ErbB4 regulation of a thalamic reticular nucleus circuit for sensory selection

Sandra Ahrens1, Santiago Jaramillo1,3,4, Kai Yu1,4, Sanchari Ghosh1, Ga-Ram Hwang1, Raehum Paik1, Cary Lai2, Miao He1,3, Z Josh Huang1 & Bo Li1

Selective processing of behaviorally relevant sensory inputs against irrelevant ones is a fundamental cognitive function whose impairment has been implicated in major psychiatric disorders. It is known that the thalamic reticular nucleus (TRN) gates sensory information en route to the cortex, but the underlying mechanisms remain unclear. Here we show in mice that deficiency of the Erbb4 gene in somatostatin-expressing TRN neurons markedly alters behaviors that are dependent on sensory selection. Whereas the performance of the Erbb4-deficient mice in identifying targets from distractors was improved, their ability to switch attention between conflicting sensory cues was impaired. These behavioral changes were mediated by an enhanced cortical drive onto the TRN that promotes the TRN-mediated cortical feedback inhibition of thalamic neurons. Our results uncover a previously unknown role of ErbB4 in regulating cortico-TRN-thalamic circuit function. We propose that ErbB4 sets the sensitivity of the TRN to cortical inputs at levels that can support sensory selection while allowing behavioral flexibility.

The TRN is a thin sheet of exclusively GABA-producing neurons located between the cortex and dorsal thalamus1,2. The TRN integrates both cortical and thalamic synaptic inputs but sends outputs only to the thalamus. This unique anatomical location and connectivity have led to the proposal that the TRN controls attention through ‘gatekeeping’ of sensory information passing through the thalamus1–4. Consistent with this hypothesis, it has been shown that the activity of TRN neurons correlates with behavioral state5, sensory detection6,7 and attention8–11 and that lesions of the TRN impair attentional orienting11. In addition, TRN dysfunction has been implicated in schizophrenia2,12–14,16,17, a mental disorder in which altered sensory processing and attentional deficit are prominent features15. Nevertheless, the cellular and synaptic mechanisms underlying TRN function remain unclear.

Recent studies suggest that the TRN may be regulated by a unique set of signaling molecules16,17. For example, TRN neurons express high levels of ErbB4 (ref. 16), a receptor tyrosine kinase that has important roles in multiple biological processes, including neurodevelopment, neuronal excitability, and the function of excitatory and inhibitory synapses18,19. Notably, ErbB4 and its ligand neuregulin-1 (NRG1) have been associated with schizophrenia and other mental disorders by human genetic studies18,19. In light of these findings, as well as those that implicate the TRN in attention and TRN dysfunction in mental disorders12,13,16,17, it is of great interest to investigate the potential role of ErbB4 in regulating TRN circuit function in behaviors that demand sensory processing and attention.

In this study, we manipulated ErbB4 content in a major population of TRN neurons in mice and assessed the effects on performance in novel sensory selection tasks. We observed robust behavioral effects that are likely to reflect changes in attention. Furthermore, by combining electrophysiological, optogenetic and molecular techniques, we identified a critical synaptic change in the cortico-TRN-thalamic circuitry that is responsible for the ErbB4 deficiency–induced behavioral phenotypes.

RESULTS

Targeting ErbB4 in the TRN and establishing behavioral tasks

To investigate the mechanisms underlying TRN function, we sought to use genetic techniques to selectively target TRN neurons. Given that a major TRN population expresses somatostatin (SOM)21,22, we used the Som-Cre mice23 that, when bred with the Ai14 reporter mice24, allow the easy identification of SOM-expressing (SOM+) TRN neurons (Fig. 1a–c). Previous studies indicate that TRN neurons express high levels of ErbB4 (refs. 16,18,25). Unexpectedly, we found that ErbB4 is primarily expressed in SOM+ neurons in the TRN (Fig. 1d). In contrast, ErbB4 is largely excluded from SOM− neurons in other brain areas, such as the cortex and hippocampus26,27 (Fig. 1d, Supplementary Fig. 1). Taking advantage of this specific coexpression profile, we manipulated ErbB4 levels selectively in SOM+ TRN neurons by breeding the Som-Cre;Erbb4lox/lox mice28, thereby generating the SomErbb4+/− or SomErbb4−/− mice (Fig. 1d).

Because the TRN is involved in sensory detection6,7 and attention8–11, we reasoned that ErbB4 deficiency in TRN neurons might affect TRN function, thereby impairing behavioral performance that relies on sensory processing and demands attention. To test this hypothesis, we examined mice in behavioral tasks based on a two-alternative choice (2-AC) paradigm29,30 (Fig. 2 and Online Methods), which engage animals in the selection of competing sensory

1Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA. 2Indiana University, Bloomington, Indiana, USA. 3Present addresses: Institute of Neuroscience and Department of Biology, University of Oregon, Eugene, Oregon, USA (S.J.); Institutes of Brain Science, Fudan University, Shanghai, China (M.H.). 4These authors contributed equally to this work. Correspondence should be addressed to B.L. (bl@cshl.edu).

Received 20 September; accepted 14 November; published online 15 December 2014; doi:10.1038/nn.3897
Figure 1 SOM⁺ neurons are a major TRN population expressing ErbB4. (a) Left, a representative image of a coronal TRN section from a Som-Cre;Ai14 mouse. SOM⁺ neurons were identified on the basis of the intrinsic fluorescence of tdTomato (SOM/Tomato). Middle, the same brain section processed for immunohistochemistry with an antibody recognizing NeuN to label all the neurons. Right, overlay; 79.89 ± 2.37% (n = 2 mice) of TRN neurons are SOM⁺. The border of the TRN is outlined. (b) Representative images (from 3 mice) of TRN from a Som-Cre;Ai14 mouse. Left, tdTomato expression in SOM⁺ TRN neurons; middle, staining with antibody to ErbB4 (anti-ErbB4). (c) High-magnification images of neurons in the TRN, showing coexpression of tdTomato (red) and ErbB4 (green) in the same cells (overlay). Arrows denote a SOM⁺ neuron that had ErbB4 staining in the soma. Arrowheads denote a SOM⁻ neuron that had no ErbB4 staining in the soma but was surrounded by fibers (presumably from other neurons) that had ErbB4 staining. ~100% of SOM⁺ TRN neurons were recognized by the ErbB4 antibody (n = 3 mice). (d) Representative images of anti-ErbB4 staining in TRN neurons in SomErbb4⁺/⁺ (WT; left) and SomErbb4−/− (KO) mice (middle) and in the hippocampus (Hipp) of the same KO mouse. Similar results were obtained in three WT and three KO mice.

inputs: an ‘auditory/auditory’ task, in which mice needed to identify the target sounds among distractor tones (Fig. 2a,c); and a ‘visual/auditory’ task, in which mice were initially trained to respond to both sound and light cues, but in subsequent test sessions were required to respond only to the light and ignore the sound (Fig. 2a,b,d and Online Methods). The visual/auditory task contains a mix of congruent trials, in which light and sound cue the same action, and incongruent trials in which the two stimuli cue conflicting actions (Fig. 2d).

Of note, the two behavioral tasks that we used—the auditory/auditory task and the visual/auditory task—are substantially different in two ways. First, in the former task, mice identify targets among within-modality distractors, whereas in the latter task, mice need to globally switch attention across modalities. Second, the effects of the distractors on performance in the auditory/auditory task are primarily sensory-driven (bottom-up)31 in nature; in contrast, the irrelevant cues (the auditory cues) in the visual/auditory task are capable of engaging goal-directed (top-down) attention31, in a manner similar to the relevant cues (the visual cues), because both the auditory and visual cues were initially associated with reward during training when the mice learned the basic 2-AC tasks (Fig. 2 and Online Methods).

ErbB4 deletion in SOM neurons alters sensory selection

All animals, including the SomErbb4⁺/⁺ (Erbb4 wild type, WT), SomErbb4−/+ (Erbb4 heterozygous, HET) and SomErbb4−/− (Erbb4 knockout, KO) mice, were able to learn the basic 2-AC tasks and reach a similar performance level (Fig. 2c; Supplementary Fig. 2). Surprisingly, both HET and KO mice showed better performance compared with the WT mice in the auditory/auditory task (Fig. 3a; note 50% is chance level). It is noteworthy that the KO mice were faster at learning the basic 2-AC task than the HET and WT mice (Supplementary Fig. 2). Enhanced learning could therefore contribute to their increased performance on the auditory/auditory task. However, the HET mice also showed increased performance on the auditory/auditory task but had a learning curve similar to that of WT mice (Fig. 3a; Supplementary Fig. 2), which argues against a learning effect.

In contrast to their increased performance in the auditory/auditory task, the KO had severely impaired performance in the incongruent trials of the visual/auditory task (Fig. 3b). The HET mice showed a trend toward decreased performance that was less severe than for the KO mice (Fig. 3b). All groups performed at similarly high levels in the congruent trials of the visual/auditory task (Fig. 3c), which do not invoke conflicting actions.

The increased performance in the auditory/auditory task and decreased performance in the incongruent trials of the visual/auditory task in the ErbB4 mutant mice could be caused by enhanced auditory perception or impaired visual perception, causing these mice to rely more on auditory rather than visual cues. This hypothesis is challenged, however, by the observation that the mutant mice showed no obvious differences from WT mice in either auditory (Supplementary Fig. 3a–c) or visual (Supplementary Fig. 3d–g) perception. Furthermore, the performance of these mice was enhanced in a visual/visual task, in which they were required to identify targets among distractors in the visual field (Supplementary Fig. 4a,b), and was decreased in an auditory/visual task in which they needed to respond to the sound cues and ignore the light cues (Supplementary Fig. 4c–e). These phenotypes mirror those observed in the auditory/auditory task and visual/auditory task, respectively (Fig. 3a–c).
**Figure 2** Behavioral tasks that assess sensory selection. (a,b) The basic two-alternative choice (2-AC) tasks. (a) Auditory task: mice initiated each trial by a nose poke into the center port of the operant chamber. After a variable (200–300 ms) silent period, a frequency-modulated target sound was presented. Mice were required to stay in the port until the onset of the sound. The center frequency of the target sound (8 kHz or 20 kHz) indicated the side port where water reward would be delivered (left or right, respectively). Mice were rewarded only in trials in which they chose the correct port as their first response. (b) Visual task: same as in a, except that a nose poke into the center port turned on a light on the same side where a water reward would be delivered (left or right). (c) After learning the basic auditory 2-AC task (see a), mice were tested in an ‘auditory/auditory’ paradigm. As in the basic 2-AC task, mice initiated a trial by a nose poke into the center port. After a silent delay of 50 ms, a train of five 100-ms pure tone distractors was presented. The frequencies of each of the five distractor tones in the train were 5, 8, 12.5, 16 and 20 kHz, and the order in which the tones were presented was random for each trial.

In each trial, one of the frequency-modulated target sounds (denoted as a waveform in red), which indicated reward at one of the side ports (see Online Methods). The position of the target in a train was randomized between 100 and 300 ms after the onset of the first distractor tone. Mice were required to stay in the port until the target was presented. (d) After learning both the auditory and the visual basic 2-AC tasks, mice were tested in a ‘visual/auditory’ paradigm in which, after the nose poke into the center port, a light cue and one of the target sounds (8 kHz or 20 kHz) were simultaneously presented. However, only the light predicted reward, and the sound was random in relation to the reward. To obtain the reward, the mice had to attend to the light and ignore the sound. Congruent (top) and incongruent (bottom) trials occurred at the same frequency and were presented in a random order. (e) The WT, HET or KO mice showed similar performance in the basic auditory (left) and visual (right) 2-AC tasks (auditory: WT, 85.87 ± 0.88%, n = 33 mice, HET, 86.59 ± 0.74%, n = 28 mice, KO, 86.03 ± 0.85%, n = 30 mice, F2,88 = 0.20, P = 0.82, one-way analysis of variance (ANOVA); visual: WT, 89.9 ± 0.87%, n = 24 mice, HET, 88.47 ± 0.86%, n = 22 mice, KO, 89.95 ± 0.79%, n = 20 mice, F2,63 = 0.97, P = 0.38, one-way ANOVA). Data are presented as mean ± s.e.m.

Thus, ErbB4 deficiency in SOM* neurons profoundly and differentially alters an animal’s ability to select between competing sensory inputs. Whereas performance in the within-modality tasks was enhanced, performance in the across-modalities tasks was impaired.

**ErbB4 deletion in SOM TRN neurons alters sensory selection**

The observed behavioral phenotypes of the SomErbB4+/− or SomErbB4−/− mice could be caused by ErbB4 deficiency in the TRN. However, although the majority of cortical or hippocampal SOM* neurons do not express ErbB4 (refs. 26,27), some of them do (Supplementary Fig. 1). In addition, ErbB4-expressing SOM* neurons might exist in other brain areas. These neurons can potentially be affected in the mice affected by ErbB4 deficiency and therefore contribute to the behavioral phenotypes of these mice. To distinguish between these possibilities, we selectively deleted ErbB4 in the TRN. To this end, we bred the Som-FIp:ErbB4flox/flox mice, in which the flippase (Fli) recombinase is selectively expressed in SOM* neurons from an ErbB4flox/flox genetic background, and injected the TRN of these mice with an adeno-associated virus expressing the Cre recombinase in an Flip-dependent manner (AAV-FRT-stop-FRT-Cre-GFP). This approach allowed restricted deletion of ErbB4 in the TRN area (Supplementary Fig. 5) and, remarkably, resulted in improved learning in the basic 2-AC task—a phenotype that resembles that of the SomErbB4−/− mice (Supplementary Fig. 2).

Importantly, as compared with controls, mice with ErbB4 deletion in the TRN had enhanced performance in the auditory/auditory task but decreased performance in the incongruent trials of the visual/auditory task (Fig. 3d–f), behavioral phenotypes that mimic those of the SomErbB4+/− and SomErbB4−/− mice. These results indicate that the observed behavioral alterations in the SomErbB4+/− or SomErbB4−/− mice are caused by ErbB4 deficiency in the TRN.

ErbB4 suppresses cortical drive onto the TRN

Because ErbB4 modulates synapse development and function in a number of brain areas and cell types18,19,32–36, we next investigated the effects of ErbB4 deficiency on excitatory synaptic transmission onto TRN neurons. We recorded pairs of adjacent SOM* (red-fluorescent) and SOM− (nonfluorescent) TRN neurons in acute brain slices (Figs. 1a and 4a). Excitatory postsynaptic currents (EPSCs) were evoked by a stimulating electrode placed at the border between the TRN and the internal capsule. In WT mice, EPSCs recorded from SOM* cells were significantly smaller than those from SOM− neurons (Fig. 4b), indicating that the strength of excitatory synapses onto SOM* neurons is weaker than that of excitatory synapses onto SOM− neurons. Unexpectedly, in the SomErbB4+/− (HET) and SomErbB4−/− (KO) mice, the strength of synapses onto SOM* TRN neurons was markedly enhanced, such that EPSCs mediated by either AMPA receptors (AMPARs) or NMDA receptors (NMDARs) were much larger in SOM* neurons than in SOM− neurons (Fig. 4b). These results indicate that ErbB4 signaling normally dampens the excitatory synapses onto SOM* TRN neurons, in contrast to what is seen in other brain areas, where ErbB4 most often acts to strengthen synapses18,19,32–36.

Excitatory inputs to the TRN originate either from the corticothalamic (CT) projections that impart ‘top-down’ control or from the thalamocortical (TC) projections that convey ‘bottom-up’ sensory information1. The methods used in the above experiments could not distinguish between these projections. To determine which input is
Figure 3 ErbB4 deficiency in SOM+ TRN neurons affects sensory selection. (a-c) Behavioral phenotypes of WT, HET and KO mice. (a) Reducing ErbB4 levels in SOM+ neurons impaired performance in the auditory/auditory task (WT, n = 16 mice; HET, n = 13 mice; KO, n = 14 mice; F2,40 = 11.06; KO compared with WT; session 1, *P = 0.025, session 2, ***P = 0.0008, session 3, ****P = 0.0006; HET compared with WT; session 1, **P = 0.0044, session 2, **P = 0.0048, session 3, **P = 0.0014; two-way repeated-measures (RM) ANOVA followed by Tukey’s tests). (b) Reducing ErbB4 levels in SOM− neurons impaired performance in the incongruent trials (WT, n = 16 mice; HET, n = 11 mice; KO, n = 14 mice; F2,38 = 11.38; KO compared with WT; session 1, ***P = 0.0005, session 2, ***P = 0.0004, session 3, ***P = 0.0004, session 4, ***P = 0.0008, session 5, ***P < 0.0001; KO compared with HET; session 1, P = 0.01, session 2, P = 0.017, session 3, P = 0.036, session 4, P = 0.016, session 5, P = 0.011; two-way RM ANOVA followed by Tukey’s tests), but not in the congruent trials (c) (F2,38 = 0.40; P = 0.67, two-way RM ANOVA), of the visual/auditory task. (d–f) Behavioral phenotypes of mice in which ErbB4 is selectively deleted in the TRN. “TRN KO”, Som-Flp;Erbb4lox/lox mice in which the TRN was injected with a Flp-dependent AAV expressing Cre-GFP, so as to delete ErbB4 in SOM+ TRN neurons; “Control”, Som-Flp;Erbb4lox/lox mice in which the TRN was injected with a Flp-dependent AAV expressing GFP. Selective deletion of ErbB4 in SOM+ TRN neurons improved performance in the auditory/auditory task (d; Control, n = 7 mice, TRN KO, n = 7 mice, F1,12 = 31.69; session 1, ***P < 0.0001, session 2, ***P < 0.0001, session 3, ***P = 0.0004; two-way RM ANOVA followed by Bonferroni tests). Selective deletion of ErbB4 in SOM+ TRN neurons impaired performance in the incongruent trials (e) (Control, n = 7 mice, TRN KO, n = 7 mice, F1,12 = 21.46; session 1, ***P = 0.0001, session 2, ***P = 0.006, session 3, ***P = 0.001, session 4, ***P = 0.002, session 5, **P = 0.013, two-way RM ANOVA followed by Bonferroni tests), but not in the congruent trials (f) (F1,12 = 0.06; P = 0.81, two-way RM ANOVA), of the visual/auditory task. Data are presented as mean ± s.e.m.

ErbB4 controls cortical modulation of the thalamus via the TRN

It is possible that the increased cortical drive onto SOM+ TRN neurons leads to enhanced cortical feedback modulation of thalamic function because activation of TRN neurons has been shown to potently modulate the activity of thalamic relay neurons.6,38 To test this hypothesis, we optogenetically stimulated the CT pathway and recorded the evoked synaptic responses in thalamic neurons (Fig. 5e,f). In each neuron we recorded both the monosynaptic EPSCs and the disynaptic inhibitory postsynaptic currents (IPSCs) in response to the same photostimulation (Fig. 5c,f, Supplementary Fig. 7). We found that the IPSC-to-EPSC ratio in thalamic neurons was drastically increased by ErbB4 deficiency, and the effect was stronger in the KO than in the HET mice (Fig. 5f). This increased IPSC-to-EPSC ratio could be the result of either potentiated input onto SOM+ TRN neurons or enhanced output from these neurons, as ErbB4 has been shown to modulate presynaptic GABA release.16,80 However, deletion

strengthened by ErbB4 deficiency, we injected the cortex or thalamus with an AAV-CAG-ChrR2(H134R)-YFP to express the light-sensitive cation channel channelrhodopsin-2 (ChR2) in neurons that give rise to either the CT or TC projections (Fig. 5a,b; Supplementary Fig. 6). We next used a minimal photostimulation protocol, which permits measurement of the strength of single synapses, to stimulate each pathway in acute slices while recording the ‘evoked minimal’ EPSCs (emEPSCs) in SOM+ TRN neurons. The emEPSCs driven by the CT projections, including those originating from the somatosensory, visual and auditory cortices, were much larger in the ErbB4 mutant mice than in their WT littermates (Fig. 5c,d; Supplementary Fig. 6). In contrast, emEPSCs driven by the TC pathway were similar across different genotypes (Fig. 5c,d). These results indicate that deficient ErbB4 expression in SOM+ TRN neurons causes selective strengthening of the excitatory synapses driven by cortical inputs.
Figure 5 ErbB4 deficiency in SOM+ TRN neurons selectively enhances cortical drive onto TRN. (a) Left, schematic of the recording configuration. The CT-TRN pathway is selectively stimulated by photostimulation of ChR2 (green), and EPSCs are recorded from SOM+ TRN neurons (red). Right, an image of a brain slice used in the recording. The slice was prepared from a Som-Cre:Ai14 mouse in which the AAV-CAG-ChR2(H134R)-YFP was injected into the primary somatosensory cortex (arrow). (b) Same as in a, except that the TC-TRN pathway was selectively stimulated, and the AAV-CAG-ChR2(H134R)-YFP was injected into the ventrobasal complex of the thalamus (arrow). (c) Representative emEPSC traces recorded from SOM+ TRN neurons in response to the photostimulation (blue bars) of either the CT-TRN (top row) or the TC-TRN (bottom row) pathway, using the minimal photostimulation protocol. Calibrations: 20 pA and 2 ms. (d) Left, quantification of the amplitude of emEPSCs driven by the CT-TRN pathway (WT: 25.81 ± 7.35 pA, n = 7 cells; HET: 122.8 ± 17.74 pA, n = 9 cells; KO: 129.9 ± 14.53 pA, n = 11 cells). F2,22 = 20.23, *P = 0.018, **P = 0.0027, ****P < 0.0001, one-way ANOVA followed by Tukey’s test). Right, quantification of the amplitude of emIPSCs driven by the CT-TRN pathway (WT: 25.81 ± 7.35 pA, n = 7 cells; HET: 122.8 ± 17.74 pA, n = 9 cells; KO: 129.9 ± 14.53 pA, n = 11 cells). F2,22 = 19.70, *P = 0.023, ****P = 0.0001, one-way ANOVA followed by Tukey’s test). (e) Schematic of the recording configuration, in which both the CT axons and their collaterals to TRN were photostimulated, and the synaptic responses were recorded from neurons in the thalamus. (f) Left, representative synaptic response traces recorded from thalamic neurons in response to photostimulation (blue bars) of the CT pathway. EPSCs and IPSCs in each neuron evoked by the same stimulation were recorded at the reversal potential of inhibitory and excitatory synaptic currents, respectively. Calibrations: 50 pA and 100 ms. Right, quantification of the ratio of inhibitory to excitatory charge transfer (I/E) (WT: 0.88 ± 0.59, n = 11 cells; HET: 0.87 ± 0.91, n = 16 cells; KO: 0.87 ± 0.91, n = 16 cells). F2,22 = 0.70, P = 0.5, one-way ANOVA). (g) Left, schematic of the recording configuration, in which both the CT axons and their collaterals to TRN were photostimulated, and the synaptic responses were recorded from neurons in the thalamus. (h) Left, representative synaptic response traces recorded from thalamic neurons in response to photostimulation (blue bars) of the CT pathway. EPSCs and IPSCs in each neuron evoked by the same stimulation were recorded at the reversal potential of inhibitory and excitatory synaptic currents, respectively. Calibrations: 50 pA and 100 ms. Right, quantification of the ratio of inhibitory to excitatory charge transfer (I/E) (WT: 0.88 ± 0.15, n = 9 cells; HET: 0.87 ± 0.91, n = 16 cells; KO: 0.87 ± 0.91, n = 16 cells). F2,22 = 0.70, *P = 0.023, ****P = 0.0001, one-way ANOVA followed by Tukey’s test). Data are presented as mean ± s.e.m.

Expression of GluA4-C-tail in SOM+ TRN neurons in the KO mice selectively weakened the excitatory synaptic transmission driven by the CT inputs (Fig. 7a, b), without affecting that driven by the TC inputs (Fig. 7c, d). This result is consistent with previous finding that deletion of GluA4 depresses synaptic transmission onto TRN neurons driven by the CT pathway, but not that driven by the TC pathway17. In subsequent behavioral experiments we found that expression of GluA4-C-tail in SOM+ TRN neurons reduced the speed of learning of KO mice in the basic 2-AC tasks, to a level comparable to that of WT mice (Supplementary Fig. 9a, b). Remarkably, the same manipulation decreased the performance of KO mice in the auditory/auditory task while enhancing their performance in the incongruent trials of the visual/auditory task. As a result, these mice performed similarly to WT mice in both tasks (Fig. 8a, b). The behavioral effect of GluA4-C-tail was dependent on the efficiency of viral infection in the TRN (Supplementary Fig. 8c), demonstrating the specificity and potency of this manipulation. In addition, expression of GluA4-C-tail in SOM+ TRN neurons did not affect performance in the congruent trials of the visual/auditory task (Fig. 8c), nor did it affect performance in a sensory perception test (Supplementary Fig. 9c–e).

Figure 6 ErbB4 deficiency does not affect presynaptic function of SOM+ TRN neurons. (a) A schematic recording configuration. The SOM+ TRN neurons (green) in Som-ires-Cre mice were infected with the AAV-DIO-GluA4-containing AMPARs17, we exploited the C-terminal tail of GluA4 (GluA4-C-tail), which blocks GluA4 trafficking thereby depressing synaptic transmission41. We bilaterally injected the TRN of the Som-ErbB4−/− mouse with an AAV-DIO-GluA4-C-tail-GFP that harbors a doubly loxp-flanked (double-floxed) inverted open reading frame (DIO), which expresses the GluA4-C-tail tagged with GFP in a Cre-dependent manner (Supplementary Fig. 8a–c). Expression of GluA4-C-tail in SOM+ TRN neurons of the KO mice selectively weakened the excitatory synaptic transmission driven by the CT inputs (Fig. 7a, b), without affecting that driven by the CT inputs (Fig. 7c, d). This result is consistent with previous finding that deletion of GluA4 depresses synaptic transmission onto TRN neurons driven by the CT pathway, but not that driven by the TC pathway17. In subsequent behavioral experiments we found that expression of GluA4-C-tail in SOM+ TRN neurons reduced the speed of learning of KO mice in the basic 2-AC tasks, to a level comparable to that of WT mice (Supplementary Fig. 9a, b). Remarkably, the same manipulation decreased the performance of KO mice in the auditory/auditory task while enhancing their performance in the incongruent trials of the visual/auditory task. As a result, these mice performed similarly to WT mice in both tasks (Fig. 8a, b). The behavioral effect of GluA4-C-tail was dependent on the efficiency of viral infection in the TRN (Supplementary Fig. 8c), demonstrating the specificity and potency of this manipulation. In addition, expression of GluA4-C-tail in SOM+ TRN neurons did not affect performance in the congruent trials of the visual/auditory task (Fig. 8c), nor did it affect performance in a sensory perception test (Supplementary Fig. 9c–e).
Figure 7  Blocking GluA4 trafficking in SOM+ TRN neurons in ErbB4 mutant mice reverses the enhanced cortical drive. (a) A schematic of the recording configuration. The CT-TRN pathway in Som^+/− (KO) mice is selectively stimulated by photoactivation of ChR2-EGFP (light green), and EPSCs are recorded from SOM+ TRN neurons (dark green). (b) Left, representative emEPSC traces recorded from a control SOM+ TRN neuron (“KO”) and a SOM+ TRN neuron expressing GluA4-C-tail-GFP (“KO, C-tail-GFP”). The emEPSCs were evoked by minimal photostimulation (blue bars) of the CT-TRN pathway. Calibrations: 20 pA and 2 ms. Right, quantification of the emEPSC amplitude (KO, n = 8 cells (2 mice); KO, C-tail-GFP, n = 9 cells (3 mice); DF = 15; F = 10.65, ****P < 0.0001, t-test). The KO data are the same as in Figure 5c,d. (c) Same as in a, except that the TC-TRN pathway is selectively stimulated. (d) Left, representative emEPSC traces recorded from a control SOM+ TRN neuron expressing GFP ("KO, GFP") and a SOM+ TRN neuron expressing GluA4-C-tail-GFP ("KO, C-tail-GFP"). The emEPSCs were evoked by minimal photostimulation (blue bars) of the TC-TRN pathway. Calibrations: 20 pA and 2 ms. Right, quantification of the emEPSC amplitude (KO, GFP, n = 13 cells (3 mice); KO, C-tail-GFP, n = 11 cells (3 mice); DF = 22, T = 0.25, P = 0.81, t-test). Data are presented as mean ± s.e.m.

Thus, reversal of the enhanced cortical drive by selective expression of GluA4-C-tail in SOM+ TRN neurons was sufficient to ‘rescue’ the behavioral phenotypes of the ErbB4 mutant mice, normalizing both their enhanced performance in the within-modality task and their impaired performance in the across-modalities task.

DISCUSSION

Previous studies aimed at determining the causal relationship between TRN function and behavior have relied on lesions of the TRN, which in general cause attention-related behavioral deficits. However, because of the thin and elongated shape of the TRN and its close proximity to the dorsal thalamus, off-target effects in lesion studies are almost unavoidable and can confound the explanation of results. With the advent of new technologies, in particular mouse genetic and optogenetic techniques that allow selective targeting of TRN neurons, recent studies have made important progress in clarifying the role of the TRN in sleep-wakefulness transitions and in broad shifts in arousal state.

Another important role that has been attributed to the TRN is its participation in goal-directed attention, a fundamental cognitive function whereby behaviorally relevant sensory information is selected against irrelevant sensory information for further processing and for guidance of goal-directed behavior; therefore, it is difficult to distinguish altered attention from changes in these other cognitive functions in behavioral assays. Previous studies on the TRN’s role in goal-directed attention were mainly carried out in monkeys, a species that is versatile in complex tasks designed to assess attention in isolation. However, monkeys are impervious to the molecular and neural circuit manipulations that are required for understanding the mechanisms of TRN function.

We overcame these challenges by devising behavioral tasks that engage goal-directed attention in mice and by selectively targeting and manipulating the cortico-TRN-thalamic circuit. This approach allowed us to uncover an important role of ErbB4 in shaping cortical feedback control of TRN function and in regulating performance in tasks demanding attention. Specifically, we show that ErbB4 deficiency in SOM+ TRN neurons markedly altered the performance of mice in sensory selection tasks, consistent with changes in goal-directed attention. Furthermore, ErbB4 deficiency selectively potentiates the excitatory synapses onto SOM+ TRN neurons driven by cortical inputs, thereby enhancing the TRN-mediated cortical feedback modulation of thalamic neurons. Finally, by adjusting the strength of the cortico-TRN synapses, we were able to rescue the ErbB4 deficiency–induced alterations in behavioral performance. The most parsimonious explanation for these results is that TRN circuit dysfunction driven by the enhanced cortical excitatory inputs is the major cause of the observed behavioral phenotypes.

The majority of TRN neurons do not form reciprocal connections with thalamic neurons; instead, they form ‘open-loop’ connections with thalamic neurons (see model diagram in Supplementary Fig. 10). These open loops provide an anatomical basis for lateral inhibition in the thalamus, which can suppress thalamic responses to distractors while allowing responses to the attended stimuli (the ‘targets’) to reach cortex (Supplementary Fig. 10a). This lateral inhibition is

Figure 8 Rescue of the behavioral phenotypes of ErbB4 mutant mice by reversal of the enhanced cortical drive to TRN. (a) Quantification of performance (KO, GFP, n = 8 mice; KO, C-tail-GFP, n = 8 mice; F1,14 = 20.94; session 1, **P = 0.0042, session 2, **P = 0.0075, session 3, ****P < 0.0001, two-way repeated-measures ANOVA followed by Bonferroni tests). The WT data in Figure 3a are re-plotted here for visual inspection. (b,c) Quantification of performance in the incongruent trials (b) (KO, GFP, n = 8 mice; KO, C-tail-GFP, n = 8 mice; F1,14 = 19.61; session 1, **P = 0.0007, session 2, **P = 0.0024, session 3, *P = 0.025, session 4, n.s., not significant (P = 0.074), session 5, **P = 0.0074; two-way repeated-measures ANOVA followed by Bonferroni tests) and the congruent trials (c) (F1,14 = 2.09, P = 0.17, two-way RM ANOVA), of the visual/auditory task. The WT data in Figure 3b are re-plotted here for visual inspection. Data are presented as mean ± s.e.m.
presumably enhanced in the ErbB4 mutant mice as a result of the enhancement in cortical drive, leading to increased signal-to-noise ratio in the thalamus and thus to improved performance in the auditory/auditory and visual/visual tasks (Fig. 3a; Supplementary Fig. 4a,b). Why, then, did the ErbB4 mutant mice display impaired performance in the across-modalities tasks (Fig. 3b; Supplementary Fig. 4c,d)? Unlike in the within-modality task, in an across-modalities task the mice need to globally switch attention across modalities. It is known that attending to an auditory stimulus suppresses neuronal activity in the visual TRN6,9, an effect that could be mediated by reticulo-reticular inhibition45,46. In addition, cross-modality interactions may also occur through TRN-mediated inhibition across different thalamic nuclei47–49 (see Supplementary Fig. 10b). It is possible that, in order for the mouse to select the relevant stimuli and make correct choices in our across-modalities tasks, it is necessary for neurons in the relevant TRN sector to be activated and those in the irrelevant sector inhibited (Supplementary Fig. 10b). This will lead to disinhibition and inhibition, respectively, of the corresponding thalamic areas and allow the relevant stimuli to reach the cortex (Supplementary Fig. 10b). Furthermore, one challenge of these tasks—as was already alluded to in a previous section—lies in the fact that even the irrelevant cues are capable of engaging in goal-directed (top-down) attention, thereby affecting performance, because these cues are initially used to guide behavior during training (Supplementary Fig. 10b, indicated by arrows from the cortex; also see Online Methods). This problem is worsened in the ErbB4 mutant mice, in which the aberration in cortical drive, leading to increased signal-to-noise ratio in the TRN and an apparent behavioral perseverance with the irrelevant cues (Supplementary Fig. 10b).

Together, our results indicate that the SOM+ TRN neurons have an important role in selecting targets from distractor and in preferentially processing behaviorally relevant sensory inputs in situations where competing sensory stimuli direct conflicting actions. Our results also suggest that normal levels of ErbB4, by regulating cortical excitatory synaptic transmission onto SOM+ TRN neurons, tune the function of the cortico-TRN-thalamic circuit, allowing a balance between the ability to attend to selective sensory inputs and the flexibility of refocusing attention according to behavioral needs.

Our study reveals a previously unidentified synaptic function of ErbB4 in the TRN that is distinct from the reported actions of this signaling molecule in other CNS regions18, and sheds light on a mechanism that could be involved in the pathophysiology of schizophrenia. NRG1-ErbB4 signaling in the nervous system and abnormalities in goal-directed attention. This is particularly interesting considering that both defects in NRG1-ErbB4 signaling and dysfunction of TRN circuitry have been implicated in schizophrenia, and that impairments in goal-directed attention are thought to underlie major cognitive symptoms of the disease15.

**METHODS**

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

**ACKNOWLEDGMENTS**

We thank A. Zador for help on the behavioral paradigms, J.J. Zhu for the GluA4-C-tail construct and A. Churchland for advice on the visual/visual task. We thank F. Albeau, A. Kepescs, H. Kessels, R. Malinow, L. Mei, S. Shea, P. Smith and L. Van Aelst for critical reading of earlier versions of the manuscript and members of the lab for discussions. This study was supported by a fellowship from the Deutsche Forschungsgemeinschaft (DFG, S.A.) and grants from the US National Institutes of Health (B.L. and Z.J.H.), the Dana Foundation (B.L.), NARSAD (S.A., B.L. and Z.J.H.), the Louis Feil Trust (B.L.) and the Stanley Family Foundation (B.L. and Z.J.H.).

**AUTHOR CONTRIBUTIONS**

S.A. and B.L. conceived the study. S.J., S.A., K.Y. and B.L. designed the behavioral tasks. S.A. performed the experiments with help from K.C.Y., S.G., G.-R.H. and R.P. S.A., S.J. and S.G. analyzed data. C.L., M.H. and Z.J.H. provided critical reagents and advice. S.A. and B.L. made figures. B.L. and S.A. wrote the manuscript with help from all authors.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Zikopoulos, B. & Barbas, H. Circuits for multisensory integration and attentional modulation through the prefrontal cortex and the thalamic reticular nucleus in primates. *Rev. Neurosci.* 18, 417–438 (2007).
2. Ferrarelli, F. & Tononi, G. The thalamic reticular nucleus and schizophrenia. *Schizophr. Bull.* 37, 306–315 (2011).
3. Crick, F. Function of the thalamic reticular complex: the searchlight hypothesis. *Proc. Natl. Acad. Sci. USA* 81, 4586–4590 (1984).
4. Pinault, D. The thalamic reticular nucleus: structure, function and concept. *Brain Res. Brain Res. Rev.* 46, 1–31 (2004).
5. Halassa, M.M. et al. State-dependent architecture of thalamic reticulospinal subnetworks. *Cell* 158, 808–821 (2014).
6. Yu, X.J., Xu, X.X., He, S. & He, J. Change detection by thalamic reticular neurons. *Nat. Neurosci.* 12, 1165–1170 (2009).
7. Krause, M., Hoffmann, W.E. & Hajas, M. Auditory sensory gating in hippocampus and reticular thalamic neurons in anesthetized rats. *Biol. Psychiatry* 53, 244–253 (2003).
8. McLellan, K., Cavanaugh, J. & Wurtz, R.H. Guarding the gateway to cortex with attention in visual thalamus. *Nature* 456, 391–394 (2008).
9. McLellan, K., Cavanaugh, J. & Wurtz, R.H. Attentional modulation of thalamic reticular neurons. *J. Neurosci.* 26, 4444–4450 (2006).
10. McLellan, K., Brown, V.J. & Bowman, E.M. Thalamic reticular nucleus activation reflects attentional gating during classical conditioning. *J. Neurosci.* 20, 8897–8901 (2000).
11. Weese, G.D., Phillips, J.M. & Brown, V.J. Attentional orienting is impaired by unilateral lesions of the thalamic reticular nucleus in the rat. *J. Neurosci.* 19, 10135–10139 (1999).
12. Pinault, D. Dysfunctional thalamic-related networks in schizophrenia. *Schizophr. Bull.* 37, 238–243 (2011).
13. Zhang, Y., Linas, R.R. & Lisman, J.E. Inhibition of NMDARs in the nucleus reticularis of the thalamus produces delta frequency bursting. *Front. Neural Circuits* 3, 20 (2009).
14. Schulman, J.J. et al. Imaging of thalamocortical dysrhythmia in neuropsychiatry. *Front. Hum. Neurosci.* 5, 69 (2011).
15. Luck, S.J. & Gold, J.M. The construction of attention in schizophrenia. *Biol. Psychiatry* 64, 34–39 (2008).
16. Woo, R.S. et al. Neuregulin-1 enhances depolarization-induced GABA release. *Neuron* 54, 599–610 (2007).
17. Paz, I.T. et al. A new mode of corticothalamic transmission revealed in the Gria4−/− model of absence epilepsy. *Nat. Neurosci.* 14, 1167–1173 (2011).
18. Mei, L. & Xiong, W.C. Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat. Rev. Neurosci.* 9, 437–452 (2008).
19. Mei, L. & Nave, K.A. Neuregulin-ERBB signaling in the nervous system and neuropsychiatric diseases. *Neuron* 83, 27–49 (2014).
20. Zikopoulos, B. & Barbas, H. Pathways for emotions and attention converge on the thalamic reticular nucleus in primates. *J. Neurosci.* 32, 5338–5350 (2012).
21. Clemence, A.E. & Mitrofanis, J. Cytoarchitectonic heterogeneities in the thalamic reticular nucleus of cats and ferrets. *J. Comp. Neurol.* 322, 167–180 (1992).
22. Bouras, C., Magistretti, P.J., Morrison, J.H. & Constantinides, J. An immunohistochemical study of pro-somatostatin-derived peptides in the human brain. *Neuroscience* 22, 781–800 (1987).
23. Taniguchi, H. et al. A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. *Neuron* 71, 995–1013 (2011).
24. Madisen, L. et al. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat. Neurosci.* 13, 133–140 (2010).
25. Neddens, J. & Buonanno, A. Expression of the neuregulin receptor ErbB4 in the brain of the rhesus monkey (Macaca mulatta). *PLoS ONE* 6, e27337 (2011).
26. You, H.J., Wang, H.F., Lai, C. & Liu, F.C. Neural development of the neuregulin receptor ErbB4 in the cerebral cortex and the hippocampus: preferential expression by interneurons tangentially migrating from the ganglionic eminences. *Cereb. Cortex* 13, 252–264 (2003).
27. Neddens, J. & Buonanno, A. Selective populations of hippocampal interneurons express ErbB4 and their number and distribution is altered in ErbB4 knockout mice. Hippocampus 20, 724–744 (2010).
28. Golub, M.S., Germann, S.L. & Lloyd, K.C. Behavioral characteristics of a nervous system-specific erbB4 knock-out mouse. Behav. Brain Res. 153, 159–170 (2004).
29. Uchida, N. & Mainen, Z.F. Speed and accuracy of olfactory discrimination in the rat. Nat. Neurosci. 6, 1224–1229 (2003).
30. Jaramillo, S. & Zador, A.M. The auditory cortex mediates the perceptual effects of acoustic temporal expectation. Nat. Neurosci. 14, 246–251 (2011).
31. Cooper, M.A. & Koleske, A.J. Ablation of ErbB4 from excitatory neurons leads to reduced dendritic spine density in mouse prefrontal cortex. J. Comp. Neurol. 522, 3351–3362 (2014).
32. Zhang, F., Wang, L.P., Boyden, E.S. & Deisseroth, K. Channelrhodopsin-2 and optical control of excitable cells. Nat. Methods 3, 785–792 (2006).
33. Halassa, M.M. et al. Selective optical drive of thalamic reticular nucleus generates thalamic bursts and cortical spindles. Nat. Neurosci. 14, 1118–1120 (2011).
34. Cruikshank, S.J., Urahe, H., Numikko, A.V. & Connors, B.W. Pathway-specific feedforward circuits between thalamus and neocortex revealed by selective optical stimulation of axons. Neuron 65, 230–245 (2010).
35. Chen, Y.J. et al. ErbB4 in parvalbumin-positive interneurons is critical for neuregulin 1 regulation of long-term potentiation. Proc. Natl. Acad. Sci. USA 107, 21818–21823 (2010).
36. Zhu, J.J., Esteban, J.A., Hayashi, Y. & Malinow, R. Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity. Nat. Neurosci. 3, 1098–1106 (2000).
37. Squire, R.L., Noudoost, B., Reiter, R.J. & Moore, T. Prefrontal contributions to visual selective attention. Annu. Rev. Neurosci. 36, 451–466 (2013).
38. Pinault, D. & Deschenes, M. Anatomical evidence for a mechanism of lateral inhibition in the rat thalamus. Eur. J. Neurosci. 10, 3462–3469 (1998).
39. Fuentealba, P. & Steriade, M. The reticular nucleus revisited: intrinsic and network properties of a thalamic pacemaker. Prog. Neurobiol. 75, 125–141 (2005).
40. Lam, Y.W., Nelson, C.S. & Sherman, S.M. Mapping of the functional interconnections between thalamic reticular neurons using photostimulation. J. Neurophysiol. 96, 2593–2600 (2006).
41. Crabtree, J.W., Collingridge, G.L. & Isaac, J.T. A new intrathalamic pathway linking modality-related nuclei in the dorsal thalamus. Nat. Neurosci. 22, 8754–8761 (2002).
42. Kimura, A., Imbe, H., Donishi, T. & Tamai, Y. Axonal projections of single auditory neurons in the thalamic reticular nucleus: implications for tonotopy-related gating function and cross-modal modulation. Eur. J. Neurosci. 26, 3524–3535 (2007).
ONLINE METHODS

Animals. Mice were housed in a temperature- and humidity-controlled environment with a 12-h light-dark cycle (9 a.m. to 9 p.m. light) in groups of 2–5 animals. All behavioral experiments were performed during the light cycle. Mice used in all behavior experiments had free access to food, but water was restricted to behavioral sessions. Free water was provided on days with no experimental sessions. For mice used in other experiments, food and water were freely available. Both male and female mice were used in all experiments, and the data were pooled, as no gender difference was observed. The Som-Cre-23, Som-Flp28, H2b-GFP34 and ErbB4△c/△c (ref. 28) mice were generated as described. The AII4 reporter mice24 were purchased from The Jackson Laboratory. All mice have been bred onto C57BL/6N background for at least 5 generations. Mice 2–4 months of age were used for all the behavioral experiments. All procedures involving animals were approved by the Institute Animal Care and Use Committees of Cold Spring Harbor Laboratory and carried out in accordance with US National Institutes of Health standards.

Viral vectors. All AAV viruses, such as AAV-CAG-ChrR(H134R)-YFP, AAV-DIO-ChrR(H134R)-YFP, AAV-DIO-GluA4-C-tail, AAV-DIO-GFP, AAV-FRT-stop-FRT-Cre-GFP and AAV-FRT-stop-FRT-GFP, were produced by the University of North Carolina Vector Core Facilities. All viral vectors were stored in aliquots at ~80 °C until use.

Immunohistochemistry. Immunohistochemistry experiments were performed following standard procedures. Briefly, mice were anesthetized and perfused with PBS and then with 4% paraformaldehyde (PFA). Brains were extracted and further fixed in 4% PFA overnight at 4 °C followed by cryoprotection in a 30% PBS-buffered sucrose solution for 36 h. Coronal sections (40–50 μm) were cut using a freezing microtome (Leica SM 2010R, Leica). Sections were first washed in PBS (3 × 5 min), incubated in PBS (0.3% Triton X-100 in PBS) for 30 min at room temperature (RT) and then washed with PBS (3 × 5 min). Next, sections were blocked in 5% normal goat serum in PBSB for 30 min at RT and then incubated with primary antibodies overnight at 4 °C. Sections were then washed with PBS (5 × 15 min) and incubated with fluorescein secondary antibodies at RT for 1 h. After washing with PBS (5 × 15 min), sections were mounted onto slides with Fluoromount-G (Beckman Coulter). Images were taken using a LSM 710 laser-scanning confocal microscope (Carl Zeiss). The primary antibodies used were: anti-ErbB4 (mouse, Fisher Scientific, MS 270P, 1:200), anti-somatostatin (rabbit, Bachem, T 4103, 1:2 000) and anti-NeuN (mouse, Millipore, MBA377, 1:100).

Behavioral tasks. Basic two-alternative choice tasks. Both an auditory and a visual two-alternative-choice (2-AC)29,30 procedure were used (see Fig. 2a,b). Mice initiated each trial by poking their nose into the center port of a three-port operant chamber. After a silent delay of random duration (200–300 ms, uniformly distributed), either a frequency-modulated target sound or a light stimulus was initiated each trial by poking their nose into the center port of a three-port operant chamber. The targets were presented. In the auditory task, the carrier frequency of the target indicated to the animal which of the two side ports would provide 10 µl of water reward. For a target carrier frequency of 8 kHz, reward was available only at the left port. For a target of 20 kHz, reward was provided at the right port. Mice were only rewarded in trials in which they chose the correct port as their first choice. Sound intensity was set at 60 dB-SPL, and sound duration was 100 ms. The modulation frequency was set at 15 Hz. In the visual task, a light signal of 500 ms duration from the left port indicated reward on the left side, and a light signal from the right port was rewarded on the right side. Mice were required to stay in the center port until the target was presented. If the animal withdrew before the onset of the target, the trial was considered invalid and was aborted. A new trial would then be initiated. Behavioral analysis included only valid trials in which the animal stayed in the center port until the time of target onset. In both the auditory and visual 2-AC, incorrect choices were punished by a 4-s timeout and a white noise.

Auditory/auditory task. As in the basic auditory 2-AC task, mice initiated a trial by a nose poke into the center port. After a silent delay of 50 ms, a train of five 100 ms pure tone distractors was presented (Fig. 2c). The frequency of each of the five distractor tones in the train was 5, 8, 12.5, 16 and 20 kHz, and the order in which the tones were presented was random for each trial. In each trial, one target (the 8-kHz or 20-kHz frequency-modulated sound in the basic 2-AC tasks described above) was embedded in the train of distractors. It should be noted that the frequency-modulated 20-kHz sound has distinct physical properties compared with the 20-kHz pure tone. The position of the target in a train was randomized between 100 and 300 ms after the onset of the first distractor tone (Fig. 2c). The performance of each mouse was monitored over three sessions.

Visual/auditory task. In this task, the light and sound stimuli were presented simultaneously, and the animal was only rewarded for correct responses to the light stimuli (Fig. 2d). Trials in which the two stimuli are congruent or incongruent were chosen randomly. The performance of each mouse was monitored over five sessions.

Visual/visual task. An LED array (3.4 cm × 3.4 cm, Linkspirtke) consisting of 8 × 8 single red LEDs was mounted above the left and right side port (Supplementary Fig. 4a). The target visual cues were continuous illumination (500 ms, indicated in red) of four LEDs in the center of the left and right arrays, signaling reward on the left and right, respectively. Mice were first trained to respond to those target cues until reaching performance criteria (above 75% correct trials for three consecutive sessions), and subsequently tested in sessions in which distractor lights were added. The distractors were lights flashing (at 20 Hz) simultaneously from both LED arrays, and were generated by two random LEDs surrounding the four center LEDs in each array. Target cues and distractor lights started 100 ms after the animal initiated the trial by nose poking into the center port. The targets stayed on for 500 ms, whereas the distractors were terminated after 400 ms.

Auditory/visual task. Same as the visual/auditory task, except that the relevant and irrelevant cues were swapped (Supplementary Fig. 4c).

Auditory discrimination task. Mice used in this experiment were first trained in the basic auditory 2-AC task to reach performance criteria. Mice initiated a trial by a nose poke into the center port. After a silent delay of random duration (200–300 ms), a frequency-modulated target sound was presented for 100 ms. The frequency of the sound was randomly selected from a group of eight frequencies (8, 9, 11, 13, 15, 17, 19 and 20 kHz). These frequencies were chosen such that they were equidistant from each other on the logarithmic scale (Supplementary Figs. 3a–c and 9c–e). All frequencies less than 12.65 kHz (the geometric mean of 8 and 20 kHz) were rewarded if the mouse chose the left water port, and those greater than 12.65 kHz were rewarded with water in the right water port. The volume of the water reward was decreased to 5 μl to ensure that the mice performed sufficient number of trials for each of the frequencies. Data from five consecutive sessions were collected (250–350 trials per session).

Data analysis. The response of a mouse to each of the eight sound frequencies was transformed into the percentage of ‘rightward selection’, which is the percentage of the trials in which the mouse chose the water port on the right side (Supplementary Figs. 3 and 9). These data were fitted using the following logistic function51:

\[
y = \frac{A1 - A2}{1 + \left(\frac{x}{X_0}\right)^p} + A2
\]

where \(X_0\) represents the median threshold and \(p\) determines the slope of the curve; \(A1\) and \(A2\) are the upper and lower bounds of the equation, respectively. A sigmoidal psychometric curve was thus generated. The median threshold \(X_0\) and parameter \(p\) of this curve were then obtained for each animal, and the data were pooled for each group.

Visual discrimination task. A horizontal panel (8 cm × 0.9 cm, Kingbright) with eight individually illuminable LEDs was mounted above the water ports (Supplementary Fig. 3d). Four LEDs were evenly distributed on either side of the midline of the center port. The center-to-center distance between adjacent LEDs was 1 cm, such that the illumination center of the individual LEDs was positioned at 0.5, 1.5, 2.5 and 3.5 cm from the midline of the center port. Mice were first trained to criteria in the basic visual 2-AC task, in which illumination of LEDs in the leftmost and rightmost indicated reward on the left and right, respectively. They were then tested for discrimination of illumination at the eight positions. In each trial, one of the eight LEDs was illuminated for 300 ms. As in the auditory discrimination task, the volume of the water reward was decreased to 5 μl, and data from five consecutive sessions were collected. Data analysis was carried out in the same way as that described for the auditory discrimination task.

Order of animal training and testing. For animals used in the auditory/auditory, visual/auditory or auditory/visual task: (1) training in the auditory basic 2-AC task until performance has reached criteria (>75% in three consecutive sessions); (2) testing in the auditory/auditory task for three sessions; (3) training in the visual basic 2-AC task until performance has reached criteria (>75%) in three
consecutive sessions); (4) repeat the auditory basic 2-AC task (step 1) for one or two sessions—this is to make sure that performance in this task has not been affected by the other tasks; (5) testing in the visual/auditory or auditory/visual task for five sessions. Note that different groups of mice were used for the visual/auditory or auditory/visual task, as these tasks may influence each other.

For animals used in the visual/visual and visual discrimination task: (1) training in the visual basic 2-AC task until performance has reached criteria (>75% in three consecutive sessions); (2) testing in the visual discrimination task for five sessions; (3) training mice to respond to the target stimuli of the visual/visual task until performance has reached criteria; (4) testing in the visual/visual task for three sessions.

During the training phase animals were trained for two 30–45 min sessions per day. In testing phase, each session was 30 min, and one session was given in each experimental day for all animals. Each animal performed on average about 200–250 valid trials per session. There were three possible behavioral outcomes: correct response, incorrect response and omission. Trials in which animal failed to make any response within 4 s after target presentation were counted as omissions, which were usually rare (<5% of all valid trials). Performance was calculated as the percentage of correct responses in all valid trials.

**Stimulus delivery.** Auditory stimuli were delivered through generic electromagnetic dynamic speakers calibrated using a pressure-field microphone (Brüel & Kjær) to reproduce 60 dB SPL in the range of 5–40 kHz at the position of the subject. Waveforms were created in software at a sampling rate of 200,000 samples per second and delivered to speakers through a Lynx L22 sound card (Lynx Studio Technology). We applied rise and fall linear envelopes of 2 ms to all sounds.

**Stereotaxic surgery.** Standard surgical procedures were followed for stereotoxic injection. Briefly, mice were anesthetized with ketamine (100 mg/kg) supplemented with xylazine (10 mg/kg) and positioned in a stereotoxic injection frame (myNeuroLab.com). A digital mouse brain atlas was linked to the injection frame to guide the identification and targeting of different brain areas (Angle Two Stereotactic System, myNeuroLab.com). Viruses were delivered with a glass micro-pipette through a skull window (1–2 mm²) by pressure application (5–12 psi, controlled by a Picospritzer III, General Valve, Fairfield, NJ, USA). The injections were performed using the following stereotoxic coordinates for TRN: −0.82/−1.34/−1.82 mm from bregma, 1.9/2.4/2.4 mm lateral from the midline and 3.6–3.2 mm vertical from the cortical surface; for thalamus: −1.70 mm from bregma, 1.45 mm lateral from the midline and 3.5 mm vertical from the cortical surface; for somatosensory cortex: −0.82 mm from bregma, 2.7 mm lateral from the midline and 2.05 mm vertical from the cortical surface; for auditory cortex: −2.80 mm from bregma, 3.88 mm lateral from the midline and 2.70 mm vertical from the cortical surface; for visual cortex: −3.28 mm from bregma, 2.30 mm lateral from the midline and 1.20 mm vertical from the cortical surface. During all surgical procedures, mice were kept on a heating pad and were brought back to their home cages after regaining movement. For postoperative care, mice were hydrated by intraperitoneal injection with 0.3–0.5 ml of lactated ringers. We used metacam (meloxicam, 1–2 mg/kg) as an analgesic and to reduce inflammation. We injected 1–1.5 µl of viral solution (−10¹² viruses/µl) bilaterally into TRN, or 0.5 µl into the cortex or thalamus, and waited approximately 2–3 weeks to allow maximal viral expression. Note that multiple locations in the TRN were targeted to ensure sufficient infection by viruses.

**Preparation of acute brain slices and electrophysiology.** Mice, 16–25 d (for electrophysiological recordings in the TRN) or 6–8 weeks (for recordings in the thalamus) of age were used. Mice were anesthetized with isoflurane and decapitated, and their brains quickly removed and chilled in ice-cold dissection buffer (110.0 mM choline chloride, 25.0 mM NaHCO₃, 1.25 mM NaH₂PO₄, 2.5 mM KCl, 0.5 mM CaCl₂, 7.0 mM MgCl₂, 25.0 mM glucose, 11.6 mM ascorbic acid and 3.1 mM pyruvic acid, gassed with 95% O₂ and 5% CO₂). Horizontal slices (300 µm) containing the TRN were cut in the dissection buffer using a HM650 Vibrating Microtome (MICROM International GmbH, Walldorf, Germany) and subsequently transferred to a storage container containing artificial cerebrospinal fluid (ACSF) ([118 mM NaCl, 2.5 mM KCl, 26.2 mM NaHCO₃, 1 mM NaH₂PO₄, 20 mM glucose, 1 mM MgCl₂ and 2 mM CaCl₂, at 34 °C, pH 7.4, gassed with 95% O₂ and 5% CO₂]). After at least 40 min recovery time, slices were transferred to room temperature and were constantly perfused with ACSF.

In acute slices the TRN can be easily identified under trans-illumination. In addition, we took advantage of the Som-Cre Ai4 line, in which the TRN had very high density of SOM⁺ neurons that are red fluorescent (Fig. 1a), to facilitate the identification of TRN under epifluorescence illumination.

**Simultaneous whole-cell patch-clamp recordings from SOM⁺/SOM⁻ neuronal pairs in TRN were obtained with Multiclamp 700B amplifiers (Molecular Devices, Sunnyvale, CA, USA).** Recordings were performed under visual guidance using an Olympus BX51 microscope equipped with both transmitted light illumination and epifluorescence illumination. The SOM⁺ cells were identified based on their red fluorescence from tdTomato. For evoked EPSCs, synaptic responses were evoked with a bipolar stimulating electrode placed in the border between the TRN and internal capsule, approximately 0.2 mm away from the recorded cell bodies in TRN. Electrical stimulation was delivered every 30 s, and synaptic responses were low-pass filtered at 1 kHz and recorded at holding potentials of −70 mV (for AMPAR-mediated responses), +40 mV (for NMDAR-mediated responses) or 0 mV (for GABA-A-receptor-mediated responses). NMDAR-mediated responses were quantified as the mean current between 50 ms and 100 ms after stimulation. Recordings were performed in the ACSF. The internal solution for voltage-clamp experiments contained 115 mM cesium methanesulfonate, 20 mM CaCl₂, 10 mM HEPES, 4 mM Na₂-ATP, 0.4 mM Na₃GTP, 10 mM Na-phosphocreatine and 0.6 mM EGTA (pH 7.2). Evoked EPSCs in certain experiments were recorded with picrotoxin (100 µM) added to the ACSF as indicated. Electrophysiological data were acquired and analyzed using pCLAMP 10 software (Molecular Devices).

To evoke synaptic transmission using the optogenetic methods, the AAV-CAG-Chr2(H134R)-YFP or AAV-DIO-Chr2(H134R)-YFP was injected into different brain regions, including cortex, thalamus and TRN and allowed to express for 10–14 d. Acute brain slices were prepared and a blue light was used to stimulate ChR2. The light source was a single-wavelength LED system (λ = 470 nm; CoolLED.com) connected to the epifluorescence port of an Olympus BX51 microscope. Light pulses of 0.2–0.5 ms, triggered by a TTL signal from the Clampex software (Molecular Devices), were used to evoke synaptic transmission. We used a minimal photostimulation protocol as previously described, in which the photostimulation resulted in 50–70% failures and low response-amplitude variability of the ‘evoked minimal’ EPSCs (emEPSCs). The emEPSCs presumably represent responses of single synapses driven by the CT or TC pathway.

**Statistics and data presentation.** Data were analyzed with GraphPad Prism Software. The sample size was estimated using power analysis based on our preliminary studies. Normality was tested by D’Agostino–Pearson or Shapiro–Wilk normality test. Statistical analysis was performed using paired or unpaired two-tailed Student’s t-test and one-way ANOVA or two-way ANOVA as indicated, followed by Tukey’s or Bonferroni’s post hoc test to correct for multiple comparisons. P < 0.05 was considered significant. No randomization was used to assign experimental groups, but mice were assigned to specific experimental groups without bias. Behavioral tests and electrophysiological data acquisition were performed by an investigator with knowledge of the identity of the experimental groups. All behavior experiments were controlled by computer systems, and data were collected and analyzed in an automated and unbiased way. No single data points were excluded. Animals that did not learn the basic 2-AC task within 35 sessions were excluded. Virus-injected animals in which the injection site was incorrect were excluded.

A Supplementary Methods Checklist is available.

50. Fenno, L.E. et al. Targeting cells with single vectors using multiple-feature Boolean logic. Nat. Methods 11, 763–772 (2014).
51. Gilchrist, J.M., Jerwood, D. & Ismaiel, H.S. Comparing and unifying slope estimates across psychometric function models. Percept. Psychophys. 67, 1289–1303 (2005).
52. Li, H. et al. Experience-dependent modification of a central amygdala fear circuit. Nat. Neuroscience 16, 332–339 (2013).