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Thalamic reticular nucleus induces fast and local modulation of arousal state

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# 1 Thalamic reticular nucleus induces fast and local modulation of arousal 2 state

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21

## 22 Abstract

23 During low arousal states such as drowsiness and sleep, cortical neurons exhibit  
24 rhythmic slow wave activity associated with periods of neuronal silence. Slow waves are locally  
25 regulated, and local slow wave dynamics are important for memory, cognition, and behaviour.  
26 While several brainstem structures for controlling global sleep states have now been well  
27 characterized, a mechanism underlying fast and local modulation of cortical slow waves has not  
28 been identified. Here, using optogenetics and whole cortex electrophysiology, we show that  
29 local tonic activation of thalamic reticular nucleus (TRN) rapidly induces slow wave activity in a  
30 spatially restricted region of cortex. These slow waves resemble those seen in sleep, as cortical  
31 units undergo periods of silence phase-locked to the slow wave. Furthermore, animals exhibit  
32 behavioural changes consistent with a decrease in arousal state during TRN stimulation. We  
33 conclude that TRN can induce rapid modulation of local cortical state.

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## 39 Introduction

40 Modulation of arousal is one of the central aspects of behavior, as sleep plays an  
41 essential role in cognitive function and survival. A key marker of decreased arousal is cortical  
42 slow wave activity (1-4 Hz), which occurs both during non-REM sleep [1-3] and in awake  
43 animals during low vigilance states and sleep deprivation [1, 4]. The slow wave marks rhythmic  
44 periods of suppression in cortical neurons (OFF periods) lasting hundreds of milliseconds [1, 5].  
45 These brief offline periods are a candidate mechanism for decreased arousal, and slow waves  
46 in local cortical regions are associated with behavioral deficits on sub-second timescales [6].  
47 Slow waves are thus correlated with both behavioral decreases in arousal and disruption of  
48 cortical activity. However, while several brainstem structures for global control of sleep states  
49 have been well characterized [7-10], no mechanism has been identified that generates the  
50 spatially isolated slow waves that occur during drowsiness, known as 'local sleep'. Slow wave  
51 activity is locally regulated both during sleep, where it plays a role in sleep-dependent memory  
52 consolidation [11], and in the awake state, where it reflects a shift in cortical processing modes  
53 [12]. Local modulation of slow waves is therefore an important element of cortical function, but  
54 the underlying mechanism is not well understood.

55 We sought to identify a forebrain structure that modulates local cortical slow wave  
56 activity. A central modulator of corticothalamic feedback that could initiate these dynamics is the  
57 thalamic reticular nucleus (TRN), a subcortical structure that provides powerful inhibition to  
58 dorsal thalamic nuclei. The TRN is a thin sheath of GABAergic neurons that surrounds the  
59 thalamus and inhibits thalamic relay cells [13, 14]. TRN has been implicated in sensory  
60 processing [15, 16], attentional gating [17-20], and sleep state modulation [21, 22] - and is  
61 uniquely positioned to selectively and rapidly modulate cortical state. TRN has a causal role in  
62 initiating sleep spindles [23-26], and molecular genetic manipulation of TRN conductances  
63 reduces EEG sleep rhythms [27, 28], indicating a role for thalamocortical feedback in cortical  
64 sleep oscillations. However, direct manipulations of thalamic activity have yielded conflicting  
65 results. Nonspecific activation of multiple thalamic nuclei (including TRN) increases time spent  
66 in sleep [29], whereas selectively stimulating thalamus induces a desynchronized cortical state

67 [30], suggesting a role for thalamus in controlling arousal states. On the other hand, directly  
68 disrupting thalamic activity does not induce slow waves or sleep states [31-33]. These mixed  
69 findings suggest a complex involvement of thalamus in regulating behavioral arousal, which  
70 could be mediated through the TRN. In addition, many sedative and anesthetic drugs act to  
71 enhance GABAergic synaptic transmission and thus potentiate the effects of TRN activity [34],  
72 further suggesting that it could be a component of the mechanism by which these drugs induce  
73 an unconscious state [35]. Neuronal activity in TRN is known to correlate with arousal [19, 36,  
74 37], but it remains unclear whether these firing patterns are a consequence of low arousal or a  
75 cause. In particular, the role of TRN in generating the low-frequency oscillatory dynamics  
76 characteristic of low arousal states is not known, and the behavioral significance of such cortical  
77 dynamics has not been causally tested.

78         Here, we optogenetically activated TRN, and found that this manipulation rapidly induces  
79 local sleep-like thalamocortical slow waves. Tonic activation of TRN in awake animals produced  
80 slow wave activity in the associated cortical region, together with phase-locked periods of  
81 silence in cortical neurons (OFF periods). This manipulation also produced a progressive  
82 decrease in arousal state: awake animals exhibited less motor activity and spent more time in  
83 non-REM sleep, and anesthetized animals exhibited a decrease in cortical activity and a shift in  
84 dynamics favoring OFF periods. We find that the net effect of TRN stimulation is to decrease  
85 thalamic firing, suggesting that TRN may modulate arousal state through selective inhibition of  
86 thalamic activity, facilitating the onset of slow waves. Furthermore, TRN and other thalamic  
87 neurons are phase-locked to the induced oscillations, suggesting that TRN, thalamus, and  
88 cortex are all engaged in the rhythm. We conclude that tonic depolarization of TRN rapidly  
89 modulates cortical state and controls the animals' arousal, by inducing suppression and  
90 rhythmic spiking in thalamus. The spatial characteristics and rapid timescale (<50 ms) of these  
91 effects show that local oscillatory dynamics between thalamus and cortex are a central  
92 mechanism for modulation of arousal.

93

94

## 95 Results

96         We sought to identify a structure that modulates local cortical slow wave activity in a  
97 rapid and spatially restricted manner. To optogenetically manipulate the TRN, we first used  
98 transgenic mice in which channelrhodopsin2 (ChR2) expression was under the control of the  
99 vesicular GABA transporter (VGAT-ChR2) [38]. In these mice, the TRN exhibits preferential  
100 expression of ChR2 compared to surrounding subcortical regions (Figure 1-figure supplement 1-

101 2) [23], allowing us to manipulate TRN activity in awake mice using chronically implanted optical  
102 fibers (Figure 1a).

103

#### 104 **Tonic activation of TRN produces cortical slow waves.**

105 We first tested how tonic activation of TRN affected neural dynamics in the cortex of  
106 awake head-fixed mice. We implanted four mice with stereotrodes distributed across cortex and  
107 an optical fiber targeting the somatosensory sector of TRN (Figure 1a). To examine the intrinsic  
108 cortical dynamics that emerge when no specific oscillation frequency is imposed, we used tonic  
109 rather than phasic stimulation, activating TRN using constant light for 30 seconds. Tonic TRN  
110 activation produced an immediate and substantial increase in low-frequency power in the local  
111 field potential (LFP) of ipsilateral somatosensory cortex (Figure 1b-d). This power increase was  
112 specific to the delta (1-4 Hz) band, which increased by 2.56 dB (95% confidence interval  
113 (CI)=[2.13 2.97]) during laser stimulation. In contrast, beta and gamma (15-50 Hz) power  
114 decreased slightly (Figure 1c, median=-1.03 dB, CI=[-1.24 -0.84]). The increase in delta power  
115 was rapid and robust: delta waves were already evident in the first second of TRN activation  
116 (change=1.12 dB, CI=[0.48 1.76]) and persisted throughout the stimulation period (Figure 1b).  
117 When individual slow wave events were detected automatically by thresholding filtered LFPs  
118 (see Methods), 0.3 slow waves per second were detected during TRN stimulation, significantly  
119 more than baseline (increase=0.13 events/s, CI=[0.10 0.17]). The amplitude of the negative-  
120 going peak was smaller during stimulation (change=-148  $\mu$ V, CI=[-244 -54]), whereas the  
121 amplitude of the positive-going peak was larger during stimulation (change=43  $\mu$ V, CI=[8.6  
122 76.7]), similar to the asymmetric waveforms typically seen during sleep slow waves [1]. The  
123 precise frequency and amplitude of slow wave events depend on the detection criteria being  
124 used, but these statistics nevertheless indicate a substantial increase in slow waves during TRN  
125 stimulation. To test whether this effect was TRN-specific rather than due to long-range  
126 GABAergic projections to thalamus, we next studied VGAT-Cre mice injected with AAV-EF1a-  
127 DIO-ChR2-EYFP specifically into the TRN and replicated the increase in delta (Figure 1-figure  
128 supplement 3-5). No such effect was observed in littermate mice that were negative for ChR2  
129 (Figure 1-figure supplement 6), indicating that the slow waves were not due to nonspecific light  
130 or heating effects.

131

132

#### 133 **TRN activation selectively controls a local ipsilateral cortical region.**

134 Cortical slow waves are observed locally in awake sleep-deprived animals, and this  
135 'local sleep' correlates with decreased performance on cognitive tasks [6]. Given that TRN  
136 establishes topographical connections with its cortical inputs and thalamic outputs, we  
137 hypothesized that TRN could support local slow wave generation. We manipulated the extent of  
138 TRN activation by varying laser power, stimulating at low (<2 mW) or high (>2 mW) power while  
139 simultaneously recording local field potentials across cortex in individual mice (Figure 1a) to  
140 investigate the spatial spread of induced slow waves. We recorded in four awake head-fixed  
141 mice with fibers targeting the somatosensory sector of TRN. This stimulation protocol is  
142 expected to stimulate local somatosensory TRN at low laser power, and stimulate broader  
143 regions of TRN at higher laser power (Figure 1-figure supplement 7, [39]). We found that low  
144 laser power consistently enhanced local delta power in ipsilateral S1 (Figure 1e). Across all  
145 electrodes in the ipsilateral posterior quadrant (Figure 1e, red circle), 9/20 recording sites (45%)  
146 showed a significant increase in delta power during tonic activation ( $p < 0.05$ , signed-rank test  
147 with Bonferroni correction). In contrast, only 2/32 recording sites (6%) in other cortical regions  
148 (e.g. contralateral or frontal) showed a significant increase in delta power, a significantly lower  
149 proportion than in the ipsilateral posterior quadrant (diff.=0.37, CI=[0.15 0.6], binomial bootstrap  
150 (see Methods)), demonstrating that slow waves were selectively induced in a local ipsilateral  
151 cortical region (Figure 1e,g). Trials using high laser power (i.e. with light spreading to larger  
152 regions of TRN) induced slow waves across a large cortical area: 10/20 (50%) of electrodes in  
153 the associated cortex and 11/32 (34%) of distant electrodes showed a significant increase in  
154 delta power (Figure 1f,h). The proportion of distant electrodes showing increased delta power  
155 was significantly higher than in the low laser power condition (diff.=0.26, CI=[0.08 0.44],  
156 binomial bootstrap (see Methods)). These data suggest that weak tonic activation of a small  
157 population of TRN neurons produces slow waves in a local ipsilateral cortical region, and that  
158 the strength of TRN activation controls the spatial spread of cortical slow waves. Local  
159 activation of TRN thus controls an aligned region of cortex, and could support the spatially  
160 restricted slow waves that occur in local sleep.

161 Global cortical slow waves could be caused by broad thalamic inhibition, or by traveling  
162 waves across cortex spreading from the local site. To investigate these possibilities, we  
163 analyzed the phase relationships between different cortical sites during global induction of slow  
164 wave activity. We selected all channels with a significant increase in delta power during the high  
165 laser power stimulation, filtered the LFP between 1-4 Hz, and quantified each electrode's phase  
166 relationship relative to the electrode closest to the optical fiber using the phase-locking value  
167 (PLV, [40]). At baseline, all channels in both the ipsilateral posterior quadrant channels and the

168 more distant channels were significantly phase-locked to the reference site (ipsilateral: mean  
169 PLV = 0.52, s.d.=0.13; distant: mean PLV = 0.43, s.d.=0.15). During TRN stimulation, the PLV  
170 decreased slightly across all electrodes ( $p=0.0065$ , signed rank test) but remained significantly  
171 larger than chance (ipsilateral: mean PLV= 0.48, s.d.=0.15; distant: mean PLV=0.39, s.d.=0.17,  
172 every channel significant in permutation test). The mean phase offset at baseline was 0.10  
173 radians (std.= 0.22 rad), and did not change significantly during TRN stimulation (mean=0.15  
174 rad, std.=0.26 rad,  $p=0.45$ , signed rank test). The mean phase offsets across channels were  
175 clustered around zero, indicating that the induced slow wave activity was generally  
176 synchronized across cortex and phase lags were relatively short. Within individual electrodes,  
177 the phase lag was strongly correlated across the baseline and stimulation conditions  
178 ( $R=0.93$ , CI=[0.82 0.98], Figure 1-figure supplement 8-9). These results suggest that TRN  
179 stimulation did not strongly affect cortical synchronization, but rather that the induced slow  
180 waves had similar phase relationships to the baseline dynamics across cortical regions. This  
181 result is consistent with previous findings that anesthesia-induced slow waves exhibit similar  
182 phase offsets to awake cortical dynamics [41]. This evidence supports the idea that TRN can  
183 induce slow waves in local or global cortical regions, with different thalamocortical loops  
184 supporting oscillations with different phase offsets across cortical sites.

185

### 186 **Cortical units rapidly phase-lock to induced slow waves and undergo OFF periods**

187 Cortical slow waves during local sleep and NREM sleep mark an alternation of cortical  
188 spiking between activated (ON) and inactivated (OFF) states [1, 42]. To investigate whether the  
189 TRN-induced slow waves reproduced this pattern, we identified 31 single units (putative single  
190 neurons) across cortex and tested whether they were modulated by local slow waves. While  
191 TRN stimulation did not significantly change firing rates in cortical units (Figure 2a, median=-  
192 0.05 Hz, CI=[-0.17 0.09]), units from electrodes with induced slow waves became strongly  
193 phase-locked, similar to cortical activity during NREM sleep and local sleep (Figure 2b,c,  
194 median change=0.044 bits, CI=[0.0012 0.112],  $n=13$  units). High gamma (70-100 Hz) power,  
195 which correlates with multi-unit spiking [43], also rapidly became phase-locked to slow waves  
196 during TRN activation (Figure 2d, median=0.0015, CI=[0.0009 0.0022]), indicating that local  
197 neuronal activity was broadly locked to the induced slow waves. In contrast, increased phase-  
198 locking was not observed in units from electrodes with no induced slow waves (Figure 2b,  
199 median=0.0002 bits, CI=[-0.004 0.007],  $n=18$  units). Across all units, the increase in phase-  
200 locking was correlated with the increase in LFP delta power ( $R=0.77$ , CI=[0.57 0.88]).

201 Phase-locking analysis does not explicitly investigate OFF states, so we next  
202 automatically detected OFF states in electrodes that contained both MUA activity and TRN-  
203 induced slow waves (Figure 2f). During TRN activation, cortical neurons spent 13.1% of the time  
204 in OFF periods, significantly more than in the awake baseline state (7.04%,  $p < 0.001$  in each  
205 mouse) and significantly more than would be expected to occur randomly (3.09%,  $CI = [0.04$   
206  $6.15]$ ). In addition, these OFF periods occurred predominantly during the negative deflection of  
207 the slow wave ( $p < 0.001$  in each mouse, Pearson's chi-square test, Figure 2e). Their average  
208 duration was 122 ms (quartiles=[69 146] ms), and their mean frequency was 0.998/second,  
209 similar to natural sleep [1]. We concluded that the induced slow waves are sleeplike, marking an  
210 oscillatory pattern in which cortical neurons undergo periods of silence lasting tens or hundreds  
211 of milliseconds.

212 To determine the timescale of the shift into sleeplike dynamics, we computed the mean  
213 LFP and spike rate locked to laser onset, across all cortical units with local slow waves. The  
214 LFP underwent a negative-going deflection for the first 100 ms of laser stimulation (Figure 2g,  
215 top), and spike rates significantly decreased (Figure 2g, bottom). The reduction in cortical  
216 spiking was significant within 20 ms, and the LFP effect by 35 ms. The effect of TRN activation  
217 was therefore rapid, driving a slow wave and cortical suppression in tens of milliseconds, and  
218 thereby inducing an abrupt transition into a new cortical state in which neurons undergo  
219 rhythmic OFF periods.

220

### 221 **Stimulation of TRN causes suppression and rhythmic firing in thalamus**

222 Tonic TRN stimulation produced striking cortical effects that were locally defined,  
223 suggesting that the key circuit mechanism was through thalamus, which is the main target of  
224 TRN outputs and has corticotopic projections that could support local control of cortex. To  
225 investigate the impact of TRN stimulation on thalamic activity, we recorded from TRN and  
226 nearby thalamus (targeting the ventral posteromedial nucleus). We isolated 28 single units from  
227 five mice and used spike waveforms to distinguish between putative TRN ('Narrow') and  
228 putative thalamocortical (TC, 'Wide') neurons (Figure 3a). The waveform distribution was  
229 bimodal (Figure 3a), with 'Narrow' units (peak-to-trough time under 200  $\mu s$ ), and 'Wide' units  
230 (peak-to-trough time above 200  $\mu s$ ). Narrow waveforms are typically characteristic of TRN  
231 GABA-ergic fast-spiking inhibitory neurons [38, 39], so we used these units ( $n=17$ ) to infer the  
232 activity of TRN, and the 'Wide' units to infer TC neuron activity [12]. Spike rates in nearly all  
233 putative TC units (9/11, 81.8%) significantly decreased during laser stimulation, whereas no



234 units significantly increased their spike rate (Figure 3b). Thalamic activity was therefore  
235 consistently suppressed by TRN activation.

236 Putative TRN units exhibited heterogeneous changes in firing rates, as expected due to  
237 the local stimulation induced by limited light spread (Figure 3c-e). A subset of units increased  
238 their spike rates as predicted (4/17 units, 23.5%), while other units had no significant change  
239 (17.7%), or decreased their firing rate significantly (58.8%). The magnitude of the increase in  
240 firing rates (Figure 3c) suggested that stimulation induced strong local excitation of TRN, and  
241 possibly led to downstream inhibition of neurons located farther from the optical fiber through  
242 either intra-TRN inhibition or through suppression of thalamic drive to TRN. Alternatively, the  
243 heterogeneous effects of stimulation on TRN neurons could reflect variable expression levels or  
244 heterogeneous cell types within the TRN with different functional properties [19, 36, 44], leading  
245 to increased spike rates in a specific subset of TRN cells due to their cell type and role in the  
246 local circuit structure. In either scenario, high firing rates in a subset of TRN neurons is sufficient  
247 to consistently inhibit thalamocortical cells. These results demonstrate that optogenetic  
248 stimulation of TRN strongly drives only a local subpopulation of TRN neurons, but nevertheless  
249 causes consistent inhibition of thalamic activity.

250 Whether and how thalamic inhibition can generate sleep states has been debated:  
251 although thalamic activation induces wake states [30], lesioning thalamus does not produce  
252 sleep states [31]. Similarly, while direct inhibition of thalamus does not induce slow waves [45],  
253 activating inhibitory brainstem projections to thalamus does [7], and thalamic stimulation can  
254 entrain cortical slow waves [33]. We therefore hypothesized that thalamus participates in  
255 generating slow waves, and tested whether these neurons engaged in the induced rhythm. We  
256 indeed observed that subcortical units increased their phase-locking to the thalamic LFP slow  
257 waves (Figure 3f). Putative TRN unit phase-locking was diverse: laser stimulation increased  
258 overall phase-locking (Figure 3c, increase=0.027 bits, CI=[0.005 0.041]), but the preferred  
259 phase varied substantially across units (Figure 3g). In contrast, putative TC neurons  
260 consistently increased their phase-locking during stimulation (Figure 3f,h, increase=0.011 bits,  
261 CI=[0.001 0.022]). Stimulation of TRN thus causes thalamic neurons to oscillate in a slow wave  
262 pattern rather than undergoing a simple decrease in activity.

263 Thalamic entrainment to slow waves can be a combination of intrinsic mechanisms [46,  
264 47] and cortical entrainment [48]. To investigate the contribution of intrinsic oscillatory  
265 mechanisms, we examined thalamic spike properties, as thalamic cells can burst at delta  
266 frequencies during hyperpolarization [47]. To test whether our manipulation affected bursting,  
267 we fit generalized linear models to the spike trains of each unit. We tested whether spike history

268 2-4 ms prior predicted an increased likelihood of spiking during TRN stimulation as compared to  
269 baseline (Figure 3m). We found a significant change in 7/11 (63%) of putative TC cells,  
270 suggesting that TRN stimulation increased the likelihood of thalamic bursting (Figure 3j,m). In  
271 addition, 5/10 of the TRN units that decreased firing rates during stimulation increased their 2-4  
272 ms history dependence, whereas 0/4 of the TRN units with increased firing did. These results  
273 suggest that the laser-driven TRN units fire tonically (Figure 3i,l), and lead to bursting and  
274 phase-locking in neighbouring TRN cells and in thalamus (Figure 3i,j,k).

275

### 276 **Tonic TRN activation decreases behavioural arousal state**

277 Slow wave activity is associated with drowsiness and sleep [49], and thalamic activity  
278 plays an important role in awake states [50, 51], so we next investigated whether strong TRN  
279 activation produced behavioral signs of decreased arousal, by recording electromyography  
280 (EMG) and frontal electroencephalography (EEG) in freely behaving mice. EMG power  
281 decreased significantly during TRN stimulation (Figure 4a, mean=-0.06, CI=[-0.08 -0.04]),  
282 indicating that stimulation caused the animals to become less active. The decrease was  
283 significant within 1 second of laser onset, demonstrating rapid modulation of behavioral state. In  
284 addition, the EEG and EMG effects were significantly negatively correlated on the single trial  
285 level (Figure 4c, correlation coefficient=-0.43, CI=[-0.53 -0.33]). This correlation was significantly  
286 stronger than at randomly shuffled times, ( $p < 0.05$ , bootstrap) demonstrating that the decrease  
287 in arousal was specifically associated with the optogenetically induced slow waves. Control  
288 experiments in ChR2 negative littermates showed no EMG effect (Figure 4-figure supplement 1).  
289 To test a more general measure of arousal, we recorded videos of behaving mice and used an  
290 automatic video scoring algorithm to quantify their motion. Motion decreased significantly during  
291 TRN activation (Figure 4a, decrease in 58.0% of trials, CI=[53.1 62.7]). These results  
292 demonstrated that TRN activation causes a rapid decrease in arousal state, evident by a decline  
293 in motor activity.

294 We next investigated whether the behavioral effect was due to a decrease in motion  
295 during the awake state, or whether the mice were also sleeping more during TRN activation. We  
296 performed semi-automated sleep scoring using EMG and frontal EEG recordings and found that  
297 TRN stimulation reduced awake time (median=-2.1 percentage points, CI=[-4.2 -0.47]) and  
298 increased NREM sleep (median=3.5 percentage points, CI=[1.68 5.44]) (Figure 4b). Tonic TRN  
299 activation thus shifted sleep dynamics, biasing animals towards NREM sleep. The change in  
300 behavioural state was subtle, corresponding to a decrease in motor activity and a small increase

301 in the probability of NREM sleep, similar to the awake but drowsy behaviour reported during  
302 local sleep.

303

### 304 **Partial inhibition of TRN decreases slow wave activity during sleep**

305         Given that stimulating TRN could rapidly and locally induce cortical slow waves, we  
306 asked whether inhibiting TRN in a sleeping animal could reduce its cortical slow wave activity.  
307 We expressed halorhodopsin in TRN neurons using local viral injections (Figure 4-figure  
308 supplement 2), resulting in widespread expression within a local region of TRN (Figure 4-figure  
309 supplement 3), and recorded cortical LFPs during partial TRN inhibition. We found that TRN  
310 inhibition reduced slow waves in mice during NREM sleep (change= -0.45 dB, CI=[-0.77,-0.13],  
311 Figure 4d). To ensure that this effect was not due to spontaneous awakenings, we shuffled the  
312 laser onset times and did not observe any effect (shuffled change=0.01, CI=[-0.39, 0.32]),  
313 suggesting that the decrease in slow wave activity was specifically due to TRN inhibition. We  
314 did not observe behavioural effects of TRN inhibition, which likely reflects that multiple powerful  
315 pathways including brainstem are acting to suppress motor activity during NREM sleep rather  
316 than TRN alone [52], but could also indicate that more extensive suppression of TRN is needed  
317 to modulate behaviour than can be achieved in this preparation. We therefore concluded that  
318 TRN can bidirectionally modulate cortical slow wave activity.

319

### 320 **Tonic TRN stimulation further increases slow wave activity during sleep**

321         For direct comparison to the halorhodopsin experiments, we also tonically stimulated  
322 TRN in VGAT-Cre mice expressing ChR2 during natural NREM sleep. Tonic TRN stimulation  
323 applied during NREM sleep increased delta power by 0.62 dB (CI=[0.27 0.97], Figure 4d),  
324 indicating a further induction of cortical slow waves even during sleep states when slow waves  
325 are already present. In contrast, spindle (9-15 Hz) power decreased significantly (median=-0.80  
326 dB, CI=[-1.17 -0.42]), likely due to increased number and prolongation of OFF periods. These  
327 dynamics are similar to those observed during transitions into deeper stages of sleep, as  
328 spindles subside and slow waves increase, suggesting that TRN stimulation can shift cortical  
329 dynamics into deeper stages of NREM.

330

### 331 **TRN stimulation modulates existing slow waves during anesthesia**

332         We next examined whether TRN can also decrease arousal in anesthetized mice; would  
333 tonic TRN activation induce slow waves when the animal is already in a state of decreased  
334 arousal and exhibits global slow waves? We recorded EEG during isoflurane anesthesia and

335 found that the baseline delta power was high, and there was no further increase during TRN  
336 activation (Figure 4e), suggesting that the ability of TRN to generate slow waves was saturated.  
337 When individual slow wave events were detected, they also showed no change in frequency  
338 during TRN stimulation (baseline=0.30 events/s, stimulated=0.29 events/s). Instead the EEG  
339 showed a broadband (0.5-50 Hz) decrease in power (-0.53 dB, CI=[-0.69 -0.37]), demonstrating  
340 a generalized quieting of cortical activity. Furthermore, the fraction of time spent in OFF periods  
341 increased by 4.02 percentage points (CI=[1.9 6.2]) and the amplitude of the positive-going LFP  
342 slow wave peak increased by 45  $\mu$ V (CI=[16 75]) Cortical units increased their phase-locking to  
343 slow waves (Figure 4-figure supplement 4-5, median=0.06 bits, CI=[0.018 0.189]), while their  
344 firing rates decreased (median=-0.09 Hz (-5.5%), CI=[-0.22 -0.02]), suggesting that cortical  
345 activity became more strongly suppressed by the existing slow waves. Similarly, firing rates in  
346 putative thalamocortical neurons were suppressed to even lower levels by TRN stimulation  
347 during anesthesia (Figure 4-figure supplement 6). We concluded that the anesthetized cortex is  
348 shifted into an even deeper state by TRN activation: not by inducing slow waves, but rather by  
349 modulating the dynamics of a slow wave that is already present and thereby prolonging the  
350 duration of the periodic suppressions.

351

352

353

## 354 Discussion

355

356 States of decreased arousal are marked by local cortical slow waves, but the circuit  
357 mechanisms that induce these states and their causal link to arousal are unknown. In this study,  
358 we identified a local thalamocortical circuit that modulates cortical arousal state. Specifically, we  
359 found that tonic TRN activation mediates an increase in thalamic inhibition and produces sleep-  
360 like cortical slow waves whose spatial spread depends on the extent of TRN activation. This  
361 electrophysiological effect is correlated with an optogenetically-induced reduction in behavioral  
362 arousal. TRN-mediated thalamic inhibition can thus serve as a mechanism for local modulation  
363 of cortical arousal state.

364

### 365 **Slow waves are generated by local corticotopic circuits**

366 We find that TRN can selectively induce slow waves in local cortical regions. This result  
367 reinforces recent findings suggesting that sleep contains dynamics that are differentiated across  
368 cortex rather than a globally homogeneous cortical state [53]. Awake sleep-deprived rats also

369 exhibit slow waves and OFF periods in local cortical areas [6], and these local dynamics are  
370 correlated with behavioural deficits. Our results show that localized depolarization in TRN can  
371 produce such local oscillations, and could therefore underlie the fragmented cortical slow waves  
372 observed during sleep as well as drowsy awake states. In addition, local cortical OFF states  
373 have been observed during sleep [54] and general anesthesia [41] in human subjects,  
374 demonstrating that OFF periods frequently occur locally even when slow-wave activity is  
375 present throughout cortex. The observed asynchronous slow waves in these unconscious states  
376 could be due to a global activation of TRN, producing slow waves throughout cortex, but  
377 different cortical regions are associated with specific thalamocortical circuits that enable them to  
378 undergo separate and asynchronous oscillations. Finally, the local control that TRN exerts over  
379 cortex provides evidence for how TRN could modulate attention across sensory modalities, by  
380 suppressing arousal in specific cortical regions. This finding thus supports the theory that TRN  
381 could function to modulate attention, not only by gating thalamic transmission of sensory  
382 information to cortex [18], but also by modulating non-sensory-driven thalamic activity, which  
383 controls the ongoing state in local cortical regions and thereby influences the structure of  
384 functional networks in cortex. The finding that TRN can independently control limited  
385 corticothalamic circuits therefore suggests it could serve as a central circuit mechanism to  
386 regulate specific cortical regions, modulating both attention and arousal.

387

### 388 **TRN-induced thalamocortical slow waves show rapid onset**

389 In natural behavior, animals can rapidly transition between arousal states. We find that  
390 the slow waves induced by depolarization of TRN are initiated abruptly, suggesting it could play  
391 a role in rapid state modulation. Cortical activity is suppressed within 20 ms of laser onset, and  
392 the deflection in the LFP can be detected within 35 ms. This pattern suggests that tens of  
393 milliseconds of TRN activation are sufficient to inhibit thalamic input to cortex and produce a  
394 cortical OFF state. The dynamics at laser offset are similarly abrupt, with slow waves vanishing  
395 within a second. TRN can therefore serve as a rapid modulator of arousal state. This finding is  
396 also compatible with established neuromodulatory sleep circuits [49], such as monoaminergic  
397 arousal pathways [55], as these neuromodulators affect TRN activity as one component of  
398 arousal regulation. TRN thus engages a fast-acting circuit for arousal control, demonstrating  
399 that thalamocortical loops can rapidly control cortical arousal state.

400

### 401 **TRN supports both slow waves and sleep spindles**

402 Here we used tonic and low-power activation of TRN, leading to decreased thalamic  
403 firing rates without complete suppression. This tonic paradigm induced slow waves and  
404 modulated cortical state in awake mice without affecting power in the spindle band (7-15 Hz). In  
405 sleeping mice, tonic activation further increased slow waves and decreased spindle power,  
406 similar to the dynamics that occur during transitions into deeper stages of sleep. Interestingly,  
407 strong phasic activation of TRN induces spindles during non-REM sleep but not in the awake  
408 state [23, 24]. Phasic and tonic modulation of TRN activity therefore produce qualitatively  
409 different sleep oscillations, suggesting that changes in the dynamics of inputs to TRN could  
410 underlie shifts between different stages of sleep. Brief activation of TRN may lead to a thalamic  
411 burst that entrains a cortical spindle, whereas prolonged activation hyperpolarizes  
412 thalamocortical cells and allows intrinsic slow waves to emerge. Interestingly, a recent study  
413 demonstrated that high-frequency vs. low-frequency corticothalamic input produces qualitatively  
414 different effects in thalamus [56], consistent with the idea that modulating the temporal  
415 dynamics of input to the thalamocortical circuit can lead to different arousal states.

416

#### 417 **Circuit mechanisms underlying the induced thalamocortical slow waves**

418 The circuit mechanism that generates slow wave activity during sleep and anesthesia  
419 remains a topic of debate [21, 57, 58]. Our results show that slow waves can be produced by  
420 depolarizing TRN, and suggest they may be generated through overall inhibition of thalamic  
421 input to cortex. Our manipulation activated a local population of TRN neurons that inhibit the  
422 associated region of thalamus. In the absence of this thalamic input to cortex, which drives the  
423 desynchronized cortical state [30], cortex and thalamus jointly enter an oscillation in which  
424 activity is periodically suppressed. Previous studies have demonstrated that cortex can maintain  
425 an awake state even when thalamus is lesioned or inactivated [31, 59]; our results therefore  
426 suggest that slow waves in the intact brain require the involvement of cortical, TRN, and TC  
427 neurons in a coordinated rhythm. Direct inhibition or lesioning of TC cells may disrupt the  
428 coordination of this rhythm, whereas physiological levels of inhibition from TRN may allow the  
429 emergence of intrinsic thalamic delta. Furthermore, TRN stimulation did not induce slow waves  
430 in the anesthetized animal, when thalamic T-type calcium channels are blocked [60], suggesting  
431 that thalamus may contribute to slow wave generation. This theory is also consistent with  
432 studies showing that anesthetic infusions directly into thalamus can induce slow waves [61], and  
433 that manipulations of thalamus affect the frequency of slow waves [33]. Taken together, these  
434 findings suggest that TRN-mediated inhibition of thalamus is a robust driver of local slow wave  
435 activity, and that slow waves in the intact brain may require both cortical and TC neurons to fire

436 in a coordinated rhythm. Hyperpolarization – but not complete suppression – of thalamus may  
437 be key to generating slow waves, as TRN induces partial suppression and bursting in TC  
438 neurons (Figure 3b,j), as opposed to the stronger suppression achieved through direct  
439 manipulation of thalamus. However, TRN, thalamus, and cortex are independently capable of  
440 generating low-frequency rhythms [2, 62-64] and our results could be consistent with any or all  
441 of these areas acting as the slow wave pacemaker. Slow waves could arise through thalamic  
442 oscillations, could be generated in cortex due to withdrawal of thalamic excitatory drive, or could  
443 be jointly driven by both structures. In each scenario, TRN may act as a local regulator that can  
444 shift the thalamocortical circuit between desynchronized and oscillatory regimes.

445

#### 446 **Neuronal activity in TRN across arousal states**

447 Due to the technical challenges in recording from TRN, only a small number of previous  
448 studies have reported single unit recordings in TRN across arousal states. Interestingly, several  
449 reports have observed heterogeneous firing properties during sleep, and have suggested the  
450 possibility of multiple types of TRN neurons that play different roles in arousal state [19, 36].  
451 Such heterogeneity could explain the variable firing properties observed in different studies.  
452 While many TRN neurons decrease their firing rates during NREM sleep, a subset maintain or  
453 increase their firing rates [36, 37]. In addition, most TRN neurons exhibit bursting properties  
454 during sleep, with brief periods of activity locked to slow wave rhythms [19, 36, 37, 65]. These  
455 observations are consistent with our results, in which we observe heterogeneous firing rates in  
456 TRN, but nearly all units exhibit phase-locking to the induced slow waves during stimulation. It  
457 may be that during natural sleep, high firing rates in TRN inhibit thalamic activity and thereby  
458 induce slow waves, but those high rates are limited to only certain phases of the slow wave  
459 (rather than tonic continuous firing) due to synchronized delta-range input from thalamus and  
460 cortex. Experiments using closed-loop control to stimulate at specific phases of slow wave  
461 activity could explore whether tonic or phase-locked activity in TRN is most effective at driving  
462 cortical slow waves. It may also be that a specific subtype of TRN neuron induces slow wave  
463 activity, and that the microcircuitry of TRN enables this subtype to fire more while suppressing  
464 other TRN neurons during optogenetic stimulation. Future studies could also examine the effect  
465 of stimulation across multiple regions of TRN, as there are distinct subnetworks within TRN that  
466 may play different functional roles in regulating arousal [19, 44].

467

#### 468 **TRN activation as a component of general anesthesia**

469 The finding that TRN activation induces slow waves and decreases arousal could  
470 contribute to a subset of the effects of GABAergic drugs used for general anesthesia, such as  
471 propofol. In human subjects, propofol induces a large increase in low-frequency (0.1-4 Hz)  
472 power [66], and this slow wave induction has been suggested as a potential mechanism for  
473 unconsciousness [41, 67]. Propofol is a GABA-A agonist [68], suggesting that it could increase  
474 low-frequency EEG power by increasing the inhibitory effects of both TRN and brainstem  
475 structures on thalamus [50]. Decreased thalamic activity has also been implicated in disorders  
476 of consciousness [69], and may be a potent mechanism for inducing decreased arousal [51].  
477 Modulation of thalamic activity may therefore be an important component of general anesthesia.

478

#### 479 **TRN as one element of arousal control**

480 Partial inhibition of TRN during NREM sleep caused a reduction in slow wave activity,  
481 suggesting that TRN plays a role in slow waves observed during natural sleep. However, the  
482 decrease in power was modest. This small effect size could be partially due to incomplete  
483 inhibition of TRN due to local light delivery and incomplete expression, as little is known about  
484 the structure of intra-TRN circuits, and inhibiting only a subset of TRN cells may have different  
485 effects than inhibiting all of them. However, the small effect size likely also reflects the fact that  
486 multiple arousal centers, including many brainstem nuclei, are modulated during NREM sleep  
487 [49, 70], leading to broad thalamic inhibition. Suppressing TRN would thus only moderately  
488 reduce inhibitory input to the thalamus as other sources of inhibition persist, leading a reduction  
489 in slow wave activity rather than complete suppression. Similarly, stimulating TRN led to robust  
490 local cortical slow waves and a relatively small decrease in behavioural arousal, suggesting  
491 TRN activity drove local sleep and drowsiness more often than a complete transition into global  
492 sleep. Our results, in combination with previous studies, suggest that TRN acts as only one  
493 element of a redundant circuit for arousal control. Brainstem structures modulate global arousal  
494 state, whereas TRN may serve as a spatially selective circuit for fine-tuning arousal state across  
495 local cortical regions, allowing flexible modulation of slow wave activity. TRN may thus play a  
496 role in the local slow waves that subserve sleep-dependent memory consolidation, whereas  
497 brainstem would regulate the presence of sleep vs. wake states at a global scale.

498

#### 499 **TRN controls local cortical arousal state.**

500 We conclude that TRN can selectively induce slow waves in local cortical regions. Taken  
501 together, our results demonstrate that TRN can control oscillatory dynamics in local



502 thalamocortical circuits and suggest it could serve as a spatially selective circuit mechanism to  
503 rapidly and independently modulate cortical arousal.

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## 511 **Materials and Methods**

512

### 513 **Optogenetic manipulation**

514 All experimental procedures were approved by the MIT Committee on Animal Care.  
515 ChR2 expression was achieved through use of either viral injections targeted at TRN in VGAT-  
516 Cre mice (n=3 mice, Figure 1-figure supplement 3-4, Figure 3d) or through expression in VGAT-  
517 ChR2 mice (n=11 mice, all remaining figures). VGAT-ChR2 mice were obtained from Prof.  
518 Guoping Feng's laboratory and VGAT-Cre mice were obtained commercially (Jackson  
519 Laboratory, stock number 106962, *Slc32a1*). For viral injections, an AAV-EF1a-DIO-ChR2-  
520 EYFP virus was injected into two sites bilaterally (A/P 0.6 mm, M/L 1 mm, D 3.75/3.25mm ; A/P  
521 1.58 mm, M/L 1.9mm, D 3mm). Halorhodopsin experiments were done through injections of  
522 AAV-EF1a-DIO-eNpHR3.0-EYFP into the same sites as described above, again using VGAT-  
523 Cre mice (n=3 mice). These viruses were produced by the vector core at University of North  
524 Carolina, Chapel Hill, with titers around  $10^{12}$  VG/ml. A volume of 100-200 nL per injection site  
525 was used. Viral injections were immediately followed by implant of electrodes and optical fibers  
526 as described below. Mice with viral injections were implanted at least 3 weeks prior to beginning  
527 experiments to allow time for viral expression to develop.

528

### 529 **Cortical Implants**

530 In order to deliver light to TRN, all VGAT-ChR2 mice were implanted with a 0.21 NA fiber of 200  
531 micron diameter targeting left TRN (1.8 mm lateral, -0.8 to -1.7 mm posterior relative to bregma;  
532 2.1 mm deep). VGAT-Cre mice received implants of 2 to 4 optical fibers to allow for  
533 simultaneous manipulation of two sites in TRN. Two types of electrode implant were performed:

534 either a cortical implant with stereotrodes [71] distributed across different cortical sites; or a  
535 subcortical implant, with moveable stereotrodes targeted to TRN. Mice did not receive both  
536 implant types; an individual mouse in which units were recorded would receive either a cortical  
537 or subcortical implant. For the cortical implants, stereotrodes were made from pairs of 12.5  
538 micron nichrome wire gold plated to ~300 kOhm (California Fine Wire, Grover Beach CA).  
539 Electrodes were attached to small sections of plastic tubing cut to defined depth offsets and  
540 inserted by hand in 11 recording sites distributed across the cortex (Figure1a) at depths of 400,  
541 500, 600, or 1300 microns, as defined by the length of the electrode extending from the plastic  
542 tubing. In one mouse with the cortical implant, subcortical recordings were also acquired  
543 simultaneously by gluing a stereotrode to the optical fiber, with the stereotrode extending 200  
544 microns beyond the optical fiber. This allowed acquisition of single thalamocortical units. To  
545 calculate laser power within the brain, the laser power was first measured outside of the brain,  
546 and then this value was scaled to account for diminished power after passing through the fiber.  
547 For surgery, mice were anesthetized with 1% isoflurane and individual holes were drilled for  
548 electrode and optical fiber insertion. Electrodes were inserted by hand and the optical fiber was  
549 placed using a stereotaxic arm.

550

### 551 **Subcortical implants**

552 Hyperdrive bodies were designed in 3D CAD software (SolidWorks, Concord, MA) and  
553 stereolithographically printed in Accura 55 plastic (American Precision Prototyping, Tulsa, OK).  
554 Each hyperdrive was loaded with 6-8 individual, independently movable microdrives made of a  
555 titanium screw cemented to a 21-gauge cannula. Each microdrive was loaded with 1-3, 12.5  
556 micron nichrome stereotrodes (California Fine Wire Company, Grover Beach, CA), which were  
557 pinned to a custom-designed electrode interface board (EIB) (Sunstone Circuits, Mulino, OR).  
558 Two EMG wires, two EEG wires and one ground wire (A-M systems, Carlsborg, WA), were also  
559 affixed to the EIB. An optical fiber targeting TRN (Doric Lenses, Quebec, Canada) was glued to  
560 the EIB. TRN targeting was achieved by guiding stereotrodes and optical fiber through a linear  
561 array (dimensions ~1.1x1.8 mm) secured to the bottom of the hyperdrive by cyanoacrylate. For  
562 surgery, mice were anesthetized with 1% isoflurane and placed in a stereotaxic frame. For each  
563 animal, five stainless-steel screws were implanted in the skull to provide EEG contacts (a  
564 prefrontal site and a cerebellar reference), ground (cerebellar), and mechanical support for the  
565 hyperdrive. A craniotomy of size ~3x2mm was drilled with a center coordinate of (M/L 2.5mm,  
566 A/P -0.5mm). The implant was attached to a custom-designed stereotaxic arm, rotated 15

567 degrees about the median and lowered to the craniotomy. Stereotrodes were lowered slightly at  
568 the time of implantation (<500 microns) and implanted into the brain.

569

### 570 **Data acquisition**

571 Electrophysiology was performed in a total of eight VGAT-ChR2 positive mice, six VGAT-Cre  
572 mice with viral injections, and three mice negative for ChR2 (total=17 mice). Electrophysiology  
573 data was acquired on a Neuralynx (Neuralynx, Bozeman MT) system with a 32 kHz sampling  
574 rate. Full sampling was used to record spikes, detected with a manually set voltage threshold.  
575 LFPs were collected with a highpass filter between 0.1 and 0.3 Hz and a lowpass between 2000  
576 and 9000 Hz. EMG was collected with a highpass filter of 10 Hz to prevent data saturation. All  
577 electrophysiology data was exported to MATLAB (Mathworks, Natick MA), and LFPs and EMGs  
578 were then lowpass filtered offline at 500 Hz and downsampled to 1000 Hz sampling rate. Spike  
579 sorting was performed with custom software (Simpleclust, [http://github.com/open-](http://github.com/open-epphys/simpleclust)  
580 [epphys/simpleclust](http://github.com/open-epphys/simpleclust)), using standard waveform features to classify spikes. Spikes that could not  
581 be assigned to a well-defined cluster were labeled as multi-unit activity, and triphasic waveforms  
582 were excluded as fibers of passage. Awake recordings were carried out in either a head-fixed  
583 setup or in a clear plastic bowl. Anesthetized recordings were performed with isoflurane in 100%  
584 oxygen, in which drug levels were increased if the animal showed any signs of motion, and  
585 decreased when the EEG showed burst suppression, for an average range of 0.6% to 1%  
586 isoflurane. Experiments in anesthetized trials were performed only after anesthesia was induced  
587 with at least 1.5% isoflurane and mice had lost the righting reflex, and isoflurane was  
588 maintained at at least 0.5% isoflurane throughout the stimulation period. Anesthetic levels were  
589 varied manually to stay within a lightly anesthetized range, by decreasing levels if the EEG  
590 showed burst suppression and increasing levels if mice showed any sign of movement. In  
591 sessions with automated motion quantification, two video cameras were mounted at two  
592 orthogonal angles to enable automated motion capture.

593

### 594 **Laser stimulation**

595 ChR2 expressing neurons were activated with a DPSS laser with a wavelength of 473  
596 nm. Halorhodopsin expressing neurons were activated with a DPSS laser with a wavelength of  
597 579 nm. In VGAT-ChR2 mice, light was delivered as 30 second stimulation periods using steady  
598 light levels (DC stimulation), followed by at least 30 seconds (typically 60-90 seconds) with no  
599 stimulation. Light was maintained at constant levels throughout a single 30 second period. For  
600 experiments comparing different laser strengths, the laser output was varied within a single

601 session, but not within a single 30 second stimulation period. Simulations for the transmission of  
602 light through tissue at these different laser strengths were performed using the calculator  
603 developed by the Deisseroth lab (<http://web.stanford.edu/group/dlab/cgi-bin/graph/chart.php>). In  
604 VGAT-Cre mice, two sites were stimulated simultaneously by using a splitter (Doric Lenses) to  
605 generate two matched light sources. Light levels were kept below 4 mW for all recordings  
606 except those mice with viral injections (Figure 1-figure supplement 3-4, Figure 4d), in which  
607 power was increased to between 4-5 mW to compensate for the lower expression levels. Most  
608 recording sessions in VGAT-Cre mice with viral injections used a 5 second DC stimulation  
609 period instead of 30 seconds as a precaution to avoid any tissue heating from the increased  
610 laser power, but the subset of sessions with 30 second stimulation periods showed similar  
611 results. Recording sessions were limited to no more than 60 stimulation trials to prevent  
612 habituation effects, although none were observed in the data, with typical sessions lasting  
613 approximately 2 hours.

614

## 615 **Histology**

616 Animals were perfused using 4% paraformaldehyde (PFA) in phosphate-buffered saline  
617 (PBS) and brains were extracted and fixed in PFA-PBST. A vibratome was used to collect 60  
618 micron coronal slices stored in PBS. Images show DAPI staining in blue and EYFP in green. 2x  
619 and 10x images were taken on a Zeiss Axio M2 microscope. In addition to the histology from  
620 mice in which we performed electrophysiological recordings (Figure 1 Supp 1, 2, 5; Figure 4-  
621 figure supplement 2), we also injected an additional VGAT-Cre mouse with the same  
622 halorhodopsin virus and performed histology at higher resolution. The same histological  
623 methods were used, except that slices were 65 microns thick. These images were taken on a  
624 Zeiss 750 confocal microscope (Figure 4-figure supplement 3). Cell counting was performed  
625 manually, and the proportion of cells that were positive (i.e. were surrounded by a fluorescent  
626 ring) was reported with error bars indicating the 95% confidence intervals calculated  
627 theoretically from the binomial distribution.

628

## 629 **Spectral analysis**

630 Spectra were computed with the Chronux toolbox using 19 tapers over 30 second  
631 windows. Spectrograms were computed with 5 tapers in 5 second sliding windows every 1  
632 second. Normalized spectrograms were computed by first taking the median power across all  
633 trials, and then dividing the power in each frequency by the mean of the power at that frequency  
634 during the 30 second pre-stimulus window. Error bars across multiple sessions (e.g. Figure 1d)

635 show the standard error of the mean across all trials. Statistical testing was done by taking the  
636 sum of power within a band of interest on individual trials, and then comparing power during the  
637 30 second stimulation against power during the 30 seconds immediately preceding TRN  
638 stimulation. Change in power is reported as the median effect size and 95% confidence  
639 intervals, computed by inverting the Wilcoxon signed-rank test. Automatic slow wave event  
640 detection was performed by bandpassing the LFP between 0.1-5 Hz using a finite impulse  
641 response filter, detecting local minima with magnitude  $>100 \mu\text{V}$ , and computing the difference  
642 between the negative trough and subsequent peak. The top 3% percentile of peak-to-peak  
643 amplitudes within each session were selected as slow wave events. Any peak within a 400 ms  
644 window with standard deviation  $>2000$  was rejected as artifact. The difference in the number of  
645 peaks was statistically tested using a Wilcoxon signed rank test to compute the median  
646 difference in slow wave event numbers in the stimulated vs. baseline periods for each trial. For  
647 analyses across electrodes, the change in power was computed for each electrode and the  
648 Bonferroni correction was applied for multiple comparisons across electrodes. To compare the  
649 number of activated electrodes in local vs. global electrodes, and across laser power conditions,  
650 we treated the number of significant electrodes as a binomial distribution. We assumed a  
651 uniform prior for the binomial parameter, obtaining a beta density as the posterior distribution for  
652 each proportion. We estimated the difference between two conditions by sampling 1000 times  
653 from the posterior distributions in each condition, and calculating the median and 95%  
654 confidence intervals as the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles of the difference between each  
655 resampled datapoint (i.e. a Monte Carlo bootstrap for the difference between two groups). To  
656 compute the PLV, each channel was filtered between 1-4 Hz and Hilbert transformed to extract  
657 the instantaneous phase. The PLV was then taken as the circular mean of the difference in  
658 phase for each electrode relative to the electrode closest to the optical fiber [40]. To test  
659 whether the magnitude of the PLV was greater than expected by chance, the trial labels were  
660 shuffled 500 times and the true PLV was compared to permuted PLV values from the shuffled  
661 trials, at  $\alpha=0.05$ . To report the confidence interval for the angle of the PLV, the trials were  
662 resampled with a bootstrap procedure and the PLV angle across the resampled data was  
663 calculated 500 times.

664

### 665 **Behavioral analysis**

666 All trials used for behavioral analysis were collected while mice behaved freely in a clear  
667 plastic bowl. Recording sessions lasted 1-2 hours and were performed during the day. Two  
668 video cameras were mounted at two orthogonal angles to enable automated motion capture.

669 EMG effects were calculated using the Chronux toolbox to determine power in the 10-200 Hz  
670 band in non-overlapping windows of 1 second width. Power was summed across all frequencies  
671 within the band to obtain a single measure of EMG power. Statistical testing was performed with  
672 the Wilcoxon signed-rank test, comparing EMG power within each laser trial with the EMG  
673 power in the associated pre-stimulus period. The rapid onset of the EMG effect was assessed  
674 by comparing EEG power in the second prior to laser onset with power in the second following  
675 laser onset. Overall changes in behavior were calculated by comparing the [-30 0] baseline  
676 period to the [0 30] laser-induced period.

677 The correlation between EEG and EMG power was calculated by computing the  
678 correlation coefficient between the change in delta (1-4 Hz) power in the EEG, calculated as the  
679 difference in the [-30 0] and [0 30] periods relative to laser onset, and correlating it with the  
680 change in EMG power across those same periods. Statistical significance was tested by  
681 performing a bootstrap with 1000 iterations on the paired power data, taking the 97.5%  
682 percentile of these resampled values, and testing whether its absolute value was larger than the  
683 correlation computed on a randomly shuffled set of times. This provided a test at significance  
684 level  $\alpha=0.05$  of whether the correlation between EEG and EMG effects was significantly  
685 higher during laser trials than would be expected during baseline conditions. Automated motion  
686 scoring was computed using automated custom software written in Matlab (to appear at  
687 [http://github.com/jvoigts/optical\\_flow\\_analysis](http://github.com/jvoigts/optical_flow_analysis)) that calculated the optical flow for each frame in  
688 the video via the Horn–Schunck method [72]. The points of maximal motion in each camera  
689 view were used to compute a motion vector in the horizontal plane. The motion vector was  
690 normalized by its mean to avoid artifact due to variations in lighting conditions and camera  
691 placement. The magnitude of the motion vector for each frame was then smoothed using a  
692 moving average filter ( $\sigma=200$  frames/6.67 s) and used as a proxy for the magnitude of  
693 animal's overall motion. In order to ensure objective assessment of sleep, semi-automated  
694 sleep scoring was performed using an algorithm that first detects wake states as periods with  
695 heightened muscle tone by computing the instantaneous amplitude between 60-200 Hz of the  
696 EMG or cranial screw recording motor activity, smoothing with a Gaussian filter of 50 ms, and  
697 then using a manually entered threshold to identify wake states as those with at least 5 seconds  
698 of data above the threshold. Second, it computes the ratio of <4 Hz and 4-16 Hz power in the  
699 EEG, smooths with a Gaussian filter, and uses a manually entered threshold to segment non-  
700 REM and REM states. Sessions where the automated algorithm could not achieve a separation  
701 of sleep and wake states were not included in the analysis (1 session out of 13 total was  
702 excluded). The user selecting the EEG and EMG thresholds was blind to the timing of laser

703 stimulation during the sleep scoring procedure. As with spectral effects, changes were reported  
 704 as the median power change in dB, and 95% confidence intervals were computed by inverting  
 705 the Wilcoxon signed-rank test. The shuffled control to test whether halorhodopsin-driven  
 706 decreases in slow wave activity were larger than would be expected by chance was performed  
 707 by pseudorandomly selecting an equivalent number of laser onset times during spontaneous  
 708 NREM sleep and recalculating the change in slow wave power. This shuffling procedure was  
 709 repeated 400 times to produce confidence intervals.

710

### 711 **Single unit analysis**

712 Statistical comparisons of firing rates were performed using confidence intervals derived  
 713 from the Wilcoxon signed-rank test to quantify the median difference between each neuron's  
 714 pre-stimulus ([-30 0] s) and stimulus-induced ([0 30] s) spike rates. To compute phase  
 715 modulation, the instantaneous phase was calculated from the local LFP channel by bandpass  
 716 filtering the LFP between 1-4 Hz with a finite impulse response filter, taking the Hilbert transform,  
 717 and then extracting the angle. The phase distribution of individual units relative to the delta  
 718 phase was quantified using the modulation index (MI), adapted from phase-amplitude  
 719 measurements [73], which measures the Kullback-Liebler distance between the observed phase  
 720 distribution and a uniform distribution. The MI for spikes was computed over 10 phase bins as

721  $\sum_{i=1}^{10} p_i \log_2 p_i + \log_2 10$ , where  $p_i$  is the proportion of spikes falling within a given phase bin. The

722 MI for gamma power was computed over 100 phase bins using a sinusoid fit to model the  
 723 amplitude of the gamma oscillation. The effect of TRN activation on unit phase modulation was  
 724 assessed as in the firing rate case, with median effect sizes and 95% confidence intervals  
 725 derived from a Wilcoxon signed-rank test comparing each neuron's MI values in the [-30 0] and  
 726 [0 30] periods. When comparing cortical units on channels with and without a slow wave effect,  
 727 normalized delta power changes were computed by dividing delta (1-4 Hz) power by total 0-50  
 728 Hz power in the [-30 0] and [0 30] periods. Electrodes with a normalized delta power increase of  
 729 at least 2% during TRN activation were labeled as having a delta effect. Subcortical units were  
 730 divided into two categories based on the time between the peak and trough of the waveform.  
 731 Narrow (<200 ms peak-to-trough) units were further subdivided into three categories based on  
 732 their spike rate response to the laser, computed by taking the difference of their spike rates in  
 733 the [0 30] s period vs. the [-30 0] period. Phase modulation was computed relative to the local  
 734 LFP for each unit. To account for sign reversals due to electrode placement or referencing and  
 735 ensure consistent phase measurements across units, thalamic LFPs were flipped such that the

736 laser-induced deflection was negative across all channels. The fraction change in phase-locking  
737 strength for each unit was calculated by subtracting the MI during the stimulated period from the  
738 MI during baseline, and then normalizing by the MI during baseline. The peak phase was  
739 selected by dividing spikes into 10 phase bins and identifying the bin containing the most spikes.  
740 The narrowness of the phase distribution was tested by computing the kurtosis separately for  
741 putative TC and putative RE units using the CircStat toolbox [74]. The difference between the  
742 two unit types was then calculated. To test whether this difference was significant, the difference  
743 in kurtosis was bootstrapped 1000 times with random resampling of units, shuffling the unit type  
744 assignment, and then the original difference in kurtosis was compared to the 95% confidence  
745 interval derived from the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentile of the resampled differences. To test the  
746 timing of the rate decrease relative to laser onset, 10 ms windows in the 2 second pre-stimulus  
747 period were used to create a distribution of baseline values, which was bootstrapped 1000 times  
748 to determine a threshold for significant change (the 0.25<sup>th</sup> percentile,  $\alpha=0.005$ ). 10 ms  
749 windows after laser onset were then compared to this threshold to assess timing of a significant  
750 change. Triggered LFP analysis was done analogously, averaging the mean LFP value in 10 ms  
751 bins. For LFP and spike timing analyses, the alpha level was set at 0.005 to correct for multiple  
752 comparisons across time bins (10 bins of 10 ms width to span the 100 ms deflection interval).

753

#### 754 **OFF period analysis**

755 To detect OFF periods, we combined all multi-unit and single-unit activity on a single  
756 channel into a point process representation, and then smoothed with a Gaussian kernel with a  
757 standard deviation of 20 ms to approximate an instantaneous firing rate in the units surrounding  
758 that electrode. OFF periods were labeled as any period of at least 50 ms with a firing rate of  
759 zero. To verify that OFF periods were occurring at a greater rate than would happen by random  
760 chance, we also computed OFF periods on simulated data with the same mean firing rate as the  
761 experimental data. The simulated data was generated by taking the interspike intervals  
762 throughout the recording period, fitting a gamma distribution to these intervals, and then  
763 generating a new spike train from that gamma distribution with the same number of spikes as  
764 the original dataset. The OFF periods were then calculated with the same method for the  
765 simulated data. Statistical testing for OFF periods was performed across trials within each  
766 session: the percent of time spent in an OFF period during laser stimulation was compared to  
767 the percent of time spent in an OFF period in the 30 seconds preceding laser stimulation with  
768 the Wilcoxon signed-rank test. The significance of this difference within each animal is reported.  
769 The percent of time in OFF periods was compared to simulated data by running the simulation



770 1000 times and testing whether the experimental value was greater than the 97.5<sup>th</sup> percentile of  
771 the simulated value. Statistical testing for the phase distribution of OFF periods was computed  
772 by splitting the data into 10 phase bins and testing for uniform distribution of OFF periods using  
773 Pearson's chi-square test – a significant result indicated that OFF periods were not uniformly  
774 distributed across the LFP slow wave phases, but rather appeared predominantly at specific  
775 phases.

776

### 777 **GLM analysis**

778 Temporal firing rate patterns were quantified using a generalized linear model, in which  
779 a unit's spike rate over time was modeled as a Poisson process with rate as a function of  
780 previous spike history. The model covariates consisted of either a 1 or a 0 to indicate whether a  
781 spike was observed in any given preceding time bin. The model used 50 bins of 2 millisecond  
782 width each. GLMs were fit in Matlab and confidence intervals were calculated using 'glmfit',  
783 which were then used to determine for each cell whether the parameter estimate for the [2 4] ms  
784 was significantly different during TRN stimulation as compared to baseline, with alpha=0.05.  
785 The proportion of cells with a significant change in model parameter estimates is reported.

786

787

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793

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991

992

## 993 Figure Legends

994

995 **Figure 1: Tonic optogenetic stimulation of thalamic reticular neurons produces local**  
 996 **cortical slow waves.** A) Diagram of surgery: fiber is implanted into left TRN, and stereotrodes  
 997 are implanted in multiple sites across cortex. B) Spectrogram showing average effect in  
 998 ipsilateral somatosensory cortex across 168 trials (4 mice): TRN stimulation causes a rapid  
 999 increase in delta (1-4 Hz) power that persists throughout the stimulation period. Power is  
 1000 normalized to the 30 second pre-stimulus period. C) Average spectrum of LFP in  
 1001 somatosensory cortex: during tonic optogenetic activation of TRN, this cortical site  
 1002 demonstrates an increase in delta (1-4 Hz) power and a decrease in beta and gamma (12-50  
 1003 Hz) power. Gray region shows zoomed-in plot of delta power increase. D) Example trace from a  
 1004 single trial, showing the LFP filtered between 1-4 Hz (black line), and the instantaneous delta  
 1005 amplitude (red line). E and F) Circles represent single electrodes, and their color indicates the  
 1006 size of the delta (1-4 Hz) power increase when laser is on (total n=136 trials, 4 mice, 12-14  
 1007 electrodes per mouse). At low powers (<2 mW), slow waves are induced only in electrodes near  
 1008 ipsilateral somatosensory cortex (red dashed circle). At high powers (>2 mW) that activate  
 1009 larger regions of TRN, slow waves appear across multiple cortical areas, including frontal cortex  
 1010 and contralateral cortex (red dashed circle). Distances are jittered so that electrodes from all  
 1011 mice can be displayed in a single schematic. Blue 'X' indicates placement of laser fiber. G)  
 1012 Example spectra from one mouse at low laser power in electrodes ipsilateral and contralateral  
 1013 to the laser fiber (n=10 trials): slow waves are induced in ipsilateral cortex but not in  
 1014 contralateral cortex. H) Example spectra from same mouse at high laser power (n=9 trials): slow  
 1015 waves are generated in both ipsilateral and contralateral cortex.

1016

1017 **Figure 2: Cortical units undergo OFF periods that are phase-locked to the slow waves**  
 1018 **during TRN activation.** A) Rate effect across all cortical units, categorized by strength of delta  
 1019 power increase in that channel. There is no significant change in spike rate for either group.  
 1020 Error bars show interquartile range. B) Phase-locking effects across all cortical units show that  
 1021 units on channels with induced slow waves become phase-locked to the slow waves during  
 1022 TRN stimulation. Error bars show interquartile range. Each dot is one unit (sites with slow  
 1023 waves: 13 units, 4 mice; sites without slow waves: 18 units, 4 mice.) C) Phase distribution of  
 1024 spikes from an example cortical unit recorded on a channel with a 3.4 dB delta power increase  
 1025 during TRN activation: unit becomes phase-locked to the slow wave. D) Phase distribution of  
 1026 normalized high gamma (70-100 Hz) power shows that high gamma power becomes rapidly  
 1027 phase-locked to slow waves during TRN stimulation. Gamma power is normalized to have a  
 1028 mean of 1 at each time point, so brightness indicates the strength of phase-locking. E) Phase  
 1029 distribution of all OFF periods shows that they occur during the trough of the slow waves. F)  
 1030 Example trace from somatosensory cortex: optogenetic TRN stimulation rapidly induces slow  
 1031 waves that are associated with OFF periods in cortical activity (gray shaded regions mark  
 1032 automatically detected OFF periods). G) Mean spike rate and LFP locked to laser onset in

1033 channels with induced delta: the induced slow wave trough and phase-locked cortical inhibition  
 1034 are observed within 100 ms of laser onset. Stars indicate timing of significant ( $\alpha=0.05$ ) decrease  
 1035 in LFP voltage and mean spike rate; the decrease persists throughout the first 100 ms.  
 1036 Triggered LFP and units are averaged across cortical electrodes with a delta power increase  
 1037 ( $n=14$  channels, 4 mice), shaded region is std. err.

1038

1039 **Figure 3: Optical stimulation strongly activates a subset of TRN neurons and induces**  
 1040 **periodic suppression of thalamic firing.** A) Histogram of waveform parameters from single  
 1041 units recorded in freely behaving mice show a bimodal distribution of peak-to-trough time across  
 1042 subcortical units ( $n=28$  units, 5 mice). Units with peak-to-trough times under  $200\ \mu\text{s}$  were  
 1043 categorized as Narrow (putative TRN), and units over  $200\ \mu\text{s}$  were categorized as Wide  
 1044 (putative thalamocortical (TC)). B) Putative thalamocortical (Wide) units consistently decrease  
 1045 their firing rates during laser stimulation. Mean firing rate in 500 ms bins, shaded region is std.  
 1046 err. across units. C-E) Heterogeneous firing rates in TRN during stimulation: 4 units strongly  
 1047 increase their firing rates, whereas 10 units decrease their firing rates. The modulation in firing  
 1048 rate is strongly time-locked to laser onset and offset. Shaded regions are std. err. across units.  
 1049 F) Phase-locking effects across all subcortical units show that most become phase-locked to the  
 1050 slow waves during TRN stimulation. Circles mark the change in phase-locking for each unit;  
 1051 error bars show median change with 25th and 75th quartiles. G) The phase distribution of  
 1052 putative TRN neurons is broad, with different neurons exhibiting different preferred phases. H)  
 1053 Peak phase-locking values of putative TC neurons show a tight distribution (Kurtosis=3.99,  
 1054  $n=11$  units), indicating that nearly all putative TC neurons show similar phase-locking to the LFP.  
 1055 Putative TC phase-locking is more consistent across units than putative TRN phase-locking (in  
 1056 B; Kurtosis=0.24,  $n=17$  units, group difference=3.74, significant at  $\alpha=0.05$  from bootstrap  
 1057 resampling). I) Example spike rasters around laser onset from 3 single units. Units were not  
 1058 recorded simultaneously; each raster is an independent example. J-L) Example ISI histograms  
 1059 in single units. M) Example of parameter estimates from generalized linear model for one unit  
 1060 shows the contribution of recent ( $<10$  ms) spike history increases during stimulation. Shaded  
 1061 regions are std. err.

1062

1063 **Figure 4: TRN modulates arousal state in a bidirectional and state-dependent manner.** A)  
 1064 Top panel: Mean EMG power locked to laser onset shows that EMG power decreases  
 1065 significantly during unilateral TRN stimulation in freely behaving mice ( $n=315$  trials, 8 sessions,  
 1066 2 mice). Bottom panel: Mean smoothed motion (6.67 second moving average) detected in  
 1067 video: animals' motion decreases significantly during optogenetic stimulation ( $n=421$  trials, 7  
 1068 mice). B) Mean change in arousal state during TRN activation: mice spend significantly more  
 1069 time in non-REM sleep and significantly less time in the awake state ( $n=560$  trials, 3 mice).  
 1070 Stars indicate significant effects at  $\alpha=0.05$ . C) Individual trial correlation shows that the  
 1071 decrease in EMG power is correlated with the TRN-induced increase in EEG delta power  
 1072 ( $n=315$  trials, 2 mice). D) Delta power increases in VGAT-Cre mice expressing ChR2 during  
 1073 TRN stimulation, whether awake or in NREM at time of stimulation. In VGAT-Cre mice  
 1074 expressing halorhodopsin, TRN inhibition has no effect in awake mice, whereas it decreases the  
 1075 delta power that is present in sleeping mice.  $N=3$  mice expressing ChR2 (160 wake trials; 192  
 1076 NREM trials),  $n=3$  mice expressing Halo (459 wake trials; 211 NREM trials), stim. duration= 5  
 1077 seconds. All recordings were in freely behaving mice. Dots show mean power  $\pm$  std. err; stars  
 1078 indicate a significant effect of the laser on the median power, computed with the Wilcoxon  
 1079 signed-rank test. E) Cortical recordings in VGAT-ChR2 mice ( $n=186$  trials, 3 mice). During



1080 isoflurane anesthesia, the slow waves appear to be saturated and are not increased by TRN  
1081 stimulation. Instead, broadband power decreases, suggesting a shift in dynamics that favours  
1082 the inactivated state.

1083  
1084 **Figure 1 - figure supplement 1: Selective TRN stimulation causes the induction of cortical**  
1085 **slow waves.** VGAT-ChR2 mouse histology: blue channel is DAPI, green channel is EYFP, at  
1086 2x.

1087  
1088 **Figure 1 - figure supplement 2: Example of VGAT-ChR2 mouse histology at 10x.** Around  
1089 the optical fiber, expression is limited to TRN.

1090  
1091 **Figure 1 - figure supplement 3: Spectra of cortical LFPs recorded in VGAT-Cre mice**  
1092 **expressing ChR2 selectively in TRN through local injections.** These mice also show a TRN-  
1093 induced selective increase in slow wave power (median=0.73 dB, CI=[0.47 0.99], n=370 trials, 3  
1094 mice).

1095  
1096 **Figure 1 - figure supplement 4: Normalized spectrogram recorded in VGAT-Cre mice**  
1097 **expressing ChR2 selectively in TRN through local injections.** These mice also show a TRN-  
1098 induced selective increase in slow wave power that is locked to laser onset. Spectrogram is  
1099 normalized to baseline within each frequency band, using same data as in Figure 1-figure  
1100 supplement 3..

1101  
1102 **Figure 1 - figure supplement 5: After viral injections, ChR2 expresses selectively in TRN.**  
1103 Example of histology at 10x, large-scale and zoomed-in, from a VGAT-Cre mouse with ChR2  
1104 viral injections. Blue channel is DAPI and green channel is EYFP, showing selective TRN  
1105 expression.

1106 **Figure 1 - figure supplement 6: Slow wave induction depends on ChR2 expression.**  
1107 Control mice that are negative for ChR2 do not exhibit slow waves during laser stimulation  
1108 (n=494 trials, 3 mice).

1109 **Figure 1 - figure supplement 7: Simulation of light transmission through tissue at**  
1110 **different laser powers.** Simulations using Yizhar et al. 2011 to predict irradiance with  
1111 increasing distance. The higher laser power would stimulate a larger volume of tissue (e.g. if the  
1112 threshold for stimulation is 1 mW/mm<sup>2</sup>, 0.4 mm would be stimulated when applying 1mW, and  
1113 0.75 mm would be stimulated when applying 3 mW, nearly twice the distance.

1114 **Figure 1 - figure supplement 8: Phase offsets across cortex during TRN stimulation.**  
1115 Phase offsets are strongly correlated across baseline and stimulation conditions in electrodes  
1116 with induced slow waves, indicating that the induced slow waves maintain similar phase offsets  
1117 to the baseline cortical dynamics. Each dot is one electrode, n=17 electrodes, 4 mice, black line  
1118 is correlation.

1119 **Figure 1 - figure supplement 9: Phase offsets across cortex are not correlated with**  
1120 **distance to the electrode.** Phase offsets in cortical electrodes were not correlated with their  
1121 distance from the electrode local to the stimulation site (R=-0.31, p=0.22). This would be  
1122 consistent with local thalamocortical oscillations, but correlations may also be weakened  
1123 because many regions that are synaptically close are still geometrically distal to the local  
1124 stimulation site.

1125 **Figure 3 – figure supplement 1:** Example waveforms for putative TC and TRN neurons. A

1126 representative set of waveform shapes is presented. The putative TRN units have a narrower  
1127 trough than the putative TC units.

1128 **Figure 4 - figure supplement 1: Laser-induced behavioural decreases in arousal depend**  
1129 **on Chr2 expression.** EMG power does not decrease during laser stimulation in control mice  
1130 that are negative for Chr2, confirming that the behavioral effect is not due to a nonspecific  
1131 effect of light (n=494 trials, 3 mice).

1132 **Figure 4 - figure supplement 2: Halorhodopsin expresses in TRN.** Example of histology at  
1133 10x, large-scale and zoomed-in, from a VGAT-Cre mouse with NpHR viral injections. Blue  
1134 channel is DAPI and green channel is EYFP, showing selective TRN expression.

1135 Figure 4 – figure supplement 3: **Halorhodopsin expresses in most cell bodies within the**  
1136 **locally injected region of TRN, and not in thalamic cell bodies outside TRN.** A) Higher-  
1137 resolution image of expression within TRN in a VGAT-Cre mouse with NpHR viral injections.  
1138 The striped fluorescence pattern is due to the anatomical structure of TRN, which is a netlike,  
1139 reticulated structure. Dense rings of fluorescence appear around the TRN cell bodies. Right  
1140 panels: Zoomed-in images demonstrate that TRN neuronal cell bodies are encircled by bright  
1141 fluorescence from membrane-bound EYFP, indicating NpHR expression. Bottom panels:  
1142 Zoomed-in images in thalamus demonstrate that expression is only in projections from TRN,  
1143 and is not in the thalamic cell bodies (no ring of fluorescence surrounds the cells). B) Cell  
1144 counting in dorsal and ventral TRN shows that the majority of cells in both regions were positive  
1145 for EYFP expression. Error bars are 95% confidence intervals.

1146 **Figure 4 - figure supplement 4: TRN stimulation further increases cortical neuronal phase**  
1147 **modulation during isoflurane anesthesia.** Phase-locking effects across all cortical units  
1148 during isoflurane anesthesia: units become significantly more modulated by slow waves when  
1149 TRN is activated. Error bars are st. dev, (n=15 units, 4 mice).

1150 **Figure 4 - figure supplement 5: Example of state-dependent increases in cortical phase-**  
1151 **locking during isoflurane anesthesia.** TRN activation in an awake mouse causes a cortical  
1152 single unit to become phase-locked to the induced slow waves. When the mouse is under  
1153 isoflurane anesthesia, the cortical unit is already phase-locked to slow waves at baseline, and  
1154 TRN activation causes the phase-locking to become even sharper, with some phases  
1155 associated with complete suppression of firing.

1156 **Figure 4- figure supplement 6: TRN stimulation deepens thalamic neuronal suppression**  
1157 **during isoflurane anesthesia.** Putative thalamic units in the awake mouse are inhibited during  
1158 TRN stimulation. Putative thalamic units in the anesthetized mouse have a baseline firing rate  
1159 slightly lower than the awake, TRN-stimulated mouse. TRN stimulation induces an even larger  
1160 suppression of thalamic activity.

1161

1162 **Video 1:** Laser stimulation in a mouse expressing NpHR during NREM sleep. The mouse  
1163 maintains NREM sleep during the stimulation and the light alone does not cause visible  
1164 behavioural changes.

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