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2 **Cortical drive and thalamic feed-forward inhibition control**
3 **thalamic output synchrony during absence seizures**
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35 CMcC, F David, MLL, RCL, GD, NL and VC designed research and experiments; CMcC, F
36 David, MV, MLL, GO, F Delicata, ZA, GR, GD and NL performed experiments and analysed
37 data; CMcC, F David and VC wrote the manuscript with critical review by other authors.

38

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48 **Competing financial interests**

49 The authors declare no competing financial interests.

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54 **ABSTRACT**

55 **Behaviorally and pathologically relevant cortico-thalamo-cortical oscillations are driven**
56 **by diverse interacting cell-intrinsic and synaptic processes. However, the mechanism that**
57 **gives rise to the paroxysmal oscillations of absence seizures (ASs) remains unknown. Here**
58 **we report that during ASs in behaving animals, cortico-thalamic excitation drives**
59 **thalamic firing by preferentially eliciting tonic rather than T-type Ca²⁺ channels (T-**
60 **channels)-dependent burst firing in thalamocortical (TC) neurons, and by temporally**
61 **framing thalamic output via feed-forward reticular thalamic (NRT)-to-TC neuron**
62 **inhibition. In TC neurons, overall ictal firing is markedly reduced and bursts rarely**
63 **occur. Moreover, block of T-channels in cortical and NRT neurons suppresses ASs, but**
64 **in TC neurons has no effect on seizures or on ictal thalamic output synchrony. These**
65 **results demonstrate ictal bidirectional cortico-thalamic communications and provide the**
66 **first mechanistic understanding of cortico-thalamo-cortical network firing dynamics**
67 **during ASs in behaving animals.**

68

69 INTRODUCTION

70 Cortico-thalamo-cortical circuits process behaviourally relevant internal and external
71 information, determine vigilance states, and elicit diverse forms of neuronal network
72 oscillations during sleep¹⁻³. Reciprocal excitation between neocortex and thalamus allows the
73 synchronization of these brain regions, while cortico-thalamic afferents also excite GABAergic
74 nucleus reticularis thalami (NRT) neurons, thus driving feed-forward inhibition of
75 thalamocortical (TC) neurons⁴. Inhibition of TC neurons can evoke a rebound T-type Ca²⁺
76 channel (T-channel)-mediated burst of action potentials that in turn excites cortical targets, as
77 well as NRT neurons leading to intrathalamic feed-back inhibition⁴. This thalamic
78 rhythmogenesis contributes to or determines many physiological cortico-thalamo-cortical
79 oscillations⁵, in particular those underlying spindle, slow-wave and delta oscillations of non-
80 REM sleep that require T-channel bursts in both TC and NRT neurons^{1,5,6}.

81 The T-channel bursts of these thalamic neuronal populations are also considered essential for
82 the thalamic rhythmogenesis of typical absence seizures (ASs), a type of non-convulsive
83 seizure characterized by sudden loss of consciousness and spike-wave-discharges (SWDs) in
84 the EEG⁷⁻⁹. Indeed, currently accepted AS mechanisms, supported by *in vitro*^{10,11} and *in*
85 *silico*^{12,13} studies, require the presence of bursts in both TC and NRT neurons at each SWD
86 cycle, with GABA_A/GABA_B receptors and intrinsic Ca²⁺-/Na⁺-activated K⁺ currents
87 contributing to the rhythmicity of these neuronal populations, respectively. However, these
88 studies are limited by the compromised network integrity and absence of behavioural correlates
89 inherent to *in vitro* preparations. In contrast, studies that examined the activity of thalamic
90 neurons in intact animals under fentanyl or anesthesia report either a paucity of T-channel
91 bursts in TC neurons¹⁴ or the presence of two populations of TC neurons, one almost fully
92 silent and the other with occasional bursts intermixed with single action potentials¹⁵, whereas
93 NRT neurons show bursts at each SWD cycle^{15,16}. The relevance of these, and other *in vivo*
94 investigations using multi-unit activity¹⁷, is also limited given the lack of behavioural seizures
95 and the profound effects of fentanyl and anesthesia on SWD properties^{18,19}. Thus, we still have
96 no knowledge of the TC and NRT neuron firing dynamics and interactions during behavioral
97 ASs.

98

99 Here, using ensemble recordings of single somatotopically-aligned thalamic and cortical
100 neurons during genetically-determined or pharmacologically-induced ASs in freely moving

101 rats, we found complex temporal dynamics of TC and NRT neuron firing, with TC neurons
102 exhibiting a marked decrease in overall firing and a paucity of burst firing. Selective block of
103 T-channels in NRT and cortical neurons decreased ASs, whereas their block in TC neurons did
104 not affect seizure expression. Cortical excitatory and NRT inhibitory input to TC neurons
105 during seizures, as well as cortical excitation of NRT neurons, suggest a role for ictal feed-
106 forward inhibition of TC neurons. Taken together, these results demonstrate that during
107 behavioural ASs the synchronous, seizure-perpetuating output of TC neurons is not determined
108 by the dynamics of their T-channel-mediated burst firing but rather is driven by top-down
109 cortical excitation and sharpened by cortically driven, T-channel-dependent NRT inhibition.

110

111 RESULTS

112 Ensemble recordings of single thalamic neurons during ASs

113 Using microdrive-mounted silicon probes (Supplementary Fig. 1d), alone or with microdialysis
114 probe(s)²⁰, we studied under freely moving conditions the interictal (between seizures) and ictal
115 (during seizures) firing dynamics of TC neurons of the ventrobasal thalamic nucleus (VB) and
116 NRT neurons in two well-established AS models: the Genetic Absence Epilepsy Rats from
117 Strasbourg (GAERS)¹⁹ and the pharmacological γ -hydroxybutyrate (GHB) model²¹ (Online
118 Methods). The VB was chosen because the somatosensory system has a crucial role in ASs of
119 these models where SWDs originate from a cortical initiation site, the peri-oral region of
120 primary somatosensory cortex (S1pO)^{22,23}, before spreading to the VB and then to other cortical
121 regions (Supplementary Fig. 2). Individual action potential waveforms were isolated from the
122 full-band raw signal, semi-automatically clustered following systematic visual inspection²⁴
123 (Fig. 1) (Supplementary Fig. 1a) and classified as originating from excitatory (i.e. TC) or
124 inhibitory (i.e. NRT) neurons based on standard criteria²⁵ (Fig. 1b-d). Reliable identification of
125 thalamic T-channel bursts during ASs was demonstrated by i) the agreement of burst signatures
126 (Fig. 1c) and action potential waveforms (Fig. 1d) with those currently used in TC and NRT
127 neuron classification²⁵, and ii) the similarity of burst features of each isolated neuron during
128 periods of varying synchrony, i.e. seizures and sleep (compare Fig. 1c and Supplementary Fig.
129 1e) (Supplementary Fig. 3d) (Online Methods).

130

131 TC neuron ictal activity

132 We recorded 139 TC neurons in 6 GAERS during 4,214 ASs (duration: 11.5 ± 4.4 s,
133 mean \pm SEM) with a SWD frequency of 7.1 ± 0.5 Hz. Single TC neurons were markedly less
134 active (52%) ictally than interictally (5.44 ± 0.03 vs 11.4 ± 0.04 Hz, $p < 0.001$) (Fig. 2a-c)
135 (Supplementary Table 1), an effect driven by a reduction in tonic firing (2.8 ± 0.01 vs 9.3 ± 0.03
136 Hz, $p < 0.001$) (Fig. 2e) and a large incidence of electrical silence: 45% of TC neurons (62 out
137 of 139) were silent for $>50\%$ of spike-and-wave complexes (SWCs), the individual cycles of
138 SWDs (Fig. 3a). The decrease of total and tonic firing started 2 s before SWD detection in the
139 EEG (Fig. 2c,e). By contrast, the interictal rate (0.40 ± 0.01 Hz) of T-channel bursts started to
140 increase rapidly 1 s before seizure onset, peaked (1.8 ± 0.22 Hz) just after seizure onset (400
141 ms) and then decayed within the next 2 s to a stable level (0.9 ± 0.01 Hz, $p < 0.05$ compared to
142 interictal) for the remainder of a seizure (Fig. 2d,f-h). Thus, the ictal incidence of bursts was
143 low ($15.7 \pm 0.1\%$ of SWCs) (Fig. 3b,e) while tonic firing occurred during $30.1 \pm 0.1\%$ of SWCs

144 (Fig. 3c,f), leading to a total firing rate of 0.85 ± 0.01 spikes/SWC (Fig. 3d). Notably, despite
145 the scarce ictal firing of individual TC neurons, there was a strong and rhythmic ictal thalamic
146 output as indicated by the cross-correlograms (XCors) of simultaneously recorded TC neurons
147 (Supplementary Fig. 4a). Moreover, the absolute and relative rates of tonic and burst firing of
148 individual TC neurons could greatly vary between seizures (Supplementary Fig. 5).

149 In summary, the firing of single TC neurons during ASs in behaving GAERS is characterized
150 by i) multi-staged temporal dynamics, ii) a marked decrease in total and tonic firing, and iii)
151 an increase in burst rate, though bursts only occur at every 7th SWC.

152

153 **NRT neuron ictal activity**

154 Two classes of NRT neurons could be identified on the basis of their firing during
155 awake/interictal periods²⁶: wake-active (WA, >10 Hz) and wake-quiescent (WQ, ≤ 10 Hz)
156 (Supplementary Fig. 1b). The total firing (47 ± 1.8 Hz) of WA NRT neurons ($n=13$) in GAERS
157 started to decrease 3 s prior to SWD detection, reached a minimum of 31 ± 0.9 Hz (500 ms prior
158 to SWD detection) ($p<0.01$), and then, in contrast to TC neurons, sharply increased to a peak
159 of 62.1 ± 0.2 Hz ($p<0.01$) at interictal-to-ictal transition (Fig. 2c,i,j) (Supplementary Table 1).
160 Then, it slowly decreased to a stable ictal level of 52 ± 2 Hz ($p<0.05$ compared to interictal)
161 (Fig. 2c). In contrast, tonic firing (25 ± 1 Hz) started to decrease 3 s prior to SWD detection,
162 reaching a stable value of 14 ± 2 Hz ($p<0.001$) (Fig. 2e). This decrease in WA NRT neuron tonic
163 firing was overcompensated for by an enhancement of burst firing, which started to increase
164 (from 0.31 ± 0.01 Hz interictally) at the same time as TC neuron bursts, peaked (2.4 ± 0.02 Hz,
165 $p<0.05$) about 2.5 s after seizure onset and then slowly decreased for the remainder of a seizure
166 (1.4 ± 0.03 Hz at ictal-to-interictal transition) (Fig. 2d,i-j). Although bursts in WA NRT neurons
167 occurred on average during $25\pm 2\%$ of SWCs, a group of neurons showed bursts in about 50%
168 of SWCs and another group in $<1\%$ (Fig. 3g). Tonic firing was present during $53\pm 2.5\%$ of
169 SWCs, and the ictal total firing of WA NRT neurons was 6.2 ± 0.3 spikes/SWC (Fig. 3g,h).

170 Since WQ NRT neurons ($n=12$) did not show any significant AS-associated variation in firing
171 (Fig. 2), or any significant AS-linked feature in subsequent analyses (Supplementary Fig. 6),
172 they will not be discussed hereafter, and WA NRT neurons will be referred to as NRT neurons.

173 In summary, the firing of GAERS NRT neurons during ASs exhibit i) multi-staged temporal
174 dynamics, and ii) increased total and burst firing rates. However, in individual NRT neurons
175 bursts do not occur at every SWC.

176

177 **Synchrony and coherence of ictal thalamic neuron firing**

178 Having characterized the firing dynamics of individual thalamic neurons during ASs, we
179 investigated the synchrony and communication strength between TC and NRT neuronal
180 ensembles. SWC-spike-triggered averages indicated that GAERS TC and NRT neuron total
181 firing peaked 15 ms before and 9 ms after the SWC-spike, respectively (Fig. 4a)
182 (Supplementary Table 2), though the initial ascending phase of the two population curves
183 showed a strong overlap. Burst and tonic firing peaks had similar shape and latency (to the
184 SWC-spike) to those of total firing (Fig. 4a).

185 However, SWC-spike-triggered averages are affected by the coherence and the amount of
186 neuronal firing, an issue of critical importance here in view of the marked firing differences
187 between the two thalamic populations. Interestingly, cumulative distribution functions of peak
188 firing times relative to the SWC-spike, which are unaffected by these properties, were similar
189 for TC and NRT neurons (Fig. 4b) ($p=0.631$; Kolmogorov-Smirnov test). This suggests that
190 the TC and NRT populations are excited by a common source.

191 To understand interactions between the firing mode and the EEG paroxysmal oscillation, we
192 analysed the coherence of TC and NRT neuron firing with the SWC-spike. TC neurons whose
193 total firing peak was just before the SWC-spike (-20 to 0 ms) were highly coherent compared
194 to those whose peak was outside this window (Fig. 4c). In contrast, NRT neurons with a firing
195 peak before the SWC-spike showed very low coherence whereas those that preferentially fired
196 after the SWC-spike had high coherence (Fig. 4c). Moreover, TC neuron coherence was little
197 influenced by firing type (Fig. 4e), whereas NRT neurons showed significantly higher
198 coherence ($p<0.001$) when they fired bursts (Fig. 4f). Consistently, NRT neurons with more
199 overall burst firing were more coherent with respect to the SWC-spike (Fig. 4d), whereas for
200 TC neurons a high burst rate was not necessary for high coherence (not shown).

201 In summary, GAERS TC and NRT neurons show synchronous activation with respect to the
202 SWC-spike. Furthermore, whereas NRT burst firing is more coherent than tonic, TC neurons
203 have similar coherence for both firing types, suggesting that TC neuron bursts are not essential
204 for paroxysmal synchrony.

205

206 **Recruitment of intrathalamic inhibition**

207 The paucity of TC neuron bursts, the high coherence of their tonic firing with SWDs, and the
208 overlap of TC and NRT cumulative firing probabilities challenge the view that TC neuron
209 bursts are necessary to drive cortical and NRT populations during seizure^{10,11,13}. Thus, it was
210 important to investigate the synaptic interactions between the two thalamic neuronal
211 populations during ASs. XCors of the ictal firing of simultaneously recorded TC and NRT
212 neuron pairs (n=14) had broad peaks, often before time-zero (Fig. 5a, Supplementary Fig. 6a),
213 indicating an increased probability of NRT neurons to fire after TC neurons. Those peaks were
214 absent during interictal periods (Fig. 5f) and reduced during non-REM sleep (Fig. 5g).

215 Thirty-six percent (5 out of 14) of GAERS TC-NRT pairs showed a significant post-zero trough
216 indicating a decreased TC neuron firing probability following an NRT action potential (Fig.
217 5a), and thus an ictally active NRT-TC inhibitory connection. These inhibitory patterns were
218 still present in XCors between TC neuron total firing and NRT neuron bursts (Fig. 5b), but
219 were absent in XCors calculated using NRT neuron tonic firing (Fig. 5d), demonstrating that
220 the decreased firing probability of TC neurons depends on NRT neuron bursts¹⁰. In contrast,
221 the troughs were still present when TC neuron tonic firing was used for the XCors, indicating
222 that TC neuron bursts are not required for these ictal intrathalamic interactions (Fig. 5c).
223 Notably, NRT neurons that had relatively flat autocorrelograms showed neither significant
224 peaks nor troughs in their XCors with TC neurons (Supplementary Fig. 6a-e).

225 In summary, ASs involve intrathalamic inhibition via the recruitment of NRT-TC neuron
226 inhibitory assemblies, which are dependent on NRT but not TC neuron burst firing.

227 TC and NRT neurons of the GHB model had similar ictal firing dynamics, distributions and
228 XCors to GAERS (Supplementary Figs. 7 and 8; Supplementary Tables 1 and 2).

229

230 **Cortico-thalamo-cortical drive and feed-forward inhibition**

231 The lack of evidence for a TC neuron burst-dependent excitatory drive of NRT neurons (Fig.
232 5e), together with the similar ictal firing distributions of TC and NRT neurons (Fig. 4b),

233 suggests that the corticofugal input may be a key source of excitation of both thalamic neuron
234 populations, thus leading to intrathalamic feed-forward inhibition. To directly investigate this
235 possibility, we next simultaneously recorded thalamic and somatotopically aligned (Online
236 Methods) putative pyramidal neurons in the S1pO cortical region of 3 GAERS (see
237 Supplementary Fig. 9 for cortical neuron isolation and classification). Multiple pairs (13/58)
238 of TC and cortical excitatory neurons showed two peaks in their ictal total firing XCors,
239 suggesting bidirectional excitation (Fig. 6a). XCors that only included cortical burst firing (Fig.
240 6a) had a larger post-zero peak compared to those of tonic (Supplementary Fig. 10a) and total
241 firing (Fig. 6a), indicating enhanced cortical to TC neuron excitation when cortical neurons
242 fired bursts. Conversely, TC burst firing XCors had almost no post-zero component (Fig. 6a),
243 indicating that cortical excitation selectively drives TC neuron tonic firing. Moreover, XCors
244 associated with the largest SWC-spikes (i.e. 4th quartile of amplitude) showed enhanced TC-
245 cortex and cortex-TC excitation compared to those with smaller SWC-spikes (i.e. 1st quartile)
246 (Fig. 6d,e) (Supplementary Fig. 10b), indicating that TC and cortical neurons are more
247 effectively recruited during cycles with higher cortical synchrony. Notably, the gain in strength
248 of the cortex-TC interactions was higher than that of TC-cortex interactions (Fig. 6f), indicating
249 that increased SWC-spike amplitude is accompanied by enhanced cortical (over thalamic)
250 influence on network dynamics.

251 XCors of pairs of simultaneously recorded NRT and cortical neurons had only post-zero peaks
252 for any type of firing, indicating a clear excitatory drive from cortical to NRT neurons, which
253 was strongest when cortical neurons fired bursts (Fig. 6b) and elicited both tonic and burst
254 firing in NRT neurons (Fig. 6b; Supplementary Fig. 10c). Notably, the probability of NRT
255 neuron firing was higher in the 10 ms periods after a cortical than a TC neuron action potential,
256 indicating that the cortex is a more significant source of ictal NRT excitation (Fig. 6c).

257 In summary, during ASs i) bi-directional excitation occur between cortical and TC neuron
258 populations, ii) cortical drive preferentially elicits tonic and not burst firing in TC neurons, and
259 iii) cortical firing is evoked by both tonic and burst firing of TC neurons. Finally, the presence
260 of stronger excitatory drive from cortex to NRT neurons than from TC to NRT neurons
261 suggests that the intra-thalamic inhibition recruited ictally (Fig. 5a-c) most likely represents a
262 cortex-dependent feed-forward mechanism.

263

264 **TC neuron T-channels do not contribute to ictal thalamic output synchrony**

265 Since the above results do not support a thalamo-cortical pacemaker mechanism involving TC
266 neuron T-channel-mediated burst firing^{7,10,11,13}, we directly tested the contribution of these
267 channels to the ictal TC neuron output. T-channels were blocked by administration of the
268 selective antagonist TTA-P2²⁷ via reverse microdialysis *unilaterally* into the VB of freely
269 behaving GAERS while simultaneously recording single TC neuron activity from an adjacent
270 silicone probe. TTA-P2 (300 μ M in the inlet dialysis probe) abolished high-frequency bursts
271 in all recorded TC neurons (n=7) during ASs as well as sleep, but tonic firing persisted during
272 ASs and wakefulness, though reduced by $57\pm 6\%$ and $33\pm 3\%$ of pre-drug rates ($p < 0.01$ and
273 $p < 0.05$, respectively) (Fig. 7a,b). Significantly, following TTA-P2-elicited block of bursts, TC
274 neuron total and tonic firing distributions expressed peaks with similar strength of phase
275 preference and time relative to the SWC-spike as before antagonist application (Kuiper test,
276 $n=7$, $p > 0.05$) (Fig. 7c,d).

277 These data demonstrate that TC neurons are capable of delivering strong synchronous output
278 to the neocortex during ASs even when their T-channels (and burst firing) are fully blocked
279 and, notably, that these channels are not required for dictating the ictal firing time of TC
280 neurons.

281

282 **Cortical and NRT but not TC neuron T-channels are essential for ASs**

283 Though the above results indicate that TC neuron T-channels are not necessary for the
284 synchronized ictal thalamic output to neocortex, they do not obviate a critical role for these
285 channels in ASs. Thus, we next applied TTA-P2 *bilaterally* by reverse microdialysis in freely
286 moving GAERS using a method that had established the spatio-temporal profile of TTA-P2-
287 mediated full block of thalamic T-channels²⁸ (Online Methods). Specifically, our microdialysis
288 dose-response and time-response analyses (see Fig. 3 in Ref. 28) showed that TTA-P2 applied
289 at a concentration of 300 μ M from a probe positioned in the center of the VB abolishes (in ~
290 20 minutes) T-channel bursts within a volume that entirely encompasses this thalamic nucleus
291 without affecting NRT neuron T-channels. Blocking T-channels throughout the VB with 300
292 μ M TTA-P2 in 8 GAERS using the above protocol did not affect ASs (Fig. 7e,f)
293 (Supplementary Fig. 11). In contrast, extending the block of T-channels to the NRT in 6 other
294 GAERS by increasing TTA-P2 concentration (and thus affected tissue volume²⁸) to 1 mM
295 decreased ASs (Fig. 7e,f). Since in the latter experiment TTA-P2 would have also spread

296 medially to the posterior nucleus, we then administered 300 μ M TTA-P2 750 μ m laterally from
297 the centre of the VB in 6 other GAERS, thus affecting the NRT and avoiding the posterior
298 nucleus. In this case too, the number and total duration of ASs were decreased by 50% (Fig.
299 7e,f) (Supplementary Fig. 11). In a different group of GAERS (n=9) we inserted the probe 750
300 μ m more medially from the centre of the VB, thus fully avoiding the spread of TTA-P2 into
301 the NRT. In this case, 1 mM TTA-P2 had no effect on ASs (Fig. 7e,f) (Supplementary Fig. 11).

302 Recent studies showing that brain-wide genetic knock-out or enhancement of CaV3.1 channels
303 (the main T-channel subtype present in TC neurons²⁹) abolishes³⁰ or induces³¹ ASs,
304 respectively, have been interpreted as suggesting a necessary role of TC neuron T-channels.
305 Since CaV3.1 channels are also heavily expressed in neocortex²⁹, we tested the effect on ASs
306 of their antagonism by TTA-P2 in the latter region. TTA-P2 (1 mM) applied by bilateral
307 microdialysis in the S1pO (i.e. the initiation site of genetic rat SWDs^{22,23}) of 16 GAERS
308 decreased ASs by 36% (Fig. 7b) (Supplementary Fig. 11).

309 Together with T-channel-mediated burst firing, GABA_B receptors of TC neurons were
310 suggested to be a key determinant of the temporal dynamics of TC neuron firing that underlie
311 ictal thalamic rhythmogenesis and determine SWD frequency^{10,12,13}. Thus, we next investigated
312 the effect of bilateral microdialysis (in the centre of the VB) of the GABA_B antagonist
313 CGP55845 at sub-maximal concentration (500 μ M). This drug markedly suppressed the total
314 time spent in ASs (aCSF: 87.0 \pm 18.3%; CGP55845: 5.69 \pm 1.4% of control, n=10 GAERS, p =
315 0.0156, Wilcoxon paired signed-rank two-sided test), as previously shown for other GABA_B
316 antagonists^{8,19}, but had no effect on the frequency of the SWDs of the remaining seizures
317 (aCSF: 6.88 \pm 0.1 Hz; CGP55845: 6.74 \pm 0.1 Hz, p=0.5781, n=7, Wilcoxon paired signed-rank
318 two-sided test).

319 In summary, these results demonstrate that T-channels of NRT and cortical, but not TC,
320 neurons are necessary for the expression of behavioural ASs.

321

322 **Top-down excitation enables ictal TC neuron recruitment in the absence of burst firing**

323 To test the neuronal mechanism of thalamic firing during ASs suggested by the experimental
324 results, we used a simple biophysical network model (Fig. 8a) (Online Methods)
325 (Supplementary Table 3). It includes one TC neuron that is connected only via GABA_A
326 synapses to two NRT neurons, one that preferentially fires bursts and one that has both tonic

327 and burst firing (cf. Fig. 3g) due to a more depolarized membrane potential (Fig. 8a,b). Two
328 cortical pyramidal neurons are connected to the NRT neurons, and reciprocally to the TC
329 neuron, via AMPA synapses (Fig. 8a). Simulations were driven by trains of 5 EPSPs delivered
330 only to the cortical neurons at 7 Hz (Fig. 8a,b), i.e. the frequency of GAERS SWDs¹⁹.

331 The model (Supplementary Fig. 12a) reproduced the TC and NRT neuron firing distributions
332 observed experimentally (Fig. 4a). Notably, the firing peak of the more depolarized NRT
333 neuron occurred earlier than that of the NRT neuron that preferentially fired bursts
334 (Supplementary Fig. 12a), and was coincident with the TC neuron peak firing when NRT-TC
335 synapses were blocked (Supplementary Fig. 12c). Moreover, increasing the strength of TC-
336 NRT synapses (up to 6-fold higher than the CX-NRT synapses) did not affect the firing
337 distribution (Supplementary Fig. 12b). By contrast, removing cortical-to-NRT synapses
338 (Supplementary Fig. 12f) or T-channels in NRT neurons (Supplementary Fig. 12h) resulted in
339 deviation of the distributions from both the simulated control conditions (Supplementary Fig.
340 12a) and the experimental results (Fig. 4a). Furthermore, removing inhibitory NRT-to-TC
341 synapses significantly broadened the peak of the TC firing distribution ($k=2.03$ vs $k=3.59$,
342 $p<0.001$; Kuiper test) (Supplementary Fig.12c), supporting the sharpening effect of NRT
343 inhibition on thalamic output.

344 XCors of the simulated firing closely reproduced those observed experimentally, notably in the
345 presence of a trough in TC-NRT XCors (compare Fig. 8c and 5a) and in the occurrence of two
346 peaks in the TC-CX XCors (compare Fig. 8c and 6a). Similar to the simulated firing
347 distributions, the various XCors were not qualitatively affected by a large increase in TC-to-
348 NRT synapse strength (compare Fig. 8c and d) or removal of TC T-channels (Fig. 8i). As
349 expected, removing TC-to-CX (Fig. 8f) or CX-to-TC (Fig. 8g) synapses abolished the peak
350 before or after time-zero, respectively. Removing NRT-to-TC inhibition (Fig. 8e), CX-NRT
351 excitation (Fig. 8h), or NRT T-channels (Fig. 8j) also generated XCors lacking key features of
352 simulated control conditions and experimental results: the post-zero trough in TC-NRT, the
353 post-zero peak in NRT-CX, and the narrow peaks in all XCors, respectively.

354 In summary, these simulations confirm that i) fast GABA_A-mediated NRT-to-TC inhibition is
355 sufficient to sharpen TC neuron ictal firing, ii) a strong TC-to-NRT excitatory connection is
356 not required to explain the ictal firing of NRT neurons, and iii) bidirectional excitation between
357 TC and cortical neurons coupled with cortical drive of NRT neurons can explain the

358 experimentally observed ictal interactions between the two thalamic neuronal populations and
359 among cortical and thalamic neuronal assemblies.

360

361 **DISCUSSION**

362 Neuronal ensemble activity of single thalamic and cortical neurons demonstrates synchronous
363 bidirectional cortico-thalamo-cortical communication during ASs in behaving animals. The
364 cortical and NRT components of this activity are T-channel-dependent, and required for AS
365 expression. The TC neuron population contributes synchronous volleys of, mainly tonic, firing
366 that are driven by cortical excitation and framed by, mainly feed-forward, NRT-mediated
367 inhibition.

368

369 **Thalamic firing dynamics**

370 The disagreements between our findings and previous studies indicate that the use of brain
371 slices^{10,11} or anesthetized/fentanyl conditions^{14-16,23} result in different TC and NRT neuron ictal
372 firing dynamics than those of behavioural ASs. Specifically, our results show that during ASs,
373 total firing of TC neurons is markedly decreased as well as temporally framed within each
374 SWD cycle, and that the synchrony and strength of TC ictal output to the neocortex is not a
375 feature of single, rhythmically bursting TC neurons but is an emergent property of this
376 population, as suggested originally by Buzsaki³². This contrasts with the conclusions of a
377 recent study³³, in which behavioural ASs were induced and suppressed by switching TC firing
378 between a rhythmic and non-rhythmic mode, respectively, with the former mode's synchrony
379 being indirectly attributed to T-channel-dependent bursts on the basis of *in vitro* optogenetic
380 experiments. Although the significance of thalamic synchrony to ASs is not contradicted by
381 our present work, the cellular or synaptic cause of that synchrony would not have been
382 detectable at the level of multi-unit activity used by Sorokin et al.³³. Furthermore, even if one
383 were to assume that TC neuron T-channel-dependent bursts were responsible for that study's
384 induction of ASs, this does not imply that they are an inevitable and necessary component of
385 the paroxysmal oscillation. In fact, when single-cell resolution is achieved, as in our current
386 work, ictal thalamic output synchrony is shown to be reliant not upon T-channels, but rather
387 upon excitation and feed-forward inhibition of cortical origin.

388 The ictal reduction of total firing in TC neurons of both GAERS and GHB models may result
389 from their increased tonic GABA_A inhibition^{34,35}. Such increased tonic GABA_A inhibition has
390 also been shown to prolong the decay of GABA_A IPSPs evoked in TC neurons by NRT neuron
391 single action potentials or bursts³⁶, which could explain the ability of NRT burst-induced IPSPs
392 to completely suppress TC output through most of the SWD cycle. Considering that tonic

393 inhibition provides 90% of GABA_A inhibition in TC neurons³⁷, the gain-of-function of TC
394 neuron extrasynaptic GABA_A receptors in epileptic animals³⁴ is sufficient to explain the
395 electrical silence, the marked decrease of tonic firing and the paucity of rebound bursts
396 observed ictally in GAERS TC neurons. This, in turn, may provide the thalamic basis for the
397 decreased response to sensory stimulation during ASs^{8,9}.

398 The peak in TC neuron burst firing at the start of ASs may result from the time-dependent
399 plasticity that is known to occur at both excitatory cortical-TC and inhibitory NRT-TC
400 synapses: optogenetic stimulation of cortico-thalamic afferents at 10 Hz (a frequency similar
401 to rodent SWDs) initially evokes in VB TC neurons a synaptic sequence of a small inward-
402 large outward current, which transforms into a stable large inward-small outward current within
403 the first second of stimulation³⁸.

404

405 **Role for T-channels**

406 Our findings question the view that TC neuron burst firing is critical for ASs^{7,10,12,13}. First, the
407 unaltered synchrony of ictal thalamic output to the neocortex during a block of TC burst firing
408 demonstrates that these channels do not determine SWD frequency, as was previously
409 proposed^{7,10,11}. The lack of effect on SWD frequency of blocking GABA_B receptors in TC
410 neurons further contradicts the mechanistic hypothesis in which synaptic GABA_B receptor
411 current and cell-intrinsic T-channel current interact to determine the timing of TC neuron firing
412 and, thus, SWD frequency^{10,12,13}. The partial block of thalamic tonic firing output by TTA-P2
413 may be explained by the small but significant contribution of T-channels to tonic firing and the
414 input-output function of TC neurons at depolarized membrane potentials because of the
415 physiologically significant fraction of T-channels that are de-inactivated at these potentials³⁹.
416 However, we cannot exclude that some single spikes included in our tonic firing classification
417 may be generated by a low-threshold spike.

418 Second, the lack of effect on ASs of TTA-P2 applied in the VB demonstrates that T-channels
419 of this nucleus, somatotopic to the cortical initiation site^{22,23}, are not required for the expression
420 and maintenance of these seizures. By contrast, sleep spindles, a cortico-thalamo-cortical
421 network oscillation with a well-established thalamic rhythmogenesis^{5,6}, are abolished when T-
422 channels of VB TC neurons are blocked by an identical TTA-P2 microdialysis application²⁸ as
423 in the current work.

424 Notably, the reduction in ASs following the block of T-channels in cortical neurons indicates
425 that bursts in the neocortex (and possibly other regions, that could include non-sensory
426 thalamic nuclei) underlie the effects on ASs following brain-wide genetic manipulations of
427 CaV3.1 channels^{30,31}. Meanwhile, the marked reduction in ASs following the block of NRT
428 neuron T-channels confirm that CaV3.2 and/or CaV3.3 channels, the two T-channel subtypes
429 present in these neurons²⁹, play a key role in genetically determined ASs, as indicated by
430 CaV3.2 channel mutations in GAERS NRT neurons⁴⁰ and human absence epilepsy cohorts⁴¹.
431 Notably, however, GHB-induced ASs are resistant to the knockout of both CaV3.2 and CaV3.3
432 channels⁴².

433

434 **Ictal cortico-thalamo-cortical interactions**

435 Our results provide direct evidence that NRT-TC inhibitory short-term assemblies become
436 engaged ictally in a manner that is critically dependent on NRT bursts and fast GABA_A
437 synapses. This is indicated by the lack of NRT-TC inhibition in XCors that considered only
438 NRT neuron tonic firing, and the close similarity between experimental XCors of TC and NRT
439 neuron ictal firing and those simulated using only GABA_A synapses. Thus, fast NRT inhibition
440 frames ictal TC neuron firing, and the higher coherence of NRT neuron burst firing that occurs
441 just after the SWC-spike is likely to reflect sharpening of the TC neuron response by these
442 bursts.

443 The lack of a sharp peak in TC-NRT neuron XCors does not suggest an ictal, strongly active
444 monosynaptic connection between TC and NRT neuron pairs, and modelled ictal firing and
445 interactions were not altered by varying TC-NRT synapse strength. This absence of a major
446 involvement of TC-NRT excitatory synapses during ictal firing interactions may be an
447 unexpected finding since this connection has been reported to be strong and reliable in juvenile
448 rats⁴³. However, in adult animals tonic TC neuron firing at 40 Hz evokes only small EPSPs in
449 NRT neurons, whereas TC bursts evoke larger NRT EPSPs (Fig. 9 in Ref. 44). In view of this,
450 and of our observed ictal 6 Hz tonic and 1 Hz burst ictal firing rates, relatively weak (but not
451 absent) ictal TC-NRT compared to cortex-NRT excitation is not surprising. Indeed, optogenetic
452 stimulation of cortico-thalamic afferents is not highly effectively in activating the TC-to-NRT
453 pathway *in vitro*⁴⁵, supporting the lower probability of ictal NRT firing following TC than
454 cortical neuron firing that we observed.

455 In summary, our findings show that ASs do not rely on widespread TC neuron bursts but rather
456 cortico-thalamic inputs exciting both TC and NRT neurons, and thalamic feed-forward
457 inhibition. Thus, the most parsimonious mechanism of a SWD cycle involves reciprocal
458 excitation of TC and cortical neurons, as well as excitation of NRT neurons mainly by cortico-
459 thalamic neurons. The precise timing of this excitation may depend on intra-cortical
460 rhythmogenic mechanisms as well as cortico-thalamic interactions. NRT neuron firing then
461 temporally frames TC neuron output by suppressing firing throughout most of the spike-wave
462 cycle. The framed TC output may help to re-engage, and synchronize, the cortex in the next
463 cycle, but rebound bursts are not a necessary part of this output, contrasting with thalamic slice
464 and *in silico* models^{7,10-13}. In other words, the temporal precision of TC neuron ictal firing is
465 not dictated by the interaction of T-channel activation/inactivation and GABA_A/GABA_B IPSP
466 dynamics^{10,12,13} but rather by the interplay of excitatory cortico-thalamic and fast feed-forward
467 NRT inhibitory drives, which are known to shape cortico-thalamo-cortical activity in other
468 behavioural contexts^{38,45,46}. On the other hand, the interplay of cortico-thalamic input and cell-
469 intrinsic mechanisms (including T-current and Ca²⁺-activated currents⁹) underlie NRT neuron
470 ictal excitation.

471

472 **Significance for human ASs**

473 The strong similarities in firing dynamics and neuronal interactions of ASs between a polygenic
474 (GAERS) and a pharmacological (GHB) model suggest that similar mechanisms may underlie
475 generalized non-convulsive seizures in other models with spontaneous or induced single-gene
476 mutations⁴⁷. Nevertheless, selective impairment of cortex-to-NRT transmission, leading to a
477 reduction of feed-forward inhibition of TC neurons, can induce ASs⁴⁸, and it would be of
478 interest to investigate the ictal mechanism of human R43Q knock-in mice that, in contrast to
479 the GAERS and GHB models, have decreased tonic GABA_A inhibition in TC neurons⁴⁹. These
480 different models undoubtedly offer useful insights on the mechanisms underlying the large
481 diversity in SWD waveform and AS severity encountered in childhood and juvenile absence
482 epilepsy patient⁵⁰.

483

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608

609 **ONLINE METHODS**

610 Experiments were approved under the UK Animals (Scientific Procedures) Act 1986 by Cardiff
611 University Animal Welfare and Ethics Committee and were conducted in accordance with
612 current recommendations for experimental work in epilepsy⁵¹. All data will be made available
613 on reasonable request by contacting one of the corresponding authors. Further details of
614 experimental design, software, materials, reagents, animal research participants, and data
615 availability can be found in the Life Sciences Reporting Summary.

616 No statistical methods were used to pre-determine sample sizes, but our sample sizes are similar
617 to those reported in previous publications^{25,34}. In the case of bilateral microdialysis
618 experiments, animals were randomly assigned to receive drug or control first. Experimenters
619 were blind to the group of animals when detecting time spent in seizure. Animals were excluded
620 from microdialysis experiments only in the case of visible distress, and neurons were excluded
621 from ensemble recording experiments if the reconstructed electrode position lay outside the
622 VB/NRT.

623 **Surgery and implantation**

624 Male GAERS and Wistar rats (both 4-7 month old), with access to food/water *ad libitum*, were
625 maintained on a 12:12 hour light:dark cycle (light on at 08:00 am). Surgery anesthesia was
626 maintained with isoflurane and body temperature with a homoeothermic heat blanket. EEG
627 gold-plated screws (Svenska Dentorama, UK) were implanted in fronto-parietal sites.

628 For ensemble unit recordings, four-shank, linear, 32-site silicon probes (Buzsaki32L,
629 NeuroNexus, Michigan, USA) were immersed in detergent at 60°C for 2-4 hours, before being
630 rinsed with deionized water and immersed in Vybrant Dil dye (Invitrogen) solution prior to
631 insertion (Supplementary Fig. 1c). Each silicon probe was lowered into the VB (AP: - 3.35
632 mm, ML: 2.9 mm)⁵², such that its lower end rested 4mm ventral from the brain surface, i.e. at
633 the top of the VB. A microdrive was then secured with grip cement that was also used to create
634 a mini-Faraday cage and protective structure of copper mesh⁵³. When an additional silicon
635 probe was implanted in S1pO cortex, its coordinates were AP: - 0.0 mm, ML: 5.5 mm) and its
636 tip was initially positioned in cortical layer 2/3.

637 For experiments with bilateral microdialysis, two guide cannulae for CMA 12 microdialysis
638 probes (Linton Instruments, UK) were lowered into the centre of the VB (VB (c) in Fig. 7b),
639 such that their tips rested at AP: -3.35 mm, ML: 2.8 mm, DV: -4.4 mm. To target the NRT,

640 probes were positioned adjacent to but not within the NRT so to avoid excessive damage of
641 this narrow nucleus (AP: -3.2 mm, ML: 3.5 mm, DV: -4.2 mm). The coordinates for the probes
642 positioned more medially than the centre of the VB (VB (m) in Fig. 7b) were AP: -3.35 mm,
643 ML: 2.1 mm, DV: -4.4 mm, and those targeting S1pO cortex were AP: -3.2 mm, ML: 5.5 mm,
644 DV: -0.5 mm.

645 Simultaneous unilateral implantation of a silicon probe and a microdialysis cannula was similar
646 to the one described above, with the microdialysis cannula being lowered into the brain at a
647 16° angle with respect to the vertical axis in the rostro-caudal plane such that its tip rested close
648 to the silicon probe (AP -3.5 mm, ML 2.8 mm, DV -6 mm²⁰).

649 All electrical and microdialysis components were affixed to the skull with methacrylic
650 cement, and the final cap structure incorporated all electrode wires and microdialysis guide
651 cannulae. Rats were allowed to recover for ≥ 5 days.

652

653 **Behavioral experiments**

654 For all behavioral experiments, each animal was placed individually in a purpose-built
655 plexiglass box housed within a Faraday cage. Animals were continuously monitored
656 throughout a procedure by an experimenter and by video recordings (using Spike2 video
657 software, CED, Cambridge, UK) to precisely time-match EEG and neuronal ensemble
658 recordings with behavior (see below). A counterweighted swivel arm positioned above the
659 recording box allowed free movement of the animal while connected to electrical and
660 microdialysis equipment.

661 *Neuronal ensemble recordings*

662 The head-mounted Plexon HST/32V-G20 VLSI-based preamplifier was connected to a Plexon
663 data acquisition system (sampling rate: 20 kHz). Online filtering was used to visualize the raw
664 signal as either LFP (low-pass filter at 300 Hz) or as spiking/unit activity (high-pass filter at
665 500 Hz). Upon connection to the recording apparatus, the presence or absence (on any
666 channels) of neurons suitable for recording was assessed based on the shape and amplitude of
667 any visible spikes. If no such neurons were present after 30 minutes of observation, the silicon
668 probe was lowered deeper into the brain by turning the microdrive, while recording the number
669 and degree of turns of the microdrive screw to allow post-hoc reconstruction of the recording
670 site (see below). A similar procedure was used for simultaneous recordings of thalamic and
671 cortical neurons.

672 For GAERS, each recording session lasted no more than 3 hours depending on the quality and
673 stability of the recorded neurons and the behavior of the animal during the session. In the GHB
674 model (100 mg/kg i.p. of γ -butyrolactone, a GHB pro-drug), recordings were limited by the
675 time course of the drug (about 1 hour). GAERS sessions included multiple bouts of non-REM
676 sleep, which were used to characterize the properties of the T-channel mediated high-frequency
677 bursts of each recorded thalamic neuron for subsequent comparison with the high-frequency
678 bursts recorded from the same neuron during multiple ASs (see below).

679

680 *Bilateral reverse microdialysis*

681 Dummy probes within the guide cannulae were replaced with CMA 12 microdialysis probes
682 (Linton Instruments, UK) 18 hours prior to the start of a recording session. For GAERS, each
683 experiment involved 1 hour habituation of the animal to the recording environment after the
684 inlet tubes of the microdialysis probes had been connected (via FEP tubing, 0.18 μ L/cm internal
685 volume, Linton Instruments, UK) to a 1 mL syringe placed in a 4-channel microdialysis pump
686 (CMA 400, Linton Instruments, UK). The second hour consisted of EEG recording during
687 reverse microdialysis of artificial cerebrospinal fluid (aCSF) at a rate of 1 μ L/min. At the end
688 of the second hour, either reverse microdialysis of aCSF was continued or a solution containing
689 TTA-P2 (300 μ M or 1 mM) or CGP-55845 (500 μ M) was administered via different syringes
690 for an additional 80 min.

691 A minimum delay of 6 days occurred between subsequent experiments to allow wash-out of
692 the drug. Each animal was only recorded twice, once with aCSF and once with TTA-P2
693 administration, and was randomly allocated to receive first aCSF or TTA-P2. The effect of
694 TTA-P2 was quantified during the 40-80 min period (“treatment hour”) (Fig. 7e) from the start
695 of its microdialysis since our previous experiments under similar conditions had shown that 20
696 min are necessary to achieve a steady block of TC neuron burst firing throughout the required
697 extent of the VB (see ref. 37 for additional information). Effects of microdialysis-applied TTA-
698 P2 on ASs are reported as percentage of the aCSF treatment (Fig. 7e,f).

699

700 *Simultaneous neuronal ensemble recordings and unilateral reverse microdialysis*

701 Silicon electrodes were employed as described earlier for neuronal ensemble recordings.
702 Microdialysis probe cannulae were inserted as described above under surgical procedures, in
703 order that the membrane of the microdialysis probe would extend beneath the VB thalamus,
704 allowing the silicon electrode to be moved gradually towards the membrane by microdrive

705 adjustment²⁰. Upon identification of suitable and stable neurons, a recording control period was
706 carried out before 300 μ M TTA-P2 was administered. Neuronal firing dynamics were
707 investigated as described below for the periods before and after full block of bursts by TTA-
708 P2 had been detected.

709

710 **Data analysis**

711 *AS detection and SWDs analysis*

712 The EEG was recorded using an SBA4-v6 BioAmp amplifier (SuperTech Inc., Hungary),
713 digitized at 1 kHz (by a Cambridge Electronic Design, CED, Micro3 D.130) and analyzed with
714 CED Spike2 v7.3 and Matlab (R2011b, The Mathworks Inc., USA). SWDs were identified
715 using the SeizureDetect script (Spike2, CED, Cambridge, UK). Firstly, baseline EEG
716 (desynchronized, active or resting wakefulness) was identified manually and voltage amplitude
717 thresholds set at 5-7 standard deviations (SD) above or below the mean. Secondly, the
718 following parameters for initial event detection were applied to all points that crossed this
719 threshold (crossings): maximum time between initial two crossings of 0.2 s, maximum time
720 between any two crossings within an event of 0.35 s, minimum of 5 crossings per event,
721 minimum time of 0.5 s between any two events for them to count as separate (otherwise
722 overriding 0.35 s limit and merging events), and a minimum event duration of 1 s. The final
723 component of the SeizureDetect script gated detected events by dominant frequency band
724 (calculated by time intervals between crossings rather than by spectrographic analysis). Events
725 with >75% of intervals within the 5-12 Hz range were classified as SWDs. Designated SWDs
726 were then visually inspected to ensure the above parameters and the signal-to-noise ratio were
727 appropriate for effective identification and isolation. All EEG sections identified as SWDs were
728 then crossed-checked with the corresponding video recordings: those that showed the
729 concurrent presence of behavioral arrest were then classified as ASs, while the very few that
730 did not were excluded from further analysis.

731 *Behavioral state classification*

732 EEG recordings were divided into one of three behavioral states: non-REM sleep, wakefulness,
733 and ASs. Isolation of ASs (i.e. concomitant presence of behavioral arrest and SWDs in the
734 EEG) was carried out as detailed above. Detection of non-REM sleep periods, from the sections
735 of the EEG and video recordings that did not contain ASs, was achieved by plotting cumulative
736 power over the 0.5 to 4 Hz frequency range of the EEG signal, and setting a threshold at 2.5

737 SD above the baseline value during a visually identified period of wakefulness, and post-hoc
738 analysis of the video recordings. The remaining time (i.e. neither ASs or non-REM sleep) was
739 classified as wakefulness upon confirmation of a desynchronized (low amplitude, high
740 frequency) EEG. Note that ASs only developed during wakefulness, and thus the pre-ictal
741 periods used for the analysis of interictal-to-ictal transitions only included wakefulness. Periods
742 of non-REM sleep were extremely rare in GHB injected Wistar rats due to the relatively short
743 duration (about 1 hour) of the recording period.

744 *Action potential sorting*

745 Extraction of action potentials was performed with different subroutines^{20,24}. The signal was
746 high-pass filtered and thresholded to extract action potential shapes (Fig. 1b) (Supplementary
747 Fig.1a), using the first 3 principal components of each action potential on each channel (8
748 channels per group for the Buzsaki32L probe) as features. The first stage of clustering used the
749 unsupervised KlustaKwik program⁵⁴ to group action potentials with similar features into
750 clusters based on a Classification Expectation Maximization (CEM) algorithm⁵⁵⁻⁵⁹ (Fig. 1a).
751 The second stage involved supervised refinement of appropriate clusters and elimination of
752 unsuitable clusters. Action potential waveforms were constant and distinct during non-REM
753 sleep and wakefulness but some would at times be insufficiently large, relative to the high
754 frequency multi-unit noise observed at each SWC cycle, to sort during ASs. In this case the
755 entire cluster was eliminated.

756 *Neuron and firing pattern classifications*

757 Thalamic neurons

758 Isolated action potentials recorded from locations outside the VB/NRT region were excluded
759 for further analysis. The remaining action potentials were classified as TC or (fibers belonging
760 to) NRT neurons²⁵ on the basis of i) the properties of their T-channel dependent high frequency
761 bursts during non-REM sleep (Supplementary Fig. 1e), ii) the action potential half-width (Fig.
762 1d) and iii) the ratio of the first to the shortest interspike interval (ISI) (Fig. 1d). A high
763 frequency burst was defined as three or more spikes with an ISI of ≤ 7 ms that were preceded
764 by a silent interval of ≥ 100 ms. NRT neurons were classified as those with an acceleration
765 index (ratio of first to shortest ISIs in a burst) greater than 1.4, while TC neurons have a ratio
766 < 1.3 (Refs. 60,61) (Fig. 1d). Moreover, NRT neurons tended to exhibit a smaller spike half-
767 width than TC neurons (Fig. 1d). These properties are in agreement with the previously
768 reported “accelerando-decelerando” pattern of NRT bursts^{60,61} (Fig. 1c2) (Supplementary Fig.

769 1d) and the sharper waveform of the action potentials of inhibitory compared to excitatory
770 neurons^{25,62} (Fig. 1d, inset).

771 Total firing reported in the text and figures for both types of thalamic neurons includes all
772 sorted spikes, while tonic firing includes all spikes separated by two ISI > 7 ms in order to
773 discard spike potentially belonging to bursts. Action potential doublets which did not fall into
774 the tonic firing classification were post-hoc included in all burst firing calculation because of
775 the marked similarity of their temporal dynamics before, during and after ASs with bursts of \geq
776 3 action potentials (Supplementary Fig. 1e). A proportion of spikes could not be conclusively
777 classified as being either burst or tonic firing, and amounted to the following percentages:
778 GAERS TC neurons: $10.0 \pm 8.9\%$, wake-active NRT neurons: $4.1 \pm 0.8\%$, wake-quiescent
779 NRT neurons: $2.4 \pm 1.5\%$; GHB TC neurons: $2.48 \pm 2.27\%$, wake-active NRT neurons: $9.7 \pm$
780 3.5% , wake-quiescent NRT neurons: $3.7 \pm 0.4\%$. These spikes, however, were all included in
781 the total firing.

782 Burst, tonic and total firing rates in each of the three behavioral conditions, as well as during
783 transitions between conditions, were calculated across entire recording sessions
784 (Supplementary Table 1). Transitions from wakefulness to seizure and vice versa were
785 investigated by plotting mean activity rates (\pm SEM) 5 s before and after seizure start and end
786 times, respectively. Only seizures of ≥ 5 s (for GAERS) or ≥ 2 s (for GHB) duration with
787 preceding/following (as appropriate) wake periods of at least the same length were selected.

788 Cortical neurons

789 Isolated cortical neurons were classified as putative excitatory cells and distinguished from
790 putative inhibitory cells on the basis of their trough-to-peak times and spike half-widths⁶²
791 (Supplementary Fig. 9). Functional somatotopic alignment of the thalamic and cortical
792 regions were verified by sending brief air puffs to the perioral zone of the GAERS rats. The
793 majority of neurons in both brain regions responded within 10 ms to this sensory stimulation.

794 Neuron-neuron and neuron-EEG relationships

795 These were investigated during each behavioral state, with neuron-SWC relationships also
796 studied during ASs. Auto- and cross-correlograms (XCors) were calculated using the xcorr
797 function of Matlab in 1-ms bins. SWC spike-triggered EEG averages were calculated by taking
798 mean values of ictal total, tonic and burst rates (burst time taken as time of first spike in a burst)
799 across entire recording sessions, and then averaging across all neurons of the same type (TC,

800 WA NRT, WQ NRT). SWC spike-triggered firing averages used SWC spike as reference. This
801 involved finding all local amplitude maxima more than 3 SD above the mean EEG amplitude
802 and then selecting the largest of these maxima within each SWC. Spike and burst rates were
803 averaged in 1 ms bins around these peaks. Other measures that quantified activity relative to
804 the SWC required definition of SWC epochs. These were extrapolated from spike peak
805 detections by selecting periods between adjacent peaks with intervals less than 300 ms. These
806 epochs were used to calculate per-SWC total firing, tonic firing and bursts across entire
807 recording sessions.

808

809 *Coherence*

810 Total firing, burst firing and tonic firing distribution were selected between -70 and +70ms
811 around the SWC spike time and normalized to get the density distribution binned per ms.
812 Vectors were then circularized by linearly converting the -70 to +70 ms interval into an angle
813 from $-\pi$ to π . The module of the average vector computed over all density values at each angle
814 bin was then estimated in the complex domain to give the coherence. This measure is
815 comparable to a spike-field coherence⁶³.

816

817 *SWC-spike amplitude*

818 The amplitude of the SWC-spikes was measured from the EEG recordings. Neuronal spikes
819 used for cross-correlations based on SWC-spike amplitudes were extracted from periods
820 around those SWCs of the smallest (1st) and largest (4th) quartiles (Fig. 6e,f, Supplementary
821 Fig. 10b,c). Analysis of the XCorrs were done for pairs of neurons that had an amplitude 20ms
822 around the time lag=0ms of at least 8 times the basal chance level found beyond 20ms away
823 from the lag=0. This ensured that thalamic and cortical neurons fire in a correlated manner for
824 the directional analysis. Twenty-four (out of 58) TC-cortical pairs satisfied this criterion. Then,
825 peak ratios were measured as ratios between 1) the maximum amplitudes corresponding to time
826 lags between -10ms and 0ms in the Xcors for the thalamo-cortical direction measurement and
827 to time lags between 0 and 10ms in the XCors for the cortico-thalamic direction measurement
828 for either the 1st or 4th quartile, and 2) the same measurement for all SWCs no matter their
829 amplitude. Those values were then normalized per SWC. The resulting peak ratios indicate
830 changes in the strengths of thalamo-cortical (TC) (Fig. 6d) and cortico-thalamic (CX) (Fig. 6e)
831 interactions with respect to changes in the amplitude of the SWC-spike. Lastly, a ratio
832 was computed between CX and TC unidirectional peak ratio (Fig. 6f).

833

834

835 **Histological processing**

836 For animals used in the microdialysis experiments, 1 μ L of thionine dye was administered via
837 both inlet and outlet channels of the microdialysis probes with stripped membranes using a 10
838 μ L Hamilton syringe in order to highlight the probes' tracks. At the end of all experiments,
839 animal received a lethal dose of Euthatal before being transcardially perfused with phosphate-
840 buffered saline (PBS, 0.9% NaCl) followed by paraformaldehyde (PFA, buffered in 0.9% NaCl
841 PBS). Microdialysis and/or silicon probes were then carefully withdrawn from the brain, which
842 was then immersed in 4% PFA for 4 hours. Brains were refrigerated in 0.9% NaCl PBS until
843 required. Brain sections around the region of interest were cut at a thickness of 100 μ m on a
844 Leica VT 1000S vibratome while immersed in chilled 0.9% NaCl PBS. For microdialysis
845 experiments, slices were photographed using a Nikon D90 camera (Nikon Imaging, UK) and
846 then inspected for actual site of recording. For neuronal ensemble recording experiments, slices
847 were photographed under an Olympus BX61 microscope at 4x magnification in brightfield and
848 with a TRITC filter. Images were merged using Adobe Photoshop (Supplementary Fig. 1c),
849 and the final position of the electrode tip was marked. The position of the recording site during
850 each session was then calculated from the noted microdrive movements.

851

852 **Drugs**

853 The aCSF for the microdialysis experiments was purchased from Tocris Biosciences (Bristol,
854 UK). 3,5-dichloro-N-[1-(2,2-dimethyl-tetrahydro-pyran-4-ylmethyl)-4-fluoro-piperidin-4-yl-
855 methyl]-benzamide (TTA-P2) was a kind gift from Merck Inc. (USA). TTA-P2 was dissolved
856 in DMSO (2% of final solution) before addition to aCSF. An identical concentration of DMSO
857 was added to the aCSF solutions. (2S)-3-[[[(1S)-1-(3,4-dichlorophenyl)ethyl]amino-2-
858 hydroxypropyl] (phenylmethyl) phosphinic acid hydrochloride (CGP-55845) was purchased
859 from Tocris (UK). The pH of all dialysis solutions was 7.

860

861 **Simulations**

862 Using Neuron software⁶⁴, a previously developed³⁹ single compartment conductance model of
863 a TC neuron was connected to two NRT neuron models⁶⁵ and to two cortical pyramidal neuron
864 (PY) models⁶⁶, which were also connected to the TC neuron and the two NRT neurons (Fig.
865 8a) (see Supplementary Table 3 for network description and synapse properties). T-type Ca^{2+}
866 channels had a conductance of 40 nS and a reversal potential of 120 mV in TC neurons. The

867 two NRT neurons received a different constant current injection in order to set their membrane
868 potential in a different range, thus favoring (NRT2) or not (NRT1) the emergence of T-channel
869 bursts. Single AMPA synapses from TC to NRT neurons and single GABA_A synapses from
870 NRT to the TC neuron were similar to those described in Ref. 65. AMPA synapses from TC to
871 CX, from CX to TC, and from CX to NRT were modelled as single exponential conductances
872 (ExpSyn object in Neuron with a decay time constant of 2 ms), with a transmission delay of 2
873 ms to represent inter-area communications. Their synaptic weights were either 0, when
874 connections were inactive, or as reported in Supplementary Table 3. All neurons also received
875 synaptic conductance fluctuating noisy inputs^{65,67,68}. The mean conductance of the synaptic
876 noise was set as 20% of g_{Leak} for excitatory noise and 80% of g_{Leak} for inhibitory noise for all
877 neurons³⁹. The SD deviation of the synaptic noise was set to 100%, 60%, and 150% of the
878 mean conductance for TC, NRT and PY neurons, respectively, in order to induce fluctuations
879 of spike timing that reflect those observed in the experimental data. The synaptic weight of the
880 cortico-thalamic EPSPs was adjusted to induce a spiking probability similar to that of our
881 experimental data (Supplementary Table 3). All neurons had T-type Ca²⁺ channels and thus
882 were able to generate rebound bursts after a hyperpolarizing pulse.

883 In addition to those basal conditions of the cortico-thalamic network described above, which
884 reflect the interictal neuronal state, the two PY model neurons received identical inputs
885 consisting of trains of 5 AMPA EPSPs (modelled as in Ref. 51). No other external input was
886 delivered to either the TC or the NRT neurons. The inter-EPSP interval within a train was
887 randomly drawn from a Poisson distribution with a mean of 3 ms. The inter-train interval was
888 randomly drawn from a Poisson distribution with a mean of 140 ms, which produced an
889 average frequency of 7 Hz, similar to the SWD frequency in GAERS. Twenty-second-long
890 simulations were repeated 40 times with different noise seeds. Simulated firing activity was
891 analyzed as the experimental data. Time-zero for the simulated firing distribution plots was
892 taken as the 3rd EPSPs of a train: selecting either earlier or later EPSPs in the train had no major
893 effects on the simulation results.

894

895 **Statistical analysis**

896 Paired Wilcoxon signed-rank test was used for the effect of TTA-P2 on TC neuron firing and
897 ASs. Circular statistics (Kuiper test and concentration factors) was used to compare phase
898 distributions across the SWC epoch (Fig. 7d and Supplementary fig12a,c). Kolmogorov-

899 Smirnov test was used for spike time distribution around the SWC spike (Fig 4). Normality
900 and equal variances were not assumed by the tests employed.

901 Permutations were used for non-parametric tests applied to compare experimental XCor to
902 XCor expected if spikes would occur at times drawn randomly from their distribution around
903 the SWC spike time (Figs. 5, 6, 8) (Supplementary Figs. 8, 10a). The same number of spike
904 time pairs as in the experimental XCor is drawn from the respective TC, NRT and cortical
905 neuron density distributions around the SWC-spike time. Computing their XCor allowed us
906 to generate a surrogate XCor where spikes are shuffled independently from each other but
907 respecting the probability distribution to find them at particular times around the SWC-spike
908 time. Repeating the process 1000 times allowed us to estimate the 5% and 95% limits
909 (confidence interval) for the surrogate XCor which could be directly compared to experimental
910 XCor. This method is more stringent than simple spike-time shuffling around their measured
911 times⁶⁹, since it takes into account specific spike-times associated to an external source of
912 synchronization which can generate peaks and troughs without any direct relationships between
913 neurons.

914

915 **Data and code availability**

916 All data included in this publication, and/or MatLab codes used for the analysis, will be made
917 available on reasonable request by contacting one of the corresponding authors.

918

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