalamus during ASs**

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

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

226 Simultaneous ensemble recordings in cortical neurons and VB thalamocortical (TC) and
227 nucleus reticularis thalami (NRT) neurons during spontaneous ASs in GAERS rats and γ -
228 hydroxybutyric acid (GHB)-induced ASs in Wistar rats under freely moving conditions show
229 that cortical afferent activity drives thalamic firing and temporally frames thalamic output via
230 feed-forward inhibition of TC neurons by NRT neurons (Figure 2D) (McCafferty et al.,
231 2018). This results in a marked decrease in the overall firing of TC neurons (with only rare T-
232 type Ca^{2+} channel-dependent burst firing being present ictally) (Figure 2B), which is causally
233 linked to the constitutively enhanced tonic GABA_A current of TC neurons that is present in
234 this AS model as well as in STG and LH mice (Cope et al., 2009). Nevertheless, the total
235 firing of TC (and NRT) neurons is strictly in phase with each SWD cycle (Figure 2C),
236 indicating that the synchrony and strength of the ictal output of TC neurons to the neocortex is
237 not a feature of single, rhythmically bursting TC neurons, as previously suggested (Sorokin et
238 al., 2017), but an emergent property of this neuronal population. Notably, an altered firing
239 that precedes the start of an AS is also present in WAG/Rij rats and STG mice (Sorokin et al.,
240 2017).

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

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

254 Finally, although ASs are generated by CT network, both the direct and indirect basal ganglia
255 pathways provide a fine control of the expression of these seizures (Deransart and Depaulis,
256 2002). Ictal changes in MRI BOLD signals in the basal ganglia of CAE cohorts have been
257 consistently reported (Bai et al., 2010). Moreover, genetic deletion of the Munc-18-1 protein
258 in cortico-striatal Layer 5 neurons elicits ETX-sensitive ASs, and activation of DREADD-
259 transfected striatal fast-spiking interneurons blocks the spontaneous ASs of mice with whole-
260 brain KO of Munc-18-1 (Miyamoto et al., 2019).

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

267

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

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

280 *7.1 Ligand-gated channels*

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

284 (DeLorey et al., 1998). Spontaneous ASs are observed in mice knock-in (KI) for the human
285 $\gamma 2$ subunit R43Q mutation (Tan et al., 2007) (which had been identified in probands with
286 febrile seizures and ASs (see Crunelli and Leresche, 2002), whereas whole-brain KO of
287 GABA_BR1 or GABA_BR2 lead only to rare or no spontaneous SWDs, respectively (Gassmann
288 et al., 2004; Schuler et al., 2001).

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

294 A common finding in GAERS rats and STG and LH mice is the constitutively enhanced tonic
295 GABA_A current of TC neurons that results from a non-genetic loss-of-function of GAT1 (one
296 of the GABA transporters) that is necessary and sufficient for AS expression (Cope et al.,
297 2009). In TC neurons of LH mice, GABA_B eIPSCs are decreased but there is no change in
298 GABA_A eIPSCs and baclofen-evoked currents (Caddick et al., 1999). The lack of changes in
299 GABA_A IPSCs is shared by other models since no difference was found in GABA_A miniature
300 IPSCs (mIPSCs) and eIPSCs in TC neurons of GAERS rats (Bessaïh et al., 2006; Cope et al.,
301 2009) and TG mice (Caddick et al., 1999), respectively. An increased amplitude, and
302 decreased frequency and duration of mIPSCs but no change in PPD was reported in GAERS
303 NRT neurons (Bessaïh et al., 2006).

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

311 *7.2 Voltage-gated Ca⁺² channels*

312 Selective knock-down (KD) of Cav2.1 channels in Layer 6 pyramidal neurons of normal mice
313 leads to spontaneous ETX-sensitive ASs accompanied by increased T-type Ca²⁺ currents in
314 the TC and NRT neurons and increased burst firing in the latter but not the former thalamic
315 neural population (Bomben et al., 2016) (Figure 3). Since no changes in cortical Cav2.1

316 channels expression were observed with light microscopy, T-type Ca^{2+} currents and
317 associated burst firing was not investigated in cortical excitatory and inhibitory neurons in
318 these Layer 6 pyramidal neuron-selective KD mice, making it difficult to accept the authors'
319 conclusion that changes in thalamic neuron excitability were the only ones causally linked to
320 AS expression. Indeed, ablation of $\text{Cav}2.1$ channels in adult mice brings about ASs that are
321 not associated with changes in T-type Ca^{2+} currents or burst firing in TC neurons (Miao et al.,
322 2019). Similarly, selective KO of $\text{Cav}2.1$ channels in PV+ and somatostatin-positive (SST+)
323 cortical interneurons leads to ASs with no change in T-type Ca^{2+} currents or burst firing of TC
324 neurons (Figure 3) (Rossignol et al., 2013), demonstrating that a constitutive or induced
325 increase in these TC neuron parameters, as observed in TG mice and mice with selective
326 Layer 6 pyramidal neurons KD of $\text{Cav}2.1$ channels, is not necessary for the expression of
327 ASs.

328 $\text{Cav}2.3$ channels are expressed in cortical and NRT, but not TC neurons, and their whole-
329 brain KO leads to a decrease in GHB-induced ASs (Zaman et al., 2011). However, whether
330 this pathological phenotype is due to NRT or cortical $\text{Cav}2.3$ channels remains to be
331 investigated.

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

342 Notably, elevated T-type Ca^{2+} currents (mainly due to $\text{Cav}3.1$ channels) are present in TC
343 neurons of different thalamic nuclei of four AS models (WAG/Rij rat, and STG, TG and LG
344 mice) (Broicher et al., 2008; Zhang et al., 2002). The block of ASs by whole-brain genetic
345 ablation of $\text{Cav}3.1$ channels in STG, TG and LH mice has been causally linked to the loss of
346 function of these channels in TC neurons (Song et al., 2004). However, such conclusion is
347 flawed since the block of ASs in these mice could also be due to the simultaneous ablation of
348 $\text{Cav}3.1$ channels in the cortex where they are highly expressed (Talley et al., 1999). Indeed,

349 ASs in GAERS rats are not affected by selectively blocking Cav3.1 channels and related burst
350 firing of TC neurons with a potent T-type Ca²⁺ channels antagonist (McCafferty et al., 2018).
351 Notwithstanding, TC neuron firing is necessary for AS expression since brief optogenetic
352 manipulation of TC neurons abort ASs in WAG/Rij rats and STG mice (Sorokin et al., 2017).
353 Mice with whole-brain KO or selective KD of Cav3.3 channels in the NRT do not show
354 spontaneous ASs, but an unexpected increase in GHB-induced ASs (Lee et al., 2014) (Figure
355 3). The same phenotype is observed in double KO mice of Cav3.3 and Cav3.2 channels (Lee
356 et al., 2014) where the T-type Ca²⁺ current of NRT neurons is completely abolished. Thus,
357 notwithstanding the key role of the NRT neurons T-type Ca²⁺ current (and the burst firing that
358 it generates) in physiological activities expressed by CT networks (Crunelli et al., 2018), full
359 ablation of these NRT channels leads to potentiation of pharmacologically induced ASs.
360 Whether this is due to the increased tonic firing of NRT neurons that is present in these single
361 and double KO mice (Lee et al., 2014) or results from alterations in the activity of cortical
362 neuronal populations remains to be elucidated since the analysis of the cellular and network
363 abnormalities of these mice was only carried out in the thalamus.

364 Elevated T-type Ca²⁺ currents are also present in the NRT neurons of GAERS and WAG rats
365 (Broicher et al., 2008; Cain et al., 2018) but have not been studied in the NRT of STG, TG
366 and LH mice. In GAERS rats, this increased current is due to the R158P missense variant of
367 *CACNA1H* (the Cav3.2 channel gene) and selective KD of these channels in NRT neurons
368 markedly reduces ASs and normalizes their elevated burst firing (Cain et al., 2018).
369 Moreover, KI of the human *CACNA1H* variant C456S in S1 of normal non-epileptic rats
370 elicits spontaneous ASs in >60% of the injected animals (Wang et al., 2015).

371 *7.3 Voltage-gated Na⁺ and Na⁺/K⁺ channels*

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

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

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

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

- 395 1. genetic ablations of diverse single channel abnormalities in single neuronal populations of
396 the CT network lead to spontaneous ETX-sensitive ASs (Figure 3); Cav2.1 channels in
397 cortical layer 6 neurons (Bomben et al., 2016), Cav2.1 channels in cortical PV+ and SST+
398 interneurons (Rossignol et al., 2013), Nav1.6 channels in NRT neurons (Makinson et al.,
399 2017), phospholipase C β 1 subunit in NRT neurons (Chang et al., 2022) and phospholipase
400 C β 4 subunit in TC neurons (Cheong et al., 2009), whereas selective KD of Cav3.3
401 channels in NRT neurons does not lead to spontaneous ASs but increases GHB-induced
402 SWDs (Lee et al., 2014);
- 403 2. secondary (compensatory) changes in a CT sector other than the one where the primary
404 genetic manipulation had been made may be the critical element for ASs expression
405 indicated by the increased T-type Ca^{2+} currents in TC neurons of mice with selective KD
406 of Cav2.1 channels in cortical Layer 6 neurons (Bomben et al., 2016);
- 407 3. different changes of membrane proteins expression may occur in diverse areas of CT
408 network, as indicated by the decreased and increased expression of HCN1 channels in the
409 cortex and thalamus, respectively, of WAG/Rij rats (Cain et al., 2015; Kole et al., 2007);
- 410 4. in view of the cortical location of SWDs initiation in humans and animal models (see Parts
411 4 and 5, above), it is unfortunate that almost all investigations that used whole-brain
412 genetic manipulations have studied abnormalities only in thalamic but not cortical neurons,
413 even when the manipulated channels are (highly) expressed in both the cortical and
414 thalamic sectors of CT networks.

415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437

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.

438 References

- 439 Atherton Z, Nagy O, Barcsai L, Sere P, Zsigri N, Földi T, Berényi A, Crunelli V, Lőrincz
440 ML. 2023. Higher-order thalamic nuclei facilitate the generalization and maintenance of
441 spike-and-wave discharges of absence seizures. *Neurobiol Dis* 178: 106025.
- 442 Avoli M. 2012. A brief history on the oscillating roles of thalamus and cortex in absence
443 seizures. *Epilepsia* 53:779–789.
- 444 Bai X, Vestal M, Berman R, Negishi M, Spann M, Vega C, Desalvo M, Novotny EJ,
445 Constable RT, Blumenfeld H. 2010. Dynamic Time Course of Typical Childhood
446 Absence Seizures: EEG, Behavior, and Functional Magnetic Resonance Imaging. *J*
447 *Neurosci* 30:5884–5893.
- 448 Bessaïh T, Bourgeois L, Badiu CI, Carter DA, Toth TI, Ruano D, Lambolez B, Crunelli V,
449 Leresche N. 2006. Nucleus-specific abnormalities of GABAergic synaptic transmission in
450 a genetic model of absence seizures. *J Neurophysiol* 96:3074–3081.
- 451 Bomben VC, Aiba I, Qian J, Mark MD, Herlitze S, Noebels JL. 2016. Isolated P/Q Calcium
452 Channel Deletion in Layer VI Corticothalamic Neurons Generates Absence Epilepsy. *J*
453 *Neurosci* 36:405–418.
- 454 Broicher T, Kanyshkova T, Meuth P, Pape H-C, Budde T. 2008. Correlation of T-channel
455 coding gene expression, IT, and the low threshold Ca²⁺ spike in the thalamus of a rat
456 model of absence epilepsy. *Molecular and Cellular Neuroscience* 39:384–399.
- 457 Budde T, Caputi L, Kanyshkova T, Staak R, Abrahamczik C, Munsch T, Pape H-C. 2005.
458 Impaired Regulation of Thalamic Pacemaker Channels through an Imbalance of Subunit
459 Expression in Absence Epilepsy. *J Neurosci* 25:9871–9882.
- 460 Caddick SJ, Wang C, Fletcher CF, Jenkins NA, Copeland NG, Hosford DA. 1999. Excitatory
461 But Not Inhibitory Synaptic Transmission Is Reduced in Lethargic (*Cacnb4* lh) and
462 Tottering (*Cacna1a* tg) Mouse Thalami. *Journal of Neurophysiology* 81:2066–2074.
- 463 Cain SM, Tyson JR, Choi H, Ko R, Lin PJC, LeDue JM, Powell KL, Bernier L, Rungta RL,
464 Yang Y, Cullis PR, O'Brien TJ, MacVicar BA, Snutch TP. 2018. CaV3.2 drives sustained
465 burst-firing, which is critical for absence seizure propagation in reticular thalamic
466 neurons. *Epilepsia* 59:778–791.
- 467 Cain SM, Tyson JR, Jones KL, Snutch TP. 2015. Thalamocortical neurons display suppressed
468 burst-firing due to an enhanced I_h current in a genetic model of absence epilepsy. *Pflugers*
469 *Arch - Eur J Physiol* 467:1367–1382.
- 470 Casillas-Espinosa PM, Powell KL, Zhu M, Campbell CR, Maia JM, Ren Z, Jones NC,
471 O'Brien TJ, Petrovski S. 2017. Evaluating whole genome sequence data from the Genetic
472 Absence Epilepsy Rat from Strasbourg and its related non-epileptic strain. *PLoS ONE*
473 12:e0179924.
- 474 Chang B, Byun J, Kim KK, Lee SE, Lee B, Kim K-S, Ryu H, Shin H-S, Cheong E. 2022.
475 Deletion of Phospholipase C β 1 in the Thalamic Reticular Nucleus Induces Absence
476 Seizures. *Exp Neurobiol* 31:116–130.
- 477 Cheong E, Zheng Y, Lee K, Lee J, Kim S, Sanati M, Lee S, Kim Y-S, Shin H-S. 2009.
478 Deletion of phospholipase C β 4 in thalamocortical relay nucleus leads to absence seizures.
479 *Proc Natl Acad Sci U S A* 106:21912–21917.
- 480 Cope DW, Di Giovanni G, Fyson SJ, Orbán G, Errington AC, Lorincz ML, Gould TM, Carter
481 DA, Crunelli V. 2009. Enhanced tonic GABA inhibition in typical absence epilepsy. *Nat*
482 *Med* 15:1392–1398.
- 483 Crunelli V, Leresche N. 2002. Childhood absence epilepsy: genes, channels, neurons and
484 networks. *Nat Rev Neurosci* 3:371–382.

485 Crunelli V, Lőrincz ML, Connelly WM, David F, Hughes SW, Lambert RC, Leresche N,
486 Errington AC. 2018. Dual function of thalamic low-vigilance state oscillations: rhythm-
487 regulation and plasticity. *Nat Rev Neurosci* 19:107–118.

488 Crunelli V, Lőrincz ML, McCafferty C, Lambert RC, Leresche N, Di Giovanni G, David F.
489 2020. Clinical and experimental insight into pathophysiology, comorbidity and therapy of
490 absence seizures. *Brain* 143:2341–2368.

491 D’Antuono M, Inaba Y, Biagini G, D’Arcangelo G, Tancredi V, Avoli M. 2005. Synaptic
492 hyperexcitability of deep layer neocortical cells in a genetic model of absence seizures:
493 Neocortical excitability and absence epilepsy. *Genes, Brain and Behavior* 5:73–84.

494 DeLorey TM, Handforth A, Anagnostaras SG, Homanics GE, Minassian BA, Asatourian A,
495 Fanselow MS, Delgado-Escueta A, Ellison GD, Olsen RW. 1998. Mice Lacking the $\beta 3$
496 Subunit of the GABAA Receptor Have the Epilepsy Phenotype and Many of the
497 Behavioral Characteristics of Angelman Syndrome. *J Neurosci* 18:8505–8514.

498 Deransart C, Depaulis A. 2002. The control of seizures by the basal ganglia? A review of
499 experimental data. *Epileptic Disord* 4 Suppl 3:S61-72.

500 Ernst WL, Zhang Y, Yoo JW, Ernst SJ, Noebels JL. 2009. Genetic enhancement of
501 thalamocortical network activity by elevating alpha 1g-mediated low-voltage-activated
502 calcium current induces pure absence epilepsy. *J Neurosci* 29:1615–1625.

503 Feng Y-CA, Howrigan DP, Abbott LE, Tashman K, Cerrato F, Singh T, Heyne H, Byrnes A,
504 Churchhouse C, Watts N, Solomonson M, Lal D, Heinzen EL, Dhindsa RS, Stanley KE,
505 Cavalleri GL, Hakonarson H, Helbig I, Krause R, May P, Weckhuysen S, Petrovski S,
506 Kamalakaran S, Sisodiya SM, Cossette P, Cotsapas C, De Jonghe P, Dixon-Salazar T,
507 Guerrini R, Kwan P, Marson AG, Stewart R, Depondt C, Dlugos DJ, Scheffer IE, Striano
508 P, Freyer C, McKenna K, Regan BM, Bellows ST, Leu C, Bennett CA, Johns EMC,
509 Macdonald A, Shilling H, Burgess R, Weckhuysen D, Bahlo M, O’Brien TJ, Todaro M,
510 Stamberger H, Andrade DM, Sadoway TR, Mo K, Krestel H, Gallati S, Papacostas SS,
511 Kousiappa I, Tanteles GA, Štěrbová K, Vlčková M, Sedláčková L, Laššuthová P, Klein
512 KM, Rosenow F, Reif PS, Knake S, Kunz WS, Zsurka G, Elger CE, Bauer J, Rademacher
513 M, Pendziwiat M, Muhle H, Rademacher A, van Baalen A, von Spiczak S, Stephani U,
514 Afawi Z, Korczyn AD, Kanaan M, Canavati C, Kurlemann G, Müller-Schlüter K, Kluger
515 G, Häusler M, Blatt I, Lemke JR, Krey I, Weber YG, Wolking S, Becker F, Hengsbach C,
516 Rau S, Maisch AF, Steinhoff BJ, Schulze-Bonhage A, Schubert-Bast S, Schreiber H,
517 Borggräfe I, Schankin CJ, Mayer T, Korinthenberg R, Brockmann K, Kurlemann G,
518 Dennig D, Madeleyn R, Kälviäinen R, Auvinen P, Saarela A, Linnankivi T, Lehesjoki A-
519 E, Rees MI, Chung S-K, Pickrell WO, Powell R, Schneider N, Balestrini S, Zagaglia S,
520 Braatz V, Johnson MR, Auce P, Sills GJ, Baum LW, Sham PC, Cherny SS, Lui CHT,
521 Barišić N, Delanty N, Doherty CP, Shukralla A, McCormack M, El-Naggar H, Canafoglia
522 L, Franceschetti S, Castellotti B, Granata T, Zara F, Iacomino M, Madia F, Vari MS,
523 Mancardi MM, Salpietro V, Bisulli F, Tinuper P, Licchetta L, Pippucci T, Stipa C,
524 Minardi R, Gambardella A, Labate A, Annesi G, Manna L, Gagliardi M, Parrini E, Mei D,
525 Vetro A, Bianchini C, Montomoli M, Doccini V, Marini C, Suzuki T, Inoue Y,
526 Yamakawa K, Tumiene B, Sadleir LG, King C, Mountier E, Caglayan SH, Arslan M,
527 Yapıcı Z, Yis U, Topaloglu P, Kara B, Turkdogan D, Gundogdu-Eken A, Bebek N, Uğur-
528 İşeri S, Baykan B, Salman B, Haryanyan G, Yücesan E, Kesim Y, Özkara Ç, Poduri A,
529 Shiedley BR, Shain C, Buono RJ, Ferraro TN, Sperling MR, Lo W, Privitera M, French
530 JA, Schachter S, Kuzniecky RI, Devinsky O, Hegde M, Khankhanian P, Helbig KL, Ellis
531 CA, Spalletta G, Piras Fabrizio, Piras Federica, Gili T, Ciullo V, Reif A, McQuillin A,
532 Bass N, McIntosh A, Blackwood D, Johnstone M, Palotie A, Pato MT, Pato CN, Bromet
533 EJ, Carvalho CB, Achtyes ED, Azevedo MH, Kotov R, Lehrer DS, Malaspina D, Marder

534 SR, Medeiros H, Morley CP, Perkins DO, Sobell JL, Buckley PF, Macciardi F, Rapaport
535 MH, Knowles JA, Fanous AH, McCarroll SA, Gupta N, Gabriel SB, Daly MJ, Lander ES,
536 Lowenstein DH, Goldstein DB, Lerche H, Berkovic SF, Neale BM. 2019. Ultra-Rare
537 Genetic Variation in the Epilepsies: A Whole-Exome Sequencing Study of 17,606
538 Individuals. *The American Journal of Human Genetics* 105:267–282.

539 Frankel WN. 2009. Genetics of complex neurological disease: challenges and opportunities
540 for modeling epilepsy in mice and rats. *Trends in Genetics* 25:361–367.

541 Gassmann M, Shaban H, Vigot R, Sansig G, Haller C, Barbieri S, Humeau Y, Schuler V,
542 Müller M, Kinzel B, Klebs K, Schmutz M, Froestl W, Heid J, Kelly PH, Gentry C, Jatón
543 A-L, Van der Putten H, Mombereau C, Lecourtier L, Mosbacher J, Cryan JF, Fritschy J-
544 M, Lüthi A, Kaupmann K, Bettler B. 2004. Redistribution of GABAB(1) Protein and
545 Atypical GABAB Responses in GABAB(2)-Deficient Mice. *J Neurosci* 24:6086–6097.

546 Gruenbaum BF, Sandhu MRS, Bertasi RAO, Bertasi TGO, Schonwald A, Kurup A,
547 Gruenbaum SE, Freedman IG, Funaro MC, Blumenfeld H, Sanacora G. 2021. Absence
548 seizures and their relationship to depression and anxiety: Evidence for bidirectionality.
549 *Epilepsia* 62:1041–1056.

550 Guo J, Kim R, Chen Y, Negishi M, Jhun S, Weiss S, Ryu JH, Bai X, Xiao W, Feeney E,
551 Rodriguez-Fernandez J, Mistry H, Crunelli V, Crowley MJ, Mayes LC, Todd Constable
552 R, Blumenfeld H. 2016. Mechanism of impaired consciousness in absence seizures: a
553 cross-sectional study. *Lancet Neurol* 15:1336–1345.

554 Harutyunyan A, Chong D, Li R, Shah AD, Ali Z, Huang C, Barlow CK, Perucca P, O'Brien
555 TJ, Jones NC, Schittenhelm RB, Anderson A, Casillas-Espinosa PM. 2022. An Integrated
556 Multi-Omic Network Analysis Identifies Seizure-Associated Dysregulated Pathways in
557 the GAERS Model of Absence Epilepsy. *Int J Mol Sci* 23:6063.

558 Iacone Y, Morais TP, David F, Delicata F, Sandle J, Raffai T, Parri HR, Weisser JJ,
559 Bundgaard C, Klewe IV, Tamás G, Thomsen MS, Crunelli V, Lőrincz ML. 2021.
560 Systemic administration of ivabradine, a hyperpolarization-activated cyclic nucleotide-
561 gated channel inhibitor, blocks spontaneous absence seizures. *Epilepsia* 62:1729–1743.

562 International League Against Epilepsy Consortium on Complex Epilepsies. 2018. Genome-
563 wide mega-analysis identifies 16 loci and highlights diverse biological mechanisms in the
564 common epilepsies. *Nat Commun* 9:5269.

565 Kanyshkova T, Meuth P, Bista P, Liu Z, Ehling P, Caputi L, Doengi M, Chetkovich DM,
566 Pape H-C, Budde T. 2012. Differential regulation of HCN channel isoform expression in
567 thalamic neurons of epileptic and non-epileptic rat strains. *Neurobiol Dis* 45:450–461.
568 doi:10.1016/j.nbd.2011.08.032

569 Kim D, Song I, Keum S, Lee T, Jeong M-J, Kim S-S, McEnery MW, Shin H-S. 2001. Lack of
570 the Burst Firing of Thalamocortical Relay Neurons and Resistance to Absence Seizures in
571 Mice Lacking $\alpha 1G$ T-Type Ca^{2+} Channels. *Neuron* 31:35–45.

572 Kole MH, Bräuer AU, Stuart GJ. 2007. Inherited cortical HCN1 channel loss amplifies
573 dendritic calcium electrogenesis and burst firing in a rat absence epilepsy model. *J Physiol*
574 578:507–525.

575 Kuisle M, Wanaverbecq N, Brewster AL, Frère SGA, Pinault D, Baram TZ, Lüthi A. 2006.
576 Functional stabilization of weakened thalamic pacemaker channel regulation in rat
577 absence epilepsy. *J Physiol* 575:83–100.

578 Lacey CJ, Bryant A, Brill J, Huguenard JR. 2012. Enhanced NMDA Receptor-Dependent
579 Thalamic Excitation and Network Oscillations in Stargazer Mice. *J Neurosci* 32:11067–
580 11081.

581 Lee SE, Lee J, Latchoumane C, Lee B, Oh SJ, Saud ZA, Park C, Sun N, Cheong E, Chen CC,
582 Choi EJ, Lee CJ, Shin H-S. 2014. Rebound burst firing in the reticular thalamus is not
583 essential for pharmacological absence seizures in mice. *Proc Natl Acad Sci USA*
584 111:11828–11833.

585 Ludwig A, Budde T, Stieber J, Moosmang S, Wahl C, Holthoff K, Langebartels A, Wotjak C,
586 Munsch T, Zong X, Feil S, Feil R, Lancel M, Chien KR, Konnerth A, Pape H-C, Biel M,
587 Hofmann F. 2003. Absence epilepsy and sinus dysrhythmia in mice lacking the
588 pacemaker channel HCN2. *EMBO J* 22:216–224.

589 Lüttjohann A, Pape H-C. 2019. Regional specificity of cortico-thalamic coupling strength and
590 directionality during waxing and waning of spike and wave discharges. *Sci Rep* 9:2100.

591 Makinson CD, Tanaka BS, Sorokin JM, Wong JC, Christian CA, Goldin AL, Escayg A,
592 Huguenard JR. 2017. Regulation of Thalamic and Cortical Network Synchrony by Scn8a.
593 *Neuron* 1–22.

594 McCafferty C, David F, Venzi M, Lőrincz ML, Delicata F, Atherton Z, Recchia G, Orban G,
595 Lambert RC, Di Giovanni G, Leresche N, Crunelli V. 2018. Cortical drive and thalamic
596 feed-forward inhibition control thalamic output synchrony during absence seizures. *Nat*
597 *Neurosci* 21:744–756.

598 McCafferty C, Gruenbaum B, Tung R, Li J-J, Zheng X, Salvino P, Vincent P, Kratochvil Z,
599 Ryu JH, Khalaf A, Swift K, Akbar R, Islam W, Antwi P, Johnson EA, Vitkovskiy P,
600 Sampognaro J, Freedman I, Kundishora A, Depaulis A, David F, Crunelli V, Sanganahalli
601 BG, Herman P, Hyder F, Blumenfeld H. 2023. The neuronal basis of impaired
602 consciousness in absence seizures. *Nat Commun*, in press.

603 Meeren HKM, Pijn JPM, Luijtelaa ELJMV, Coenen AML, Silva FHL da. 2002. Cortical
604 Focus Drives Widespread Corticothalamic Networks during Spontaneous Absence
605 Seizures in Rats. *J Neurosci* 22:1480–1495.

606 Meyer J, Maheshwari A, Noebels J, Smirnakis S. 2018. Asynchronous suppression of visual
607 cortex during absence seizures in stargazer mice. *Nat Commun* 9:1938.

608 Miao Q-L, Herlitze S, Mark MD, Noebels JL. 2019. Adult loss of Cacna1a in mice
609 recapitulates childhood absence epilepsy by distinct thalamic bursting mechanisms. *Brain*
610 143:161–174.

611 Miyamoto H, Tatsukawa T, Shimohata A, Yamagata T, Suzuki T, Amano K, Mazaki E,
612 Raveau M, Ogiwara I, Oba-Asaka A, Hensch TK, Itohara S, Sakimura K, Kobayashi
613 Kenta, Kobayashi Kazuto, Yamakawa K. 2019. Impaired cortico-striatal excitatory
614 transmission triggers epilepsy. *Nat Commun* 10:1917.

615 Ogiwara I, Miyamoto H, Tatsukawa T, Yamagata T, Nakayama T, Atapour N, Miura E,
616 Mazaki E, Ernst SJ, Cao D, Ohtani H, Itohara S, Yanagawa Y, Montal M, Yuzaki M,
617 Inoue Y, Hensch TK, Noebels JL, Yamakawa K. 2018. Nav1.2 haplodeficiency in
618 excitatory neurons causes absence-like seizures in mice. *Commun Biol* 1:96.

619 Panthi S, Leitch B. 2019. The impact of silencing feed-forward parvalbumin-expressing
620 inhibitory interneurons in the cortico-thalamocortical network on seizure generation and
621 behaviour. *Neurobiology of Disease* 132:104610.

622 Papp P, Kovács Z, Szocsics P, Juhász G, Maglóczy Z. 2018. Alterations in hippocampal and
623 cortical densities of functionally different interneurons in rat models of absence epilepsy.
624 *Epilepsy Research* 145:40–50.

625 Paz JT, Bryant AS, Peng K, Fenno L, Yizhar O, Frankel WN, Deisseroth K, Huguenard JR.
626 2011. A new mode of corticothalamic transmission revealed in the Gria4(-/-) model of
627 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 GM, 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 GABA
649 Responses in Mice Lacking GABA_B(1). *Neuron* 31:47–58.
- 650 Song I, Kim D, Choi S, Sun M, Kim Y, Shin H-S. 2004. Role of the α 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 $\hat{\alpha}3$ subunit-knockout mice. *Neuroscience*
676 154:595–605.

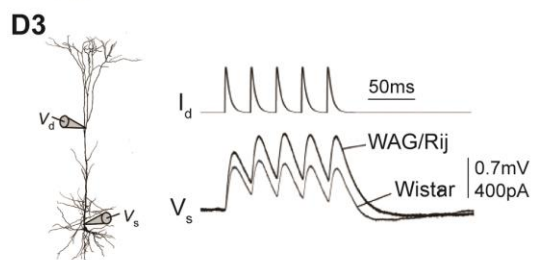
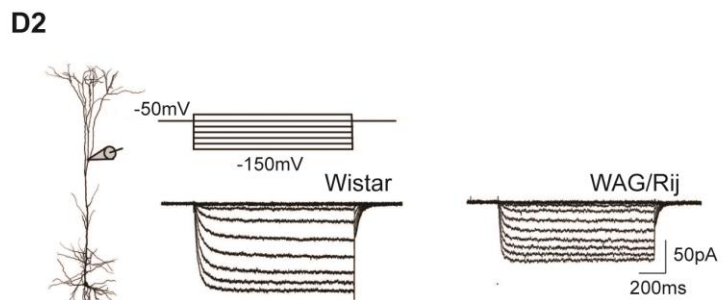
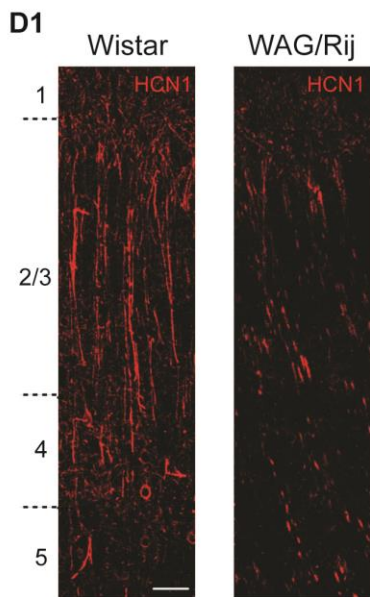
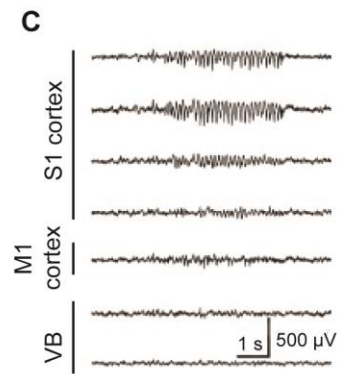
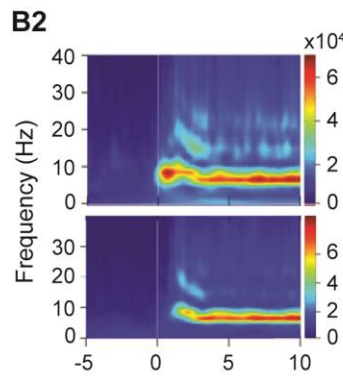
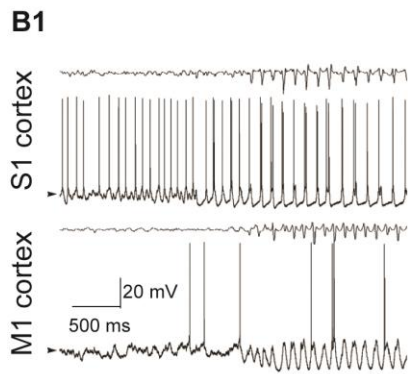
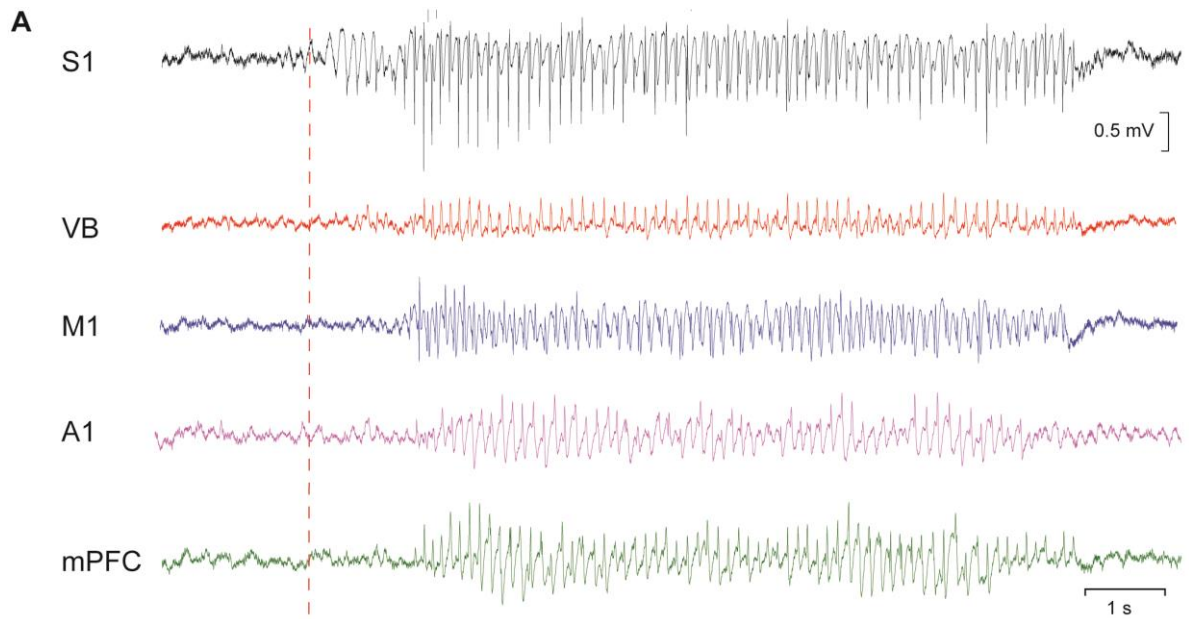
677 Xie H, Su W, Pei J, Zhang Yujia, Gao K, Li J, Ma X, Zhang Yuehua, Wu X, Jiang Y. 2019.
678 De novo SCN1A, SCN8A, and CLCN2 mutations in childhood absence epilepsy.
679 *Epilepsy Research* 154:55–61.

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



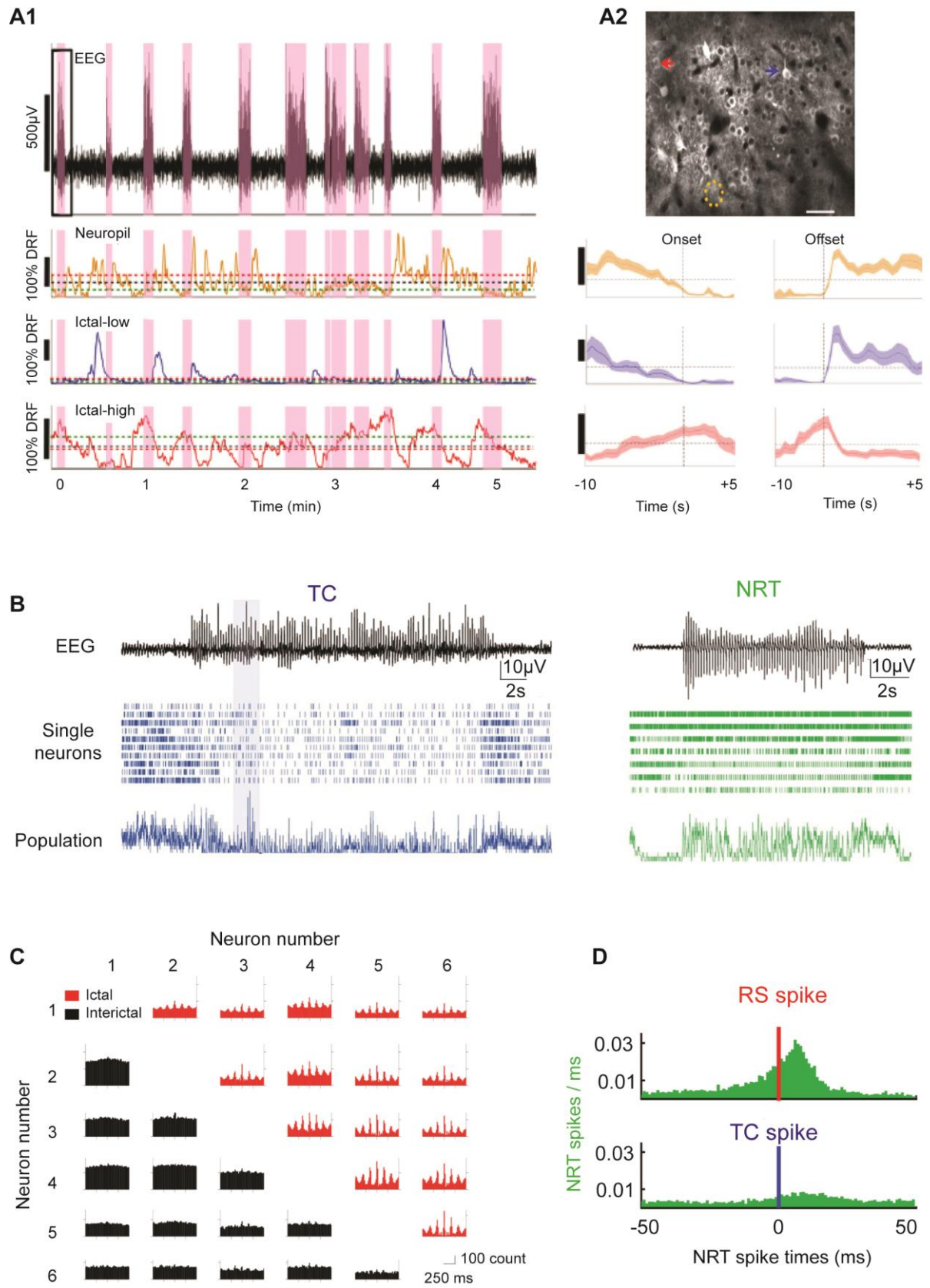
692
693

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

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

716

717



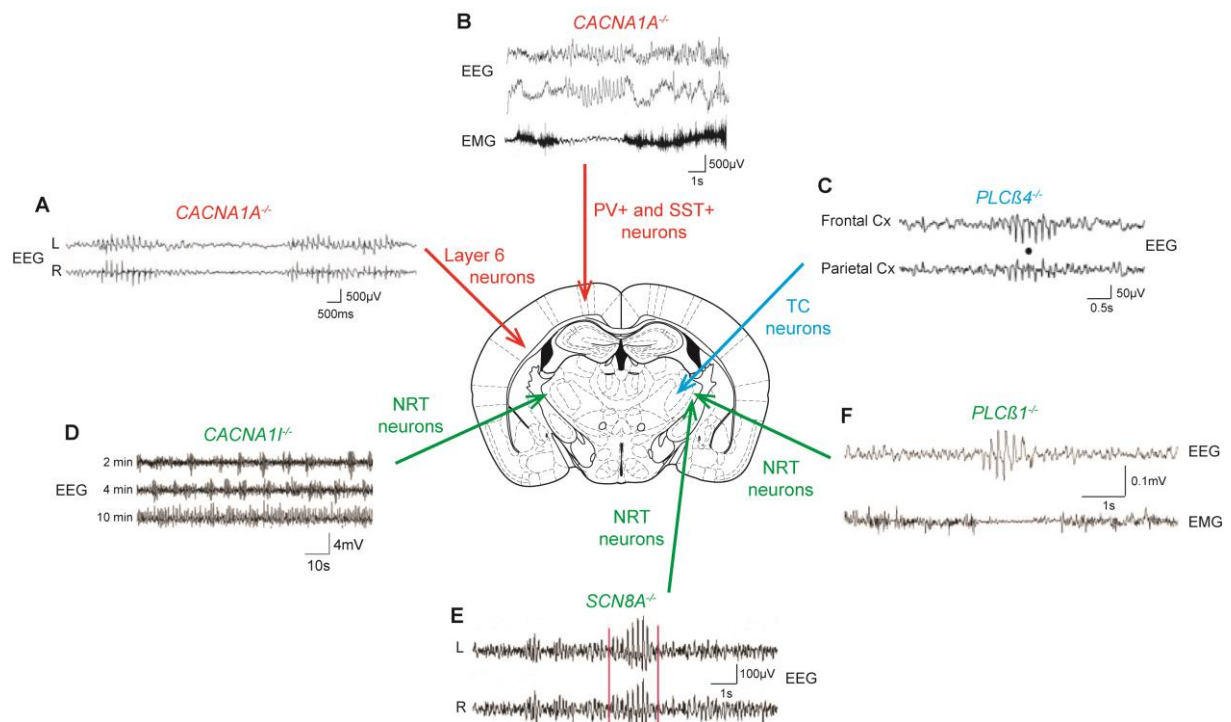
718

719

720

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

721 **(A1)** Concomitant electroencephalographic (EEG) (top trace) and intracellular Ca^{2+} changes
722 from Layer 2/3 GCaMP6-filled neurons in the visual cortex of a head-restrained STG mouse.
723 The Ca^{2+} changes of each neuron/neuropil patch over time are shown with the seizure epochs
724 highlighted in pink. Mean ictal activity (green dashed line), mean interictal activity (red
725 dashed line), and overall mean activity (black dashed line). **(A2)** The average activity (vertical
726 bar = 20% $\Delta\text{F}/\text{F}$) time-locked to the seizure onset and offset is shown for exemplary neuropil
727 (top), a neuron with low activity during ASs (ictal-low, middle) and a neuron with high
728 activity during ASs (ictal-high, bottom). The ictal-low and ictal-high neuron are marked by a
729 blue and red arrow, respectively in the 2-photon microscopy image shown at the top, with the
730 neuropil area highlighted by a yellow dotted circle. Scale bar = 50 μm . **(B)** EEG (top trace)
731 and spike-time raster plots from 10 thalamocortical (TC) neurons in the ventrobasal thalamic
732 nucleus and 7 nucleus reticularis thalami (NRT) neurons simultaneously recorded with a
733 silicon probe in a freely moving GAERS rats. Most TC neurons show a decrease in firing rate
734 during the ASs, which is also evident from the population activity (bottom trace). NRT
735 neurons show two different types of ictal firing: one group increases their firing ictally (top
736 three cells in raster-plot) and another group shows a decreased activity (bottom four cells).
737 **(C)** Cross-correlograms of the total firing of 6 simultaneously recorded GAERS TC neurons
738 during interictal periods (black plots) and ASs (red plots). Despite the overall decrease in
739 firing during ASs (red plots), there is a marked increase in neuronal synchrony. **(D)**
740 Histograms of the firing of an NRT neuron recorded simultaneously with a cortical regular
741 spiking (RS) neuron (top) or a TC neuron (bottom) in a freely moving GAERS rat. Note that
742 the ictal NRT neuron firing (green bars) is mainly driven by the cortical RS neuron spikes
743 rather than the TC neuron spikes. Time zero marks the firing of the RS and TC neurons (red
744 and blue lines, respectively). Adapted from Meyer et al. (2018) (A) and McCafferty et al.
745 (2018) (B-D).
746



747

748

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

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

760