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Thalamic inhibition: diverse sources, diverse scales

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Abstract

The thalamus is the major source of cortical inputs shaping sensation, action and cognition. Thalamic circuits are targeted by two major inhibitory systems: the thalamic reticular nucleus (TRN) and extra-thalamic inhibitory (ETI) inputs. A unifying framework of how these systems operate is currently lacking. Here, we propose that TRN circuits are specialized to exert thalamic control at different spatiotemporal scales. Local inhibition of thalamic spike rates prevails during attentional selection whereas global inhibition more likely during sleep. In contrast, the ETI (arising from basal ganglia, zona incerta, anterior pretectum and pontine reticular formation) provides temporally-precise and focal inhibition, impacting spike timing. Together, these inhibitory systems allow graded control of thalamic output, enabling thalamocortical operations to dynamically match ongoing behavioral demands.

31

32 **Introduction – General questions**

33

34 While several studies have elegantly delineated roles for thalamus in relaying sensory
35 inputs to the neocortex, the extensive reciprocal connections between thalamic nuclei and
36 all cortical regions suggest that the function of the thalamus extends well beyond sensory
37 processing and simple relay. Thalamic nuclei are integral to processes involving motor control
38 [1] memory [2] and arousal [3]. In one of the best studied cases, the visual thalamus, the
39 retinal signal experiences substantial transformation on its way to the cortex that involves
40 contrast- and context-dependent gain modulation [4] as well as temporal structuring [5].
41 Because these operations are prevalent across non-sensory systems as well, analogous
42 thalamic circuits and computations are likely to subserve multiple cognitive functions [6–8].

43 The aforementioned thalamic operations require highly complex inhibitory control.
44 Unlike cortex, striatum and cerebellum, the thalamus lacks a variety of interneuron types that
45 provides spatio-temporally diverse and precise GABAergic input to its projection neurons (see
46 Box 1). The best-studied source of thalamic inhibition derives from a thin sheet of cells, the
47 thalamic reticular nucleus (TRN) which innervates all its individual nuclei [9]. Although some
48 heterogeneity has been described among TRN neurons, their morphology and
49 neurochemistry appear to be much less diverse than those of cortical interneurons [10,11].
50 Nevertheless, thalamic operations are under similar constraints as those of the neocortex,
51 requiring inhibitory control across multiple spatial and temporal scales (see Box 1).

52 Given this challenge, *the central question* we pose here is *how does heterogeneity of*
53 *inhibition arise in the thalamus?* In other words what specialized GABAergic mechanisms
54 enable thalamic circuits to differentially process information streams in both space and time,
55 and according to ongoing behavioral demands?

56 In this review, we will discuss two putative solutions to this problem:

57

58 I Structural and physiological features of TRN will allow its circuits to shift the spatial
59 and temporal scales of inhibition in various behavioral conditions.

60 II Powerful ETI systems [12–16] will provide heterogeneous, nucleus-specific
61 inhibitory control of thalamus involved in a well-defined set of nuclei.

62 **We should note here that while many of the experimental data informing our view**
63 **of the thalamus here are derived from studies of rodent brains, we have tried to focus on**
64 **principles that are likely to be universal to mammalian thalamic function. In line with this**
65 **approach, we indicate when comparative data on rodents and primates (including humans)**
66 **are available.**

67 **We also note that thalamic interneurons as a third form of inhibition in the thalamus**
68 **are outside the scope of the present account. These cells are found in variable numbers and**
69 **distributions across distinct mammalian species [17]. Outside of their function in vision (as**
70 **local spatial contrast enhancement elements [4]), there is very little information about their**
71 **role in other parts of the thalamus. From our perspective their peculiar anatomical**
72 **connectivity pattern (forming dendro-dendritic contacts in triadic arrangements) represent**
73 **a spatially highly restricted form of inhibition acting on a single excitatory input. This may,**
74 **therefore, add yet another level of complexity to thalamic inhibition.**

75

76 **The thalamic reticular nucleus**

77 *General overview*

78 The thalamic reticular nucleus (TRN) is a shell of GABAergic neurons that covers the
79 lateral and anterior aspects of the mammalian thalamus (Figure 1A). TRN-like structures exist
80 in reptiles [18] and fish [19], suggesting an evolutionary conserved origin. Compared to the
81 knowledge we have about the development of cortical interneuron classes, we know
82 surprisingly little about the developmental origins of the TRN. Early reports referred to the
83 origin of TRN as ventral (or rostral) thalamic , but more recently the term prethalamic has
84 been introduced [20]. In adult life, the question of how exactly the TRN regulates thalamic
85 function is an open one. Our thesis is that the TRN can operate at variable spatio-temporal
86 scales to modulate thalamic processing according to behavioral needs. This would require
87 specialized TRN connectivity, intrinsic properties and synaptic outputs. In the following

88 sections, we discuss these putative mechanisms and their contribution to a spatiotemporal
89 *sliding scale* of thalamic inhibition.

90

91 *TRN connectivity that enables variable scales of action*

92 While the sole recipients of TRN output are thalamic projection neurons
93 (thalamocortical (TC) cells also called relay neurons), TRN neurons receive excitatory inputs
94 from both thalamus and cortex. Based on pioneering anatomical studies, a sectorial
95 organization of the TRN has been known for several decades [21,22]. This along with
96 physiological mapping experiments have indicated that a TRN neuron innervating a particular
97 thalamic nucleus receives its main inputs from topographically-aligned deep layer cortex and
98 the corresponding thalamic nucleus [23]. The vast majority of individual TRN cells innervate
99 a single thalamic nucleus (Figure 1B) [24]. In sensory systems, this organization would allow
100 the TRN to provide modality-specific inhibition that is impacted by both “bottom-up” inputs
101 coming through the thalamus and “top-down” feedback from cortex [9].

102 Despite this knowledge, however, the functional meaning of TRN’s topographical
103 organization was diminished by the lack of targeted physiological measurements of TRN
104 neurons that innervate different thalamic nuclei (Box 2). Simultaneous probing of
105 connectivity and function was not routinely performed, and therefore it only recently became
106 clear that the TRN is composed of functional ‘subnetworks’ based on projections to distinct
107 thalamic nuclei [25]. Such conclusions are based on recordings from optically-tagged TRN
108 neurons in the freely behaving mouse, which show that neurons that project to sensory
109 thalamic nuclei are engaged in canonical sleep rhythms known as spindles, and a substantial
110 proportion of them show elevated spiking during sleep compared to wake (Figure 1C). In
111 contrast, TRN neurons that project to limbic thalamic nuclei (associated with hippocampal
112 processing) do not engage in spindles and show reduced spiking in sleep [25]. These
113 observations suggested that in sleep, inhibition is higher for sensory thalamic processing
114 (diminishing sensory transfer) but lower for memory-associated thalamic processing
115 (allowing the replay of memory traces) matching the brain’s needs in this state. Such
116 observations also clearly demonstrate that thalamic inhibition is not globally controlled, but
117 can operate on more local scales. How local and how scalable is this type of inhibition?

118 Answering this question requires detailed information of TRN connectivity on a single cell
119 resolution, which is currently lacking (Box 2). Available data, nonetheless, make very
120 interesting predictions about the different functional architectures reticulo-thalamic circuits
121 can exhibit.

122 Behaviorally-relevant variable scales of thalamic inhibition can be concluded based on
123 recent studies of the visual TRN subnetwork (visTRN; projecting to lateral geniculate nucleus)
124 in mice performing tasks with different requirements [26]. In a simple visual detection task,
125 when there was no modality-specific sensory gating requirement, most visTRN neurons
126 exhibited a stereotyped reduction in firing rate during external visual stimulus anticipation
127 resulting in increased sensory gain of the relay cell activity (Fig 1C). **Many neurons that likely**
128 **project to other sensory thalamic nuclei, show this type of activity profile as well,**
129 **suggesting that general enhancement of sensory processing during sensory-directed**
130 **arousal may involve coordinated suppression of multiple sensory TRN subnetworks.** In
131 contrast, when behavior is controlled in a way that requires the brain to selectively gate visual
132 or auditory information, visTRN neurons displayed richer dynamics; while these neurons
133 showed the predicted reduction in firing rate when vision was favored, they showed elevated
134 firing when audition was instead favored and vision suppressed (Fig 1D). Optogenetic
135 suppression of this increased visTRN firing rate resulted in diminished performance on trials
136 where the auditory input was the target, suggesting that distractor suppression (vision in this
137 case) is required for optimal performance. It is likely that this type of activity is mirrored in
138 the auditory TRN (audTRN), and as such the level of engagement of TRN subnetworks is
139 dependent on behavioral requirements. Also, it is important to note that distractor
140 suppression is consistent with selective attentional engagement [26], and is dependent on
141 intact prefrontal activity in this task. The precise pathway that links prefrontal activity to TRN
142 control, however, is unclear.

143 Various inputs to TRN subnetworks are likely to be important for determining their
144 engagement and the scale of action. One clue comes from the aforementioned study that
145 monitored sensory and limbic TRN subnetworks in sleep [25]. Because the cortex is known to
146 oscillate between active (UP) and inactive (DOWN) states during sleep, subcortical structures
147 will be entrained to this cortical rhythm depending on how robust their cortical inputs are.
148 Sensory TRN neurons were much more likely to be entrained by the cortical slow oscillation

149 than those found in limbic subnetworks [25]. This suggest that during sleep the spatial scale
150 of TRN action can increase and can be coordinated across multiple sensory nuclei, but would
151 still not involve the entire TRN.

152 Another possible route to alter TRN engagement is the variety of inputs from
153 subcortical structures. For example, cholinergic neurons of the basal forebrain and the
154 brainstem project to TRN neurons [27], exerting a powerful postsynaptic response with a fast
155 excitatory nicotinic component and a slower muscarinic inhibitory component [28]. This same
156 logic applies to GABAergic inputs to the TRN which arise from an ill-defined zone along the
157 basal forebrain – external globus pallidus border possibly involving some lateral hypothalamic
158 regions as well [29–31]

159 In addition to extrinsic inputs, TRN neurons establish connections amongst
160 themselves which have the potential to change the scale of thalamic inhibition. Adjacent TRN
161 neurons can be electrically coupled by gap junctions, enabling coordinated firing [32].
162 Moreover gap junctions display activity-dependent plasticity which can dynamically alter
163 their scale of action [33]. **Although the available evidence suggests that gap junctions exist**
164 **only among closely spaced TRN cells [34,35] the extent to which electrical coupling varies**
165 **within and between subnetworks is unclear. In the somatosensory TRN most of gap junction**
166 **(i.e. dye-) coupled TRN cells project to a single nucleus, probably enhancing focal**
167 **subnetwork action but in certain cases dye-coupled cells can target two nuclei [36].** Thus it
168 would be extremely important to determine whether TRN neurons that project to one
169 thalamic target in general are more likely to be electrically coupled than ones that project to
170 different targets. In addition to gap junctional coupling, TRN neurons may connect to one
171 another through chemical synapses (either axo-dendritic or dendro-dendritic), establishing
172 mutual inhibitory networks [37]. **Most available evidence suggests however, that functional**
173 **axo-dendritic connections are present only in young [35,38,39] but not in adult animals**
174 **[24,32,40]. In adult rats no intra-TRN axon collaterals were revealed in case of over 100 TRN**
175 **neurons filled in vivo [24]. In a more recent study optogenetic stimulation of TRN cells or**
176 **blocking synaptic release of GABA revealed no evidence for intra-TRN inhibition in mice**
177 **after P14 [40]. Lack of extensive intra-TRN connectivity certainly limits the spatial**
178 **dimensions of interacting TRN subnetworks.**

179 TRN neurons also appear to exhibit variable patterns of connections to their thalamic
180 targets. In certain sensory systems, TRN neurons appear to receive converging inputs from
181 multiple thalamic neurons evident by their broad receptive fields compared to thalamic
182 projection neurons [41]. TRN neurons, in turn, project to their thalamic targets in a variety of
183 patterns, including axonal arbors with variable sizes [24]. In general, primary sensory nuclei
184 appear to contain more spatially compact axonal arbors compared to **nuclei that receive their**
185 **major excitatory input from cortical layer 5 rather than the periphery (i.e. higher order**
186 **nuclei)** [24]. These patterns of innervation are expected to play critical roles in determining
187 the spatial scale of action of TRN inhibition.

188

189 *Intrinsic and synaptic TRN properties that enable variable scales of action*

190 Similar to thalamocortical neurons, TRN neurons display two types of firing mode:
191 tonic and bursting [42] (Fig 2A). This dual firing mode may be yet another way for the TRN to
192 switch, in this case, the temporal scale of action. Tonic spiking refers to regular tetrodotoxin
193 (TTX)-sensitive Na⁺ spike trains with interval distributions that are poisson-like. A burst on the
194 other hand, is a large amplitude TTX-insensitive Ca²⁺ spike that is crowned by high frequency
195 Na⁺ spikes. While the Ca²⁺ spike is readily observable in intracellular recordings, a burst is
196 inferred in extracellular recordings using criteria that capture their intracellular statistics
197 (100msec silence followed by <4msec interspike intervals) [43] (Fig 2B). Thalamic Ca²⁺ spikes
198 are generated by low-threshold T-type Ca²⁺ channels (CaV 3 family) [44], which are recruited
199 at hyperpolarized membrane potentials that are thought to be more prevalent during periods
200 of behavioral quiescence and sleep [45]. For the TRN, this would mean enhanced bursting
201 during these states resulting in more widespread and longer-lasting inhibition of thalamic
202 targets. The effect of TRN bursting is hugely aggravated by a firing pattern dependent
203 transmitter release mechanism [46]. During tonic spikes GABA predominantly activates
204 synaptic GABA-A receptors, however during bursts GABA spills over to extrasynaptic
205 receptors as well resulting in large, several hundred msec long burst IPSCs [46,47] (Fig 2C-E)
206 switching the temporal scale of inhibitory action. This spill-over is permitted by the lack of
207 complete glia sheet around TRN terminals [15]. These burst IPSCs are especially suitable to
208 promote de-inactivation of T-type Ca²⁺ channels in TC neurons, resulting in reverberating

209 TRN-thalamic oscillations. The importance of extrasynaptic GABA-A receptors receptors in
210 thalamus has also been demonstrated in epileptic models [48]. **It is worth to mention here**
211 **an even slower mode of GABA action has been proposed acting via extrasynaptically**
212 **located GABA-B receptors up to tens of microns away from TRN synapses [49,50]. In our**
213 **view this represents the lower end of the variable temporal scale of TRN action.**

214 Given that recruitment of T-type channels within individual TRN neurons would
215 depend on its intrinsic properties and inputs, it is conceivable that at any given moment TRN
216 subnetworks can exhibit varying degrees of bursting vs tonic firing. This can precisely dictate
217 the spatio-temporal spread of inhibition within and between thalamic nuclei across all states
218 essentially allowing fine tuning the scale of action.

219 A striking example of how intrinsic and synaptic TRN properties and a gradual shift
220 from burst to tonic mode may contribute to a sliding scale of thalamic inhibition has recently
221 been observed during sleep spindle events [51]. Spindles are 7-14Hz phasic transients that
222 are observed in the cortical electroencephalograph (EEG) during sleep, and have been linked
223 to sensory processing and memory consolidation [45]. Spindles are known to require
224 interactions between TRN and connected thalamus, but the detailed synaptic interactions
225 have only been revealed recently. Recordings from connected TRN and thalamic pairs in the
226 somatosensory system (Fig 2B) showed that the primary determinant of spindle duration is
227 TRN engagement on a cycle-by-cycle basis [51]. The number of spikes in each TRN burst (Fig
228 2F) and the percentage of TRN neurons engaged in each spindle cycle progressively decreased
229 with spindle event progression. Thalamic projection neurons on the other hand showed the
230 opposite modulation, indicating that the reduction of TRN engagement during a spindle event
231 cannot be explained by reduction in their thalamic drive [51]. Instead, a strong possibility is
232 that changes in their intrinsic properties and/or cortical inputs explain their disengagement.
233 Progressive decrease in TRN bursting will result in a corresponding drop of TC inhibitory
234 charge likely via the diminished extrasynaptic receptor activation [46] as indicated above. This
235 will diminish TC rebound bursting and eventually terminate a spindle event. Importantly, the
236 initial level of engagement of TRN neurons in a spindle event determines its length [51],
237 indicating that whatever determines the size of TRN neuronal pool recruitment determines
238 the temporal and perhaps spatial spread of thalamic inhibition during a spindle (Fig 2G).

239 **Whether spindles can be coordinated across multiple sensory TRN subnetworks,**
240 **and thereby multiple sensory thalamo-cortical loops, is an important question that can be**
241 **answered with simultaneous optogenetic tagging of two TRN subnetworks while recording**
242 **spindles across their associated thalamo-cortical circuits. This is clearly an exciting area of**
243 **investigation that will not only help determine what the functional meaning of spindles is,**
244 **but also how they shape thalamic inhibitory dynamics. Nonetheless, we speculate that**
245 **connectivity will determine TRN engagement in thalamo-cortical network activity at the**
246 **macroscale, as opposed to intrinsic properties which will determine more microscale**
247 **engagement within individual subnetworks. This, of course, may be hijacked by**
248 **pathological conditions resulting in abnormally wide-spread thalamocortical network**
249 **synchrony [52].**

250 Given evidence for heterogeneity among TRN neurons with respect to their intrinsic
251 properties [11] and axonal arbor size [53], it is likely that a wide range of spatio-temporal
252 control of TRN inhibition can exist. Most critically, one type of inhibitory control TRN neurons
253 exert on their thalamic targets is shunting inhibition [54]. Meaning, it reduces the overall
254 probability of firing, and therefore, is more likely to control the overall firing rate of individual
255 thalamic neurons rather than individual spikes times (See next section). In summary, these
256 data together clearly demonstrated that the connectivity and intrinsic properties of TRN
257 neurons uniquely allow them to change the scales of inhibitory action to match state-
258 dependent requirements of the thalamocortical system.

259

260 **Extrathalamic Inhibitory System**

261 *General overview*

262 Extrathalamic GABAergic afferents arise in a set of GABAergic nuclei outside the
263 thalamus (Fig 3A-B). Their common, defining feature is the large, multisynaptic terminals in
264 the thalamus which are unlike any other known inhibitory terminal in the forebrain
265 [12,15,14,16] (Fig 3C). We will consider here the following ETI nuclei: the output nuclei of
266 basal ganglia (including substantia nigra pars compacta, SNR; internal globus pallidus, GPi; the
267 ventral pallidum, VP), the zona incerta (ZI), the anterior pretectal nucleus (APT) and the

268 pontine reticular formation (PRF) (Box 3). It is important to emphasize that both the
269 connectivity pattern of ETI inhibitory system with the thalamus and the ultrastructure of ETI
270 terminals are evolutionary highly conserved. For example, both the basal ganglia and the PRF
271 the ETI fibers innervate the same sets of thalamic nuclei in rodents and primates (including
272 humans) [14,16,55] and the quantitative ultrastructural features of ETI terminals in the
273 thalamus is indistinguishable between rats and macaques [14] (Fig 3C). **ET inhibition and TRN**
274 **inhibition differ conceptually regarding both connectivity (see Box 3) synaptology [15] and**
275 **function.**

276 Target regions of ETI nuclei may involve more than one thalamic nuclei but the
277 innervation always remain selective for a well-defined thalamic territory which indicates well-
278 focused action of ETI nuclei on the thalamus. Furthermore, any given part of an ETI nuclei will
279 provide afferents only to a restricted part of its thalamic target nuclei [13,14]. The axons
280 forms clusters of large terminals interspersed with bouton free regions [13].

281 The embryonic origins of these nuclei are quite variable and include subpallial,
282 telencephalic (GPi, VP), rostral diencephalic (recently referred to as prethalamic) (ZI), caudal
283 diencephalic (APT) or tegmental (SNR, PRF) structures [20]. In the adult brain ETI nuclei cover
284 the ventral (ZI) or caudal (APT) or latera (Gpi) aspects of the thalamus, located in the basal
285 forebrain (VP) or in the tegmentum (PRF and SNR) (Fig 3A). ETI nuclei are known to be
286 involved in various distinct large scale neuronal circuits. We group them here based on their
287 shared properties of the thalamic afferents. ETI nuclei are known to have significant role in
288 cortical development [56] motor control [1] and are involved in major neurological diseases
289 [57–61] but with the exception of basal ganglia very little data is available about their exact
290 function.

291

292 *ETI connectivity and scales of thalamic action*

293 Unlike TRN which innervates all thalamic nuclei, ETI nuclei display selective
294 innervation of well-defined thalamic territories. While each ETI nucleus has its own specific
295 projection patterns, one common feature among all is the lack of innervation of primary
296 sensory thalamus. Instead, they innervate higher order sensory nuclei (ZI, APT) [12,13] ,

297 motor territories (SNR, GPi) [14], midline and intralaminar nuclei (PRF) [16] or the
298 mediodorsal nucleus (VP) [62]. As a consequence they don't influence the classical thalamic
299 function, sensory relay, rather they are likely to be involved in sensory-motor integration,
300 executive and motor control or decision making.

301 Since ETI nuclei, even collectively, do not innervate the entire thalamus they are not
302 able to exert global thalamic influence. Even though some ETI nuclei are interconnected,
303 which potentially synchronize their activity [63] their spatial scale of action will still be
304 restricted to their target nuclei. Since they don't receive thalamic feedback (Box 3), they may
305 initiate but can't maintain reverberating thalamic activity. Their influence over global brain
306 dynamics is likely the result of inhibition of thalamic nuclei with widespread cortical
307 projections, in contrast to the recurrent dynamics that can be established between thalamus
308 and TRN. Such global state control has been recently demonstrated by selective optogenetic
309 stimulation of PRF afferents to the intralaminar thalamus. Activating PRF afferents resulted
310 in suspension of all behavior and the emergence of cortical slow oscillations with extremely
311 short latency [16] (Fig 3).

312 Cortical input reaches ETI nuclei either directly [64] or, in case of BG nuclei, via the
313 subthalamic nucleus. A given cortical region receives inputs from the thalamic nuclei where
314 its ETI target terminates (e.g S1 innervates ZI, this in turn projects to posterior thalamic
315 nucleus, which projects back to S1). This allows the emergence of a cortico-ET-TC loop,
316 parallel to the cortico-TRN-TC loop. However, beside the difference in cortical origin (layer 5
317 vs layer 6 respectively) there is a conceptual difference between the two loops. As indicated
318 above the TRN loop allows the emergence of local reverberating oscillations
319 (alpha/mu/spindles) that may be dependent on the previous synaptic history of the circuit.
320 The ETI loop on the other hand may communicate long term *changes* in cortical outputs as
321 an inhibitory signal back to widespread cortical regions via the highly divergent axonal arbor
322 of their thalamic targets.

323 *Intrinsic and synaptic properties of ETI – precise temporal scales of action*

324 The major features of ETI afferents in the thalamus is that a single axon terminal
325 contacts the postsynaptic elements via several active zones [12,13,15,14,16] (Fig 3C). Almost
326 all other inhibitory terminals, studied so far, including TRN, establish one or maximum two

327 synapses on their target [15,65]. Indeed, ETI boutons are the largest and most complex
328 inhibitory terminals of the brain described so far (with respect to size and number). On
329 average one terminal establishes 7-9 synapses but this number can reach 16. All these active
330 zones always converge on a single postsynaptic TC element [15,14,16]. The entire terminal is
331 wrapped by glial sheets which probably restricts spillover of GABA, unlike in case of TRN
332 where the glial cover is incomplete [15]. The terminals preferentially innervate thick proximal
333 dendrites, electrotonically close to the soma [12,15,14,16]. As such, the activation of a single
334 fiber is quite powerful and able to elicit rebound bursting in the innervated TC cell [13] or
335 result in complete silencing of action potential generation. Intriguingly, despite the different
336 embryonic origin of ETI nuclei, the ultrastructure of their thalamic terminals are similar.
337 Moreover this structure is stable across different taxa (rodent vs. primates or even in birds)
338 [14,16], suggesting conserved functional principles.

339 Unlike TRN terminals which display pronounced short term depression if activated by
340 repetitive stimulation ETI fibers display little evidence for short term plasticity even above 50
341 Hz. [15,16] (Fig 3H). Modelling studies indicated that this is probably due to the overflow of
342 GABA from one presynaptic active zone to all postsynaptic specializations belonging to one
343 axon terminal [66]. By reaching several synapses, the release of GABA via any of the many
344 active zones can result in similar postsynaptic response, thus the chance of release failure is
345 minimal. This mechanism allows faithful inhibitory transmission even at high presynaptic
346 firing rates. Indeed, the available in vivo data demonstrate high frequency firing activity of
347 ETI cells (Fig 3D), (approaching the theoretical maximal firing rate of 1000 Hz) [13,16,64,67]
348 where non-depressing IPSPs can maintain a constant inhibitory bombardment of the
349 postsynaptic TC cells [67]. Precise control of thalamic outputs may be established by the
350 reduction of firing in the sensory [67,68] or motor [69] systems studied so far or changing the
351 exact pattern of inhibitory activity which vetoes TC firing in a well-defined time window [1].

352 The above mentioned cellular features of ETI systems suggest that their predominant
353 action is a strong and focal thalamic inhibition. It also indicates the primary importance of
354 disinhibition i.e. mechanisms that results in the cessation of ETI firing consequently a
355 temporal removal of a strong inhibition from TC cells. **Indeed, disinhibition has been**
356 **suggested as a primary mode of action in case of BG via an elaborate system of direct and**
357 **indirect pathways both in case of dorsal and ventral striatum [1,70]. In addition the**

358 **significance of ETI disinhibition in the thalamus was best studied in the somatosensory**
359 **system [68,71]. Trigeminal inputs exert a strong feed-forward inhibition on posterior**
360 **thalamic nucleus (Po) via collaterals to ZI. (see Box 3) [71]. The disynaptic inhibition via ZI**
361 **is so fast and powerful that it overtakes monosynaptic trigeminal excitation of Po and able**
362 **to completely block sensory transmission in this nucleus. Chemical lesion of ZI reveal a full-**
363 **blown sensory response in Po to whisker stimulation [71]. So what mechanisms may**
364 **“lesion” ZI influence on Po in real situations? Up till now three non-exclusive disinhibitory**
365 **mechanisms have been suggested; i; presynaptic muscarinic receptors on ZI terminals**
366 **inhibit GABA release during focused attention [72] ii, intra-incentral inhibition driven by**
367 **descending cortical inputs temporally suspend inhibitory ZI output in the somatosensory ZI**
368 **sector [73] iii; ZI inhibition is overcome when powerful ascending and descending inputs**
369 **converge within a narrow time window on Po cells [74]. Note that in this case ETI inhibitory**
370 **influence is tightly regulated both in spatial and temporal domains. Spatially by the limited**
371 **extent of the thalamic axon arbor, temporally by the precise disinhibitory mechanisms.**

372 **By emphasizing the strength of ETI inhibition, we do not suggest that TRN inputs to**
373 **be universally weak or modulatory. In fact, paired recordings show that some TRN neurons**
374 **are capable of exerting strong and reliable inhibition of their thalamic targets [75–77]**
375 **especially in case of TRN bursts [46] (see above). Moreover, it has been shown that the axon**
376 **of a single TRN neuron also establishes multisynaptic contacts with the TC cells. The main**
377 **difference, however, is that in case of TRN, the postsynaptic cell is contacted by single**
378 **synapses of small terminals, not by many synapses of few large terminals, like ETI contacts**
379 **[15]. Thus, the major difference between TRN and ETI unitary IPSCs on TC cells is not so**
380 **much in their amplitude but rather in their short term dynamics [15]. Still only ETI, not TRN,**
381 **inputs can provide an extremely fast inhibition that could eliminate individual spikes from**
382 **a spike train without impacting the overall spike rate of a target TC neuron [1]. In contrast**
383 **to TRN, ETI nuclei are unlikely to exhibit major shifts in spatial or temporal scale of action.**
384 **The paucity of T-type Ca²⁺ channels in ETI neurons and the complete glial wrap around the**
385 **terminals indicate that low threshold burst mediated phasic activation of extrasynaptic**
386 **receptors are unlikely to occur.**

387 **Finally, it must be emphasized that ET inhibitory control is likely perturbed in certain**
388 **disease states, for example in Parkinson’s disease, epilepsy or chronic pain [57,58,60,78–80]**

389 where aberrant synchrony among ETI nuclei and changes in their output properties may
390 contribute to spatially extensive aberrant oscillatory patterns.

391

392 **Conclusions**

393 In summary, all available data suggest that the two inhibitory systems of the thalamus
394 have complementary roles. ETI signals are able to exert strong, rapid and focal effects on their
395 thalamic targets which will impact timing of individual spikes and therefore the content of
396 the signals these thalamic cells transmit. Such precise control of content is in contrast to TRN
397 inhibitory control, which appears to be largely involve gain control (signal amplification) and
398 rhythm genesis. By exerting a relatively weaker and slower type of inhibitory control overall
399 their thalamic targets, TRN subnetworks can modulate the rate of spike trains while minimally
400 impacting their overall temporal structure. Such features allow the TRN to control the
401 magnitude of cortical input while preserving its content.

402 It must be emphasized, however, the understanding of thalamic (both TRN and ETI)
403 inhibition is very limited in terms of both structure and function. Concerted efforts using the
404 most up-to-date techniques are required to approach the level of understanding we achieved
405 in case of cortical, striatal or cerebellar inhibition.

406

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415

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625

626 **Outstanding questions box**

627

628 To what extent cortical and thalamic inputs of a given TRN neuron correspond to its output
629 (i.e. to what extent are TRN loops closed vs open)?

630 To what extent openness/closeness vary across TRN subnetworks?

631 Do TRN neurons that project to different thalamic nuclei exhibit systematic differences in
632 their inputs, synaptic or intrinsic properties?

633 Can TRN neurons projecting to different thalamic nuclei interact via gap-junctions?

634 How TRN neurons shape the activity of non-sensory thalamus?

635 What is the entire extent of the ETI system?

636 How do ETI inputs shape the output of thalamic cells?

637 What is the information content and behavioral correlates of ETI spiking activity? How it is
638 shaped by cortical and subcortical inputs?

639 How do different ETI systems interact with one another?

640 How do TRN and ET inhibition interact on thalamic cells?

641 How does impaired ETI function contribute to aberrant thalamocortical activity in
642 neurological diseases?

643

644

645 **BOX 1 Scales of inhibition**

646 The nervous system needs to organize neuronal activity across multiple spatial and temporal
647 scales. The spatial domain ranges from local cell populations to the entire brain, the time
648 domain from slow oscillations (0.1 - 1Hz) to high gamma activity (up to 250Hz) [81]. In several
649 brain regions (cortex, hippocampus, striatum, cerebellum) one solution to cope with these
650 the wide range of scales is the emergence of distinct interneuron classes [82–85] . E.g. the
651 size of axon arbors in cortical interneurons ranges from small, very dense (e.g neurogliaform
652 cells) through mid-range, covering roughly a cortical column (e.g. basket cells) to long range
653 interneuron selective cells which can simultaneously affect the activity of large cortical
654 territories [86]. The size of axon arbor, thus, will physically determine the spatial scale of
655 action. Along the temporal dimension, the firing rate of the interneurons as well as the exact
656 mechanism of GABAergic action will determine the scale of action in time. The slow firing
657 rate, sluggish kinetic of receptor activation as well as the extrasynaptic mode of action will
658 enable neurogliaform cells to act in the time domains of slow oscillations [87]. On the other
659 hand, high firing rate and fast GABA-A receptor mediated inhibition of basket cells allows
660 them to control gamma oscillations [86].

661 The question we pose here is how can the thalamus cope with these variable spatio-temporal
662 scales? Since a substantial proportion of cortical operations involve interactions with
663 thalamus, thalamic inhibition needs to operate across a similar spatio-temporal range to that
664 of the cortex. In addition, various inputs parcellate the thalamus into distinct nuclei and
665 subnuclei which, in some situation, need to be controlled separately while in others
666 synchronously. So what are the inhibitory mechanisms that enable different scales of control
667 in the thalamus?

668

669

670 **BOX 2 Open vs. closed loop organization of TRN**

671 The TRN is composed of individual parallel networks, each innervating different thalamic
672 target (Pinault & Deschenes, 1998, Halassa 2014). The detailed anatomical connectivity of
673 TRN neurons is unknown and we think that this will be a critical determinant of how wide-
674 spread thalamic inhibition will be in space and time. The most important question is to what
675 extent TRN forms open or closed loop connections with their thalamic targets. An open loop

676 here refers to an arrangement in which a TRN neuron projecting to a thalamic neuron would
677 not receive input back from that neuron, whereas a closed loop indicates that the TRN neuron
678 innervates only those TC cells that it receives input from. Closed loop organization may allow
679 more restricted spatial scale of inhibition whereas open loop connections (depending on their
680 degree of divergence) may involve larger part of the thalamus. In addition open loop
681 organization would allow exerting lateral inhibition within thalamic circuits and ascending
682 sensory signals therefore, likely to show a winner-take-all feature [88]. It is intriguing to
683 speculate that activating subsets of TRN neurons by top-down control can result in biasing
684 thalamic processing towards particular sensory features based on this open-loop
685 organization.

686 Due to technical challenges detailed investigation of the openness/closeness of the TRN-TC
687 loops has not been performed. Closed loop organization is suggested by the TRN axon arbors
688 restricted mainly to single nuclei [24], (or to a single subregion e.g barreloids [89], the medio-
689 laterally strictly organized “tier” system of TRN [90–92], rebound activity even in in vitro slice
690 preparation [75], spatially restricted oscillatory (spindle) activity in vivo under anesthesia
691 [51]. **Open loops were first indicated by a mismatch between the position of TRN axon
692 arbors and location of their presumed TC cells [93], however, the method used here did not
693 allow positive identification of the synaptic contacts established by the back-filled TC cells
694 on the labeled TRN neurons. Subsequent physiological data, such as crossmodal projections
695 [94] and interactions [95] as well as cross-nuclear inhibitory modulations [96,97] strongly
696 supported the presence of open loops.**

697 Open and closed loop organization is clearly the two ends of a spectrum and perhaps a more
698 realistic scenario is a certain degree of openness in the TRN-TC circuits. The spatial scale of
699 openness (i.e. within a subregion, within a nucleus, or open circuits among different nuclei)
700 needs to be defined since these will results in distinct TRN gating functions ranging from
701 lateral inhibition to selective attention. Finally, the degree of openness will likely differ in
702 various sectors of TRN innervating different sets of thalamic nuclei. Combination of classical
703 labeling and novel viral tracing methods will hopefully soon provide insights into these
704 important questions which largely determine the spatial scale of TRN action.

705

706 **BOX 3 Connectivity of TRN vs ETI nuclei**

707 Inhibitory inputs to thalamus arise from two major sources; the TRN and a set of subcortical
708 nuclei located outside the thalamus which we jointly refer to as extrathalamic inhibitory (ETI)
709 system due their shared morphological and physiological properties (see main text). These
710 two systems not only differ in the organization of their thalamic outputs but display
711 distinctively different connectivity with the rest of the brain suggesting that the regulation of
712 their activity can be completely independent. We list here major differences:

- 713 1. Unlike TRN, ETI nuclei have no thalamic inputs. Therefore, they display a
714 unidirectional, feed forward inhibitory control over thalamus which is not influenced
715 by thalamic activity. Consequently they are not able to participate in reverberating,
716 oscillatory activity with thalamus.
- 717 2. Unlike TRN, ETI neurons receives substantial and widespread inputs from subcortical
718 glutamatergic centers. These include e.g trigeminal nuclei [71], cerebellum or the
719 subthalamic nucleus. ZI is known to receive glutamatergic inputs from almost the
720 entire neuroaxis [98]. Interestingly, many of these glutamatergic centers also project
721 directly to the thalamus, where their terminals overlap with those of ETI cells [67]. As
722 a consequence the ETI activity can temporally limit the impact of these glutamatergic
723 afferents on the thalamus through di-synaptic, feed-forward inhibition. [67,68]. This
724 form of inhibition is entirely missing in case of TRN.
- 725 3. TRN is known to receive inputs from the layer 6 of all cortical areas. In contrast ETI
726 nuclei are innervated by layer 5 of a well-defined cortical regions (mostly frontal,
727 motor, premotor cortical areas) or in case of basal ganglia output nuclei cortical inputs
728 may be entirely absent. These L5 fibers are the collaterals of descending corticofugal
729 pathways [99].
- 730 4. The sole output of TRN is the thalamus. In contrast, without exception all ET nuclei
731 have extensive axon arbors in wide variety subcortical centers [98]. This creates a
732 unique opportunity to synchronize thalamic activity with other functionally related
733 subcortical nuclei.
- 734 5. In contrast to TRN, ETS cells provide extensive intranuclear axon arbors [13] . In
735 addition, APT, ZI and PRF are mutually linked [63]. This allows various forms of

736 interaction within ETI sectors and/or among ETI nuclei, which may involve
737 disinhibition as well as synchronization [73].

738

739

740 **Figure legends**

741

742 **Figure 1)** Heterogeneity, subnetworks and gating by TRN. A. Parasagittal section of the mouse
743 brain, highlighting TRN in blue. B. Juxtacellular filling of two neighboring TRN neurons in the
744 rat (red and green). Although the cell bodies are in close proximity, each neuron projects to
745 a distinct thalamic target (AD; anteriodorsal thalamus associated with mnemonic processing
746 and LD; laterodorsal associated with sensory processing). C. TRN neurons display distinct
747 physiological phenotypes. In relation to spindle power, one TRN neuron (blue trace) shows
748 positive whereas the other (red) negative firing rate correlation. Subsequent optogenetic
749 tagging showed that these physiological phenotypes map onto anatomical projections.
750 Consistent with this notion, several visual TRN neurons show enhanced firing rate in sleep
751 compared to wake, while limbic neurons are exclusively suppressed during sleep (bottom
752 right in C). D. Cross-modal divided attention in the mouse shows TRN recruitment by
753 attentional allocation. Example raster plot and corresponding peristimulus time histograms
754 of two visual TRN neurons when the animal is instructed to attend to vision (red) or audition
755 (blue). Grey shading depicts TRN activity during the stimulus anticipation period following the
756 presentation of the instruction signal. Note that visual TRN activity is reduced during visual
757 trials but augmented during auditory trials, resulting in a corresponding decreased and
758 increased inhibitory output to visual thalamic cells. This is consistent with a gating role of TRN
759 during selective attention to a given modality and focal subnetwork specific TRN action Figure
760 B is based on [24] C on [25], D on [26]

761

762 **Figure 2)** Heterogeneous scales of action by TRN. A) Tonic (black) and burst (gray) response
763 of a TRN neuron to depolarizing and hyperpolarizing current step, respectively. B) Rhythmic
764 burst activity of interconnected TC (black) and TRN (purple) cells during sleep spindles in
765 freely sleeping animals. C) TRN bursts generate large, slow burst IPSC in TC cells in control
766 condition in vitro (VB control, black). Substantial amount of this burst IPSC persists after the
767 total removal synaptic GABA-A receptors (VB AAV-Cre, red) indicating that a significant
768 portion of the inhibitory charge is carried via extrasynaptic receptors. D) However, during
769 single spike TRN activity only synaptic receptors are activated resulting in an order of
770 magnitude faster response. This indicates that changes in firing pattern alter the temporal

771 scale of TRN action D) In the absence of synaptic inhibition, phasic extrasynaptic burst IPSCs
772 is sufficient to pace normal spindle oscillations E) Cycle-by-cycle decrease in the number of
773 spikes/TRN bursts during sleep spindles with variable duration (5-14 cycles). Change from
774 burst to tonic IPSCs alter the temporal scale of action, resulting in a drop in the inhibitory
775 which leads to termination of spindles F) Participation probability of TRN cells (purple) in the
776 first cycle of the sleep spindles display a strong correlation with spindle length. This indicates
777 that the duration of spindles is determined at the onset by the state of the network and this
778 state is coded best by the activity level of TRN cells. Figures B,F,G from [51], C-E from [46]

779

780 **Figure 3)** Extrathalamic inhibition in the thalamus A) Parasagittal section of the brain
781 highlighting the position of ETI nuclei (blue) around the thalamus. B) An ETI neuron in the
782 APT. The cell have spiny dendrites, profuse local axon collaterals (left) and two ascending
783 main axons ramifying in n.posterior of the thalamus (arrow, right). The cell also display a
784 descending main axon (double arrow). C) Comparison of ETI and TRN terminals on the same
785 scale. 3D reconstructions from serial electron microscopic sections. Yellow, active zones;
786 blue, puncta adhaerentia; red, membrane of the terminal; green, glia. All active zones of ETI
787 terminals converge on the same TC cell. Almost all TRN terminals have a single active zone
788 per target. If they form two (arrows on the right) synapses they are separated by glia and
789 innervate different dendrites. Note the similarity of ETI terminals among structures and taxa
790 D) Firing activity of an APT cell in vivo with concurrent EEG recording. Note high frequency
791 action potential clusters (inset). E-G) Activation of ETI terminals originating from PRF in the
792 intralaminar nucleus leads to the disruption of all ongoing behavior and global alteration of
793 the EEG activity. E) Experimental arrangement. F) Normalized travelled distance, before,
794 during and after the stimulation. G) Wavelet spectrum of the cortical LFP. Warm color depicts
795 higher power. Grey bars indicate the time of raw cortical LFP shown above. H) Difference in
796 charge transfer during high frequency stimulation between ETI (APT) and TRN terminals. ETI
797 transmission is stable at high presynaptic firing rates as well. Figures B, D from [13], C from
798 [15,14], E-G from [16] and H from [15].

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