

A gain in GABA_A receptor synaptic strength in thalamus reduces oscillatory activity and absence seizures

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Neural inhibition within the thalamus is integral in shaping thalamocortical oscillatory activity. Fast, synaptic inhibition is primarily mediated by activation of heteropentameric GABA_A receptor complexes. Here, we examined the synaptic physiology and network properties of mice lacking GABA_A receptor $\alpha 3$, a subunit that in thalamus is uniquely expressed by inhibitory neurons of the reticular nucleus (nRT). Deletion of this subunit produced a powerful compensatory gain in inhibitory postsynaptic response in nRT neurons. Although, other forms of inhibitory and excitatory synaptic transmission in the circuit were unchanged, evoked thalamic oscillations were strongly dampened in $\alpha 3$ knockout mice. Furthermore, pharmacologically induced thalamocortical absence seizures displayed a reduction in length and power in $\alpha 3$ knockout mice. These studies highlight the role of GABAergic inhibitory strength within nRT in the maintenance of thalamic oscillations, and demonstrate that inhibitory intra-nRT synapses are a critical control point for regulating higher order thalamocortical network activity.

benzodiazepine | epilepsy | inhibition | knockout

Neural networks within the thalamocortical system are capable of generating rhythmic activity. These oscillations are correlative to normal behaviors, such as 7–14 Hz spindles displayed during sleep, but can be hallmarks of neurological disorders, such as the bilateral and hypersynchronous 3 Hz spike and wave discharge (SWD) produced during absence epileptic seizures (1, 2). The cellular and molecular basis for both normal and pathological thalamic oscillations has been extensively studied, and demonstrated to rely upon the balance of excitatory and inhibitory inputs between populations of reciprocally interconnected neurons (3). The thalamic reticular nucleus (nRT) contains GABAergic neurons that surround and innervate the dorsal thalamus, providing synaptic inhibition onto excitatory thalamocortical relay neurons (TC) (4). The firing of nRT neurons produces IPSPs on TC neurons, and this hyperpolarization deactivates low threshold T-Type calcium channels and evokes TC rebound bursting (5). TC neurons, in turn, project collaterals back to the nRT, and reexcite nRT neurons leading to intrathalamic oscillations that integrate with and sustain thalamocortical network oscillations.

An integral component of thalamocortical circuitry is fast inhibition, primarily mediated by activation of postsynaptic GABA_A receptors (GABA_ARs). These receptors are heteropentameric ligand-gated chloride channels formed from an array of 16 identified subunits ($\alpha 1$ – $\alpha 6$, $\beta 1$ – $\beta 3$, $\gamma 1$ – $\gamma 3$, δ , ϵ , π , and θ) (6). At synapses, GABA_ARs likely contain 2 α 's: 2 β 's: 1 γ , and inclusion of different subunits into the pentamer can confer unique biophysical and pharmacological properties, including: conductance, gating, affinity, and sensitivity to allosteric modulators (7). Immunohistochemical localization studies have shown that the thalamus displays nucleus-specific differences in GABA_AR subunits (8, 9). Neurons in the nRT primarily express $\alpha 3$, $\beta 3$, and $\gamma 2$ subunits, whereas TC neurons, for example those in the ventrobasal nucleus (VB), contain $\alpha 1$, $\alpha 4$, $\beta 2$ $\gamma 2$ and δ . These

subunits have been proposed to assemble into 3 physiologically distinct receptor subtypes: $\alpha 3\beta 3\gamma 2$ receptors mediate phasic inhibition in nRT neurons (10), $\alpha 1\beta 2\gamma 2$ receptors phasic inhibition in TC neurons, and $\alpha 4\beta 2\delta$ containing receptors a tonic inhibition (11) in TC neurons. This heterogeneity provides an opportunity to investigate the physiology of inhibition mediated by specific subunits in discrete nuclei in the thalamus, and reveal their corresponding roles in the generation of rhythmic activity.

Recently, a mouse line with the GABA_AR $\alpha 3$ subunit gene deleted ($\alpha 3$ KO) has been generated and described (12–14). Behaviorally, $\alpha 3$ KO mice present a mild phenotype, with no overt disturbances to thalamocortical function, despite a complete loss of $\alpha 3$ subunit protein in the nRT. Because the $\alpha 3$ subunit is the only α subunit detectable in the nRT and is not localized elsewhere in the thalamus (9) we hypothesize that these mice would show disturbances in synaptic inhibition in the nRT and provide a model system to study the physiology of $\alpha 3$ mediated synaptic inhibition within thalamocortical circuitry, as predicted from previous studies in $\beta 3$ subunit mutants (15).

Results

Inhibitory Postsynaptic Currents in the Thalamus Are Altered in the nRT of $\alpha 3$ KO Mice. To investigate the impact of deletion of the GABA_AR $\alpha 3$ subunit gene on neurotransmission in the thalamus, we performed whole-cell patch clamp recordings on wild-type (WT) and $\alpha 3$ KO brain slices. Despite the loss of this major subunit, electrophysiological recordings detected robust spontaneous inhibitory postsynaptic current (IPSC) events in $\alpha 3$ KO nRT neurons (representative trace Fig. 1B) in all neurons examined (26/26 cells from 15 animals). Events were clearly GABA_AR mediated, as demonstrated by their characteristic chloride conductance and complete blockade by 20 μ M the specific GABA_AR antagonist SR-95331 (Fig. 1C). The GABA_AR antagonists bicuculline and picrotoxin also abolished IPSCs in the nRT of $\alpha 3$ KO mice and did not shift the baseline holding current, indicating that there were not compensatory changes in the GABA tonic conductance. This was further examined through analysis of the variance of the baseline noise current, which was similar between genotypes (WT = 2.2 ± 0.2 pA, $n = 14$; $\alpha 3$ KO = 2.4 ± 0.1 pA, $n = 14$, $P > 0.05$).

We next examined the parameters of isolated IPSC events and compared these between WT and $\alpha 3$ KO. The kinetics of IPSCs in nRT of WT rodents have been described, and are characterized by a long-lasting, slowly deactivating current (Fig. 1A, D)

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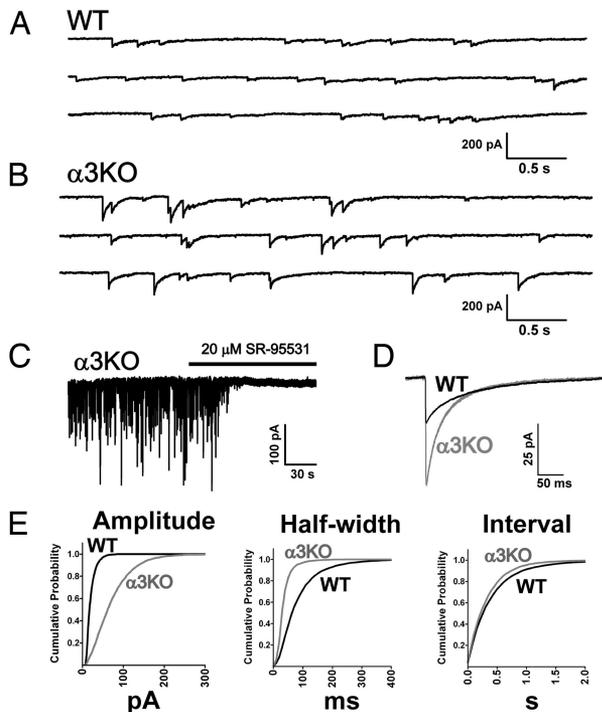


Fig. 1. Deletion of the GABA_A receptor $\alpha 3$ subunit alters synaptic inhibition in the thalamus. Ten seconds of IPSC recordings from representative nRT neurons from (A) WT and (B) $\alpha 3$ KO mice. Events in $\alpha 3$ KO nRT were GABA_A receptor mediated, as demonstrated by (C) complete blockade of events by 20 μ M the specific antagonist SR-95531. (D) Ensemble averaged IPSCs from WT (black, $n = 25$ cells) and $\alpha 3$ KO (gray, $n = 26$ cells) nRT neurons, plotted on the same time scale to illustrate the differences in amplitude and kinetics. (E) Cumulative probability histograms of >5000 isolated events from WT ($n = 5$ neurons) and $\alpha 3$ KO ($n = 5$) demonstrate changes in amplitude and kinetics across all populations of events.

(16). We analyzed the mean IPSC response from multiple nRT neurons from WT ($n = 25$) and $\alpha 3$ KO ($n = 26$). In both genotypes, IPSC events activated in a rapid manner (10–90% rise times: WT = 1.2 ± 0.1 ms; $\alpha 3$ KO = 1.2 ± 0.1 ms, $P > 0.05$). Most significantly, IPSCs in $\alpha 3$ KO displayed a large increase in amplitude compared with wild type (WT = 23 ± 1 pA; $\alpha 3$ KO = 60 ± 4 pA, $P < 0.001$). Additionally, decay kinetics were significantly faster in $\alpha 3$ KO, measured as both the event half-width (WT = 60 ± 3 ms; $\alpha 3$ KO = 32 ± 1 ms, $P < 0.001$) and the weighted biexponential decay time (WT $\tau_{D,W} = 112 \pm 3$ ms; $\alpha 3$ KO $\tau_{D,W} = 60 \pm 2$ ms, $P < 0.001$). These changes in amplitude and kinetics were observed across all populations of events, as demonstrated by the cumulative probability histograms (Fig. 1E). There were no differences in IPSC frequencies between genotypes (WT = 2.4 ± 0.2 Hz; $\alpha 3$ KO = 2.9 ± 0.3 Hz, $P > 0.05$). We also examined the “miniature” IPSC population using bath application of 1 μ M tetrodotoxin, and events displayed similar parameters between WT and $\alpha 3$ KO (supporting information (SI) Fig. S1) indicating that the observed gain in amplitude in $\alpha 3$ KO neurons was not attributable to an increase in the number of action potential mediated events. Additionally, we sorted and analyzed IPSCs according to different ages and sex, and found that the aforementioned alterations in IPSC parameters were not attributable to a transient developmental effect or sexual dimorphism, as we observed the characteristic large-amplitude, faster-decaying type of IPSCs in all ages of $\alpha 3$ KO mice tested (postnatal days 12 through 35) and in both males and females.

To further characterize inhibitory neurotransmission in nRT, we examined evoked IPSC events (eIPSCs) in WT and $\alpha 3$ KO neurons (Fig. S2). Using a protocol of minimal electrical stim-

ulation within the nRT, we recorded isolated eIPSC events from nRT neurons (WT $n = 8$; $\alpha 3$ KO $n = 14$). Similar to spontaneous event data, $\alpha 3$ KO eIPSCs displayed a significant increase in amplitude (WT = 62 ± 10 pA; $\alpha 3$ KO = 296 ± 43 pA, $P < 0.01$) and faster decay kinetics (WT $\tau_{D,W} = 195 \pm 11$ ms; $\alpha 3$ KO $\tau_{D,W} = 85 \pm 8$ ms, $P < 0.01$) compared with wild-type. The charge transfer per event, calculated as the integral area, was also larger in $\alpha 3$ KO neurons (WT = 8.5 ± 1.2 pC; $\alpha 3$ KO = 17.7 ± 2.5 pC, $P < 0.05$). Together, these data show that deletion of the $\alpha 3$ subunit produces a paradoxical gain in inhibitory efficacy of synaptic transmission in nRT and increases the total inhibitory current onto nRT neurons.

Changes in Sensitivity to Allosteric Modulators of GABA_A Receptors in the nRT of $\alpha 3$ KO Mice. The observed differences in IPSC amplitude and kinetics between genotypes indicate a change in the subunit composition of the postsynaptic receptor in $\alpha 3$ KO mice. To investigate this, we used subunit specific pharmacology to probe the identity of GABA_ARs in nRT. Clonazepam (CZP) is a benzodiazepine and positive allosteric modulator of GABA_ARs, which invariably requires the presence of a γ subunit in the receptor complex to exert its actions (17). In WT mice, 300 nM CZP robustly increased the amplitude (predrug: 23 ± 1 pA; CZP: 28 ± 1 pA, $n = 7$, $P < 0.01$) and decay time (predrug: $\tau_{D,W} = 107 \pm 12$ ms; CZP: $\tau_{D,W} = 156 \pm 17$ ms, $n = 7$, $P < 0.05$) of IPSCs in the nRT (Fig. 2A), a result that is consistent with postsynaptic currents in this nucleus mediated by GABA_ARs containing the $\gamma 2$ subunit (17). In $\alpha 3$ KO neurons, CZP retained its modulatory effects on IPSCs in nRT, but the major action was increasing the decay kinetics (predrug: $\tau_{D,W} = 61 \pm 2$ ms; CZP: $\tau_{D,W} = 83 \pm 5$ ms, $n = 6$, $P < 0.05$), whereas the effects on event amplitude were not significant (predrug: 49 ± 9 pA; CZP: 52 ± 9 pA, $n = 6$, $P > 0.05$). These results demonstrate that the $\gamma 2$ subunit and benzodiazepine binding site remain intact in $\alpha 3$ KO GABA_ARs, but the altered pharmacological effects of CZP on IPSC shape suggest additional changes to the postsynaptic subunit combination.

Next, we examined the activity of loreclezole (LOR), a modulator of GABA_ARs that specifically activates receptors containing either $\beta 2$ or $\beta 3$ subunit, but not receptors containing $\beta 1$ (18). 10 μ M LOR (Fig. 2B) increased the amplitude (control: 23 ± 1 pA; LOR: 27 ± 1 pA, $n = 11$, $P < 0.05$) and decay kinetics (control: $\tau_{D,W} = 112 \pm 3$ ms; LOR: $\tau_{D,W} = 183 \pm 21$ ms, $n = 11$, $P < 0.001$) of IPSCs in WT neurons, consistent with high levels of $\beta 3$ subunit expression in nRT. Similar to the effects of CZP, LOR did not alter IPSC amplitude in $\alpha 3$ KO nRT neurons (control: 60 ± 4 pA; LOR: 53 ± 4 pA, $n = 8$, $P > 0.05$), but did slow decay rate (control: $\tau_{D,W} = 60 \pm 2$ ms; LOR: $\tau_{D,W} = 132 \pm 8$ ms, $n = 8$, $P < 0.001$). These data indicate that the $\beta 3$ subunit is likely retained in postsynaptic receptors of $\alpha 3$ KO nRT neurons, and interestingly, these receptors appear more sensitive to the effects of LOR, suggesting possible increased $\beta 3$ subunit dependence.

Next, we examined the effects of the benzodiazepine site agonist zolpidem (ZOL) on IPSCs in the nRT. This compound displays high affinity for GABA_ARs containing the $\alpha 1$ subunit, intermediate affinity for receptors containing $\alpha 2$ and $\alpha 3$, and no affinity for receptors containing $\alpha 5$ (19). Accordingly, we used 1 μ M ZOL, a high concentration that activates $\alpha 1$, $\alpha 2$ and $\alpha 3$ subunits. In WT nRT neurons, ZOL (Fig. 2C) produced no increase in IPSC event amplitude (predrug: 20 ± 2 pA; ZOL: 23 ± 3 pA, $n = 5$, $P > 0.05$), but did significantly prolong decay kinetics (predrug: $\tau_{D,W} = 115 \pm 7$ ms; ZOL: $\tau_{D,W} = 135 \pm 12$ ms, $n = 5$, $P < 0.05$). On $\alpha 3$ KO nRT neurons, ZOL had no observable modulatory action, affecting neither amplitude (predrug: 56 ± 7 pA; ZOL: 54 ± 7 pA, $n = 7$, $P > 0.05$) nor decay kinetics (predrug: $\tau_{D,W} = 60 \pm 4$ ms; ZOL: $\tau_{D,W} = 62 \pm 5$, $n = 7$, $P > 0.05$). This result confirms the absence of $\alpha 3$ subunit and indicates that $\alpha 1$ or $\alpha 2$ subunits are not expressed at inhibitory

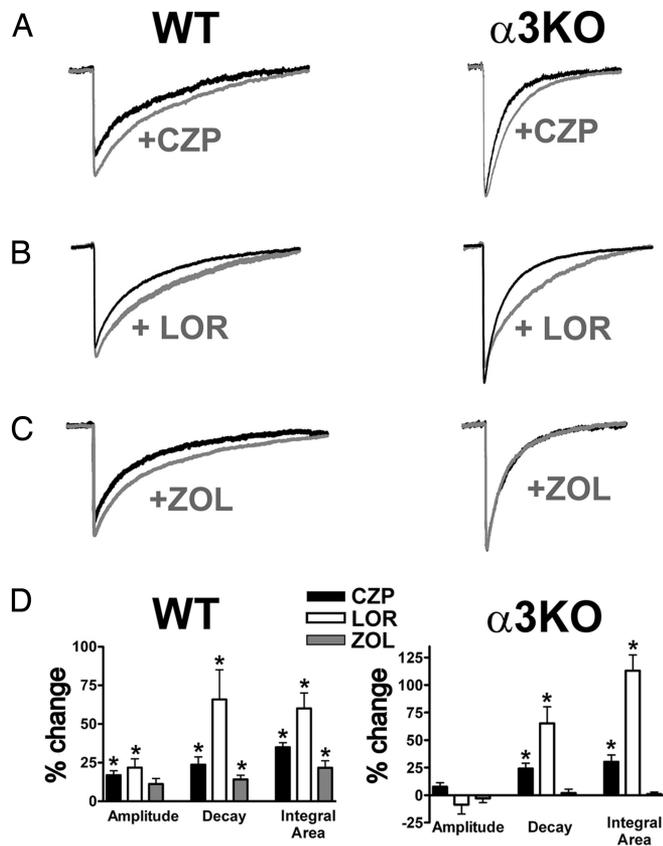


Fig. 2. Altered pharmacology at $\alpha 3$ KO nRT synapses. The benzodiazepine (A) clonazepam (CZP, 300 nM) and the $\beta 2/3$ selective modulator (B) loreclezole (LOR, 10 μ M) augment the amplitude, decay kinetics, and integral area of IPSCs in WT nRT neurons. Although effects are present in $\alpha 3$ KO neurons, the main actions are upon decay kinetics and integral area. Conversely, (C) zolpidem (ZOL, 1 μ M) increases the decay and integral area of IPSCs in WT, but has no effect on $\alpha 3$ KO currents, suggesting altered α subunit composition of GABA_A receptors in $\alpha 3$ KO mice. (D) Summaries of the changes in IPSC changed induced by CZP, LOR and ZOL (*, $P < 0.05$).

synapses in $\alpha 3$ KO nRT neurons, a result that is consistent with immunohistochemical localization studies in these mice (13).

The major GABA_AR benzodiazepine binding sites in the brain are formed by the $\gamma 2$ subunit in combination with $\alpha 1$, $\alpha 2$, $\alpha 3$ or $\alpha 5$ subunits (7, 17). Accordingly, the previous data cannot exclude the possibility that GABA_ARs in the nRT of $\alpha 3$ KO neurons contain $\alpha 5$. To examine this, we used L-655,708, a potent selective inverse agonist for the benzodiazepine binding site, which at low nanomolar concentrations displays high specificity for the $\alpha 5$ subunit (20). However, 20 nM L-655,708 had no effect on IPSCs amplitude or decay kinetics in $\alpha 3$ KO nRT neurons (Fig. S3), suggesting that the $\alpha 5$ subunit is not contained in these synaptic GABA_ARs. Our pharmacological results are consistent with a change in the subunit composition of the postsynaptic receptor of $\alpha 3$ KO mice. Although $\alpha 3$ KO GABA_ARs retain sensitivity to benzodiazepines and loreclezole, indicating β and γ are present, their major pharmacological effects were on IPSC decay kinetics, not amplitude. In addition, ZOL and L-655,708, compounds that modulate receptors containing specific α subunits, had no effect in $\alpha 3$ KO mice, suggesting an alteration of α subunits in $\alpha 3$ KO nRT.

Other Network Features Are Unaltered in $\alpha 3$ KO Mice. A caveat of gene knockout methodology is the potential for multiple in vivo compensatory changes that might obscure the precise pheno-

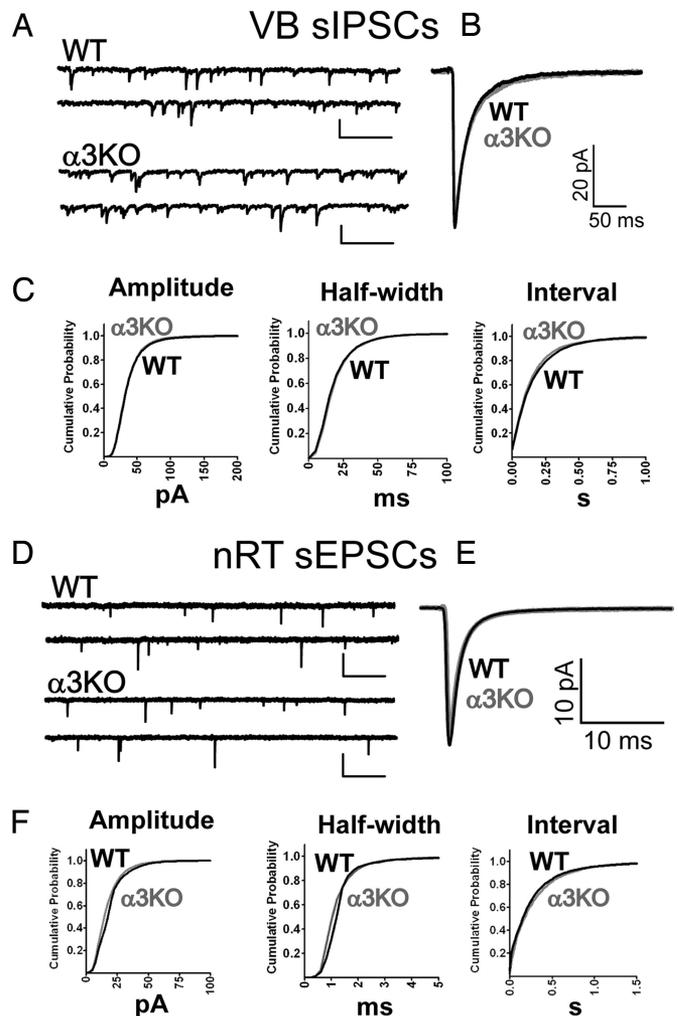


Fig. 3. Other aspects of thalamic synaptic transmission were unaltered in $\alpha 3$ KO mice. (A) Representative 10 second recordings showing IPSCs in VB in WT and $\alpha 3$ KO, scale bars indicate 100 pA; 500 ms. (B) Ensemble IPSC responses averaged across multiple WT ($n = 9$) and $\alpha 3$ KO ($n = 11$) VB neurons and (C) cumulative probability histograms of >6000 isolated events demonstrate no changes in amplitude, kinetics or frequency between genotypes. Excitatory neurotransmission in the nRT is also unaffected. (D) Isolated EPSC events from representative 5 second recordings from WT and $\alpha 3$ KO nRT neurons, scale bars 50 pA; 200 ms. (E) Ensemble EPSC responses averaged from WT ($n = 14$) and $\alpha 3$ KO ($n = 14$) nRT neurons and (F) cumulative probability histograms of >4000 isolated EPSC events from each genotype demonstrate no changes in amplitude, kinetics or frequency.

type. To determine if deletion of the GABA_AR $\alpha 3$ subunit produces other changes within the thalamic circuit, we examined 2 additional types of synaptic transmission and intrinsic excitability of nRT cells. TC relay neurons receive inhibitory input from nRT neurons, and we analyzed isolated IPSCs in VB neurons from WT ($n = 9$) and $\alpha 3$ KO ($n = 11$) mice (Fig. 3A–C). The amplitude (WT = 42 ± 5 pA; $\alpha 3$ KO = 38 ± 2 pA, $P > 0.05$), frequency (WT = 5.6 ± 0.8 Hz; $\alpha 3$ KO = 5.3 ± 0.8 Hz, $P > 0.05$), half-width (WT = 15 ± 1 ms; $\alpha 3$ KO = 15 ± 1 ms, $P > 0.05$) and decay kinetics (WT: $\tau_{D,W} = 24 \pm 2$ ms; $\alpha 3$ KO: $\tau_{D,W} = 23 \pm 3$ ms, $P > 0.05$) were not statistically significant between genotypes. This result validates the specificity of the $\alpha 3$ KO effect for the nRT, because TC neurons in the VB have been demonstrated to only express mRNA and protein for $\alpha 1$ and $\alpha 4$ subunits (8, 9).

Because neural circuitry requires a precise balance of inhibition and excitation, the increased efficacy of inhibitory neuro-

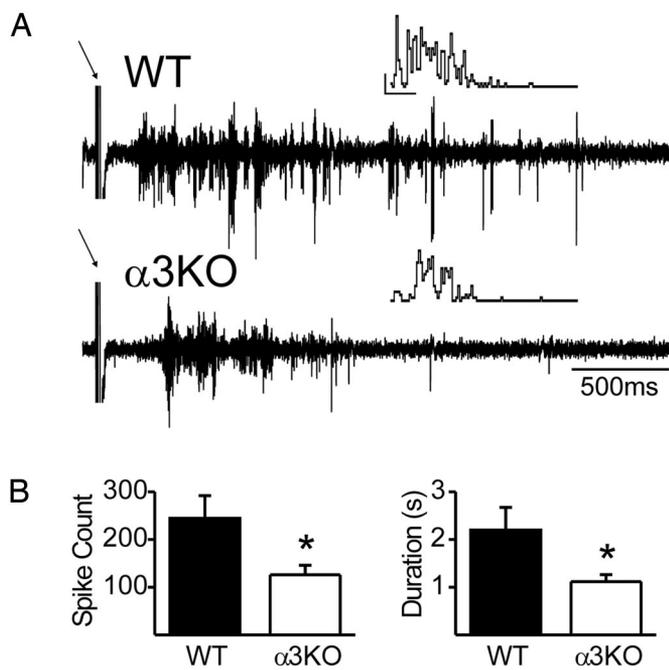


Fig. 4. Evoked oscillations are suppressed in thalamic slices from $\alpha 3$ KO mice (A) Representative multiunit recordings of intra-thalamic oscillations from WT and $\alpha 3$ KO slices elicited in brain slices by single electrical shocks (arrows indicate stimulus artifacts). Insets show a poststimulus time histograms of multiunit spike rates during the oscillations (horizontal scale: 500 ms, vertical scale: 200 Hz). (B) Summary data for evoked thalamic oscillations. The number of spikes and the duration were both significantly reduced in $\alpha 3$ KO slices (*, $P < 0.05$)

transmission in $\alpha 3$ KO mice might produce an activity dependent compensatory down-regulation of excitatory postsynaptic current events (EPSCs). To examine this, we recorded isolated sEPSCs from neurons in the nRT of WT ($n = 14$) and $\alpha 3$ KO ($n = 14$) thalami (Figs. 3D–F). Again, we observed no significant changes in the amplitude (WT = -21 ± 2 pA; $\alpha 3$ KO = -22 ± 2 pA, $P > 0.05$), frequency (WT = 4.0 ± 0.5 Hz; $\alpha 3$ KO = 4.5 ± 0.5 Hz, $P > 0.05$) or event kinetics (half-width: WT = 1.5 ± 0.1 ms; $\alpha 3$ KO = 1.4 ± 0.1 ms, $P > 0.05$). In addition, we found no changes in intrinsic excitability of nRT neurons (Fig. S4). Overall these results demonstrate the specificity of the gain in inhibitory synaptic strength in nRT in $\alpha 3$ KO and show this effect is not accompanied by concomitant changes in intrinsic excitability or excitatory neurotransmission.

Deletion of the GABA_AR $\alpha 3$ Subunit Reduces Evoked Thalamic Oscillatory Activity. The synaptic data show that $\alpha 3$ KO mice present a unique neurophysiological phenotype, in which a single component of the thalamocortical circuit has been altered in a gain of function manner, with no apparent changes to other types of synaptic transmission. To investigate the effects of increased nRT inhibitory strength on thalamocortical circuit function, we examined the properties of evoked thalamic oscillations. Delivering a single electrical shock to the internal capsule of thalamic slices elicits rhythmic spiking activity that can be detected via extracellular unit recordings within VB (21, 22). In WT slices ($n = 11$), evoked thalamic oscillations are robust (Fig. 4A), lasting several seconds (mean duration = 2.20 ± 0.25 s) and producing several hundred spikes (mean spike count = 244 ± 44). By contrast, $\alpha 3$ KO thalamic slices ($n = 18$) displayed a significant decrease in the length of evoked oscillations (mean duration = 1.12 ± 0.14 s, Fig. 4B) and total number of spikes (mean spike count = 126 ± 19 , Fig. 4B). These data support the conclusion that thalamic oscillations are modulated by

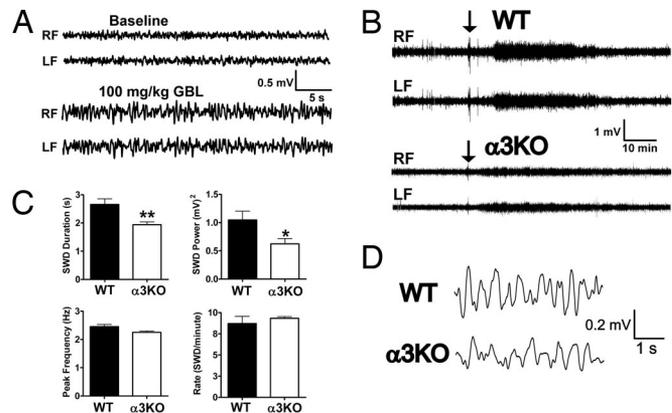


Fig. 5. Pharmacologically induced absence seizures are reduced in $\alpha 3$ KO mice. (A) Representative EEG recordings from right frontal (RF) and left frontal (LF) cortex from a WT mouse during baseline and 15 minutes after injection of GBL (100 mg/kg). SWDs are clearly detectable bilaterally synchronous 2–4 Hz waveform events that emerge 5–10 min after GBL administration. (B) Comparison of 80-min trials of continuous EEG recording from frontal cortex from representative WT and $\alpha 3$ KO mice. The arrow marks the GBL injection time point. (C) Summaries of wavelet analysis: SWD power and duration were significantly reduced in $\alpha 3$ KO mice (WT $n = 5$; $\alpha 3$ KO $n = 5$; $P < 0.05$, ** $P < 0.01$), whereas SWD peak spectral power and SWD rate were not different between genotypes ($P > 0.05$). (D) Example traces of representative SWD events in WT and $\alpha 3$ KO mice.

GABA_AR mediated inhibitory synaptic transmission within the nRT. Specifically, the duration of network activity is inversely proportional to strength of intra-nRT connectivity, which is consistent with the increased duration and strength of thalamic oscillatory activity in GABA_AR $\beta 3$ knockout mice that have reduced inhibitory transmission in nRT (15).

$\alpha 3$ KO Mice Display a Reduction in Duration and Strength of Induced Absence Seizures. Absence seizures are electrically characterized by hypersynchronous cortical activity of thalamic origin (23). The drug γ -butyrolactone (GBL) induces thalamocortical seizures characteristic of absence epilepsy, which accompany the behavioral features of seizures, including motor freezing and staring (24). We recorded cortical electroencephalogram (EEG) activity during GBL induced seizures in WT and $\alpha 3$ KO mice. During baseline awake conditions, mice produced little or no detectable SWD activity (Fig. 5A and B), and the peak power in the frequency range of 0.8 to 10 Hz was < 1.5 Hz. Subcutaneous injection of 100 mg/kg GBL induced behavioral seizures that accompanied an increase in 2–4 Hz SWD with onset 5–10 min postinjection (Fig. 5A and B). We analyzed individually isolated SWDs for the period of 60 min after GBL injections from WT ($n = 5$) and $\alpha 3$ KO mice ($n = 5$). Seizure activity was observed in both genotypes and the occurrence of seizures were similar (WT = 8.7 ± 0.9 SWDs/minute; $\alpha 3$ KO = 9.3 ± 0.2 SWDs/minute, $P > 0.05$), as was the predominant peak spectral frequency (WT = 2.5 ± 0.1 Hz; $\alpha 3$ KO = 2.3 ± 0.1 Hz, $P > 0.05$). However, $\alpha 3$ KO mice displayed a significant decrease in the average SWD duration (WT = 2.7 ± 0.2 s; $\alpha 3$ KO = 1.9 ± 0.1 s, $P < 0.01$) and average SWD power (WT = 1.05 ± 0.15 mV²; $\alpha 3$ KO = 0.62 ± 0.09 mV², $P < 0.05$). To further validate these results, we also examined absence seizures using a second pharmacological model. Pentylentetrazol (PTZ, 25 mg/kg) is a GABA_AR antagonist that evokes 3–6 Hz SWD in vivo (25). Analysis of EEG recordings (Fig. S5) showed that PTZ seizures were similarly affected by deletion of $\alpha 3$ as GBL seizures; genotype did not alter the seizure rate or peak spectral frequency, but $\alpha 3$ KO mice displayed a reduction in the power and length of PTZ seizure events. Therefore, in 2 pharmacological

models of absence seizures, the frequency and characteristic spectral power of SWDs are not affected by deletion of the $\alpha 3$ subunit, suggesting that pharmacological initiation of SWD seizures is independent of inhibitory strength in nRT. By contrast, seizure duration and power were both affected in the $\alpha 3$ KO, consistent with the hypothesis that strength of nRT inhibition strongly regulates seizure duration and severity.

Discussion

We found that mice lacking the GABA_AR $\alpha 3$ subunit display profound changes in the physiology of inhibitory synaptic currents in the nRT. These events are atypically large in amplitude and display faster decay kinetics than those present in wild-type neurons, properties observed in all spontaneous, miniature and evoked synaptic recordings. Measured as a product of the charge transfer per IPSC event and frequency, $\alpha 3$ KO nRT neurons receive more inhibitory current than wild-type neurons. At the network level, this specific increase in inhibitory strength in nRT dampens the duration of evoked isolated thalamic oscillations, and decreases the duration and power of pharmacologically induced absence seizures. These data highlight the role of GABAergic inhibition within the nRT as a critical control point for the excitability of the thalamocortical circuit.

Inhibition within thalamic circuitry is established at functionally diverse GABAergic synapses, and this study strongly supports the hypothesis that $\alpha 3$ containing receptors expressed by nRT neurons normally function in an anti-oscillatory capacity. Anatomically, nRT neurons project collaterals onto adjacent nRT neurons that form axo-dendritic and dendro-dendritic synapses, which interconnect a powerful inhibitory network of cells that control the output of the dorsal thalamus to the cortex (26, 27). Previous studies have provided the initial evidence that inhibitory intra-nRT synapses are integral in the oscillatory process. First, it was demonstrated that increasing inhibition within the nRT pharmacologically can dampen thalamic oscillations (28), and genetically engineered knock-in mice, in which the $\alpha 3$ subunit was rendered insensitive to benzodiazepines, failed to display a reduction in evoked thalamic oscillation duration when treated with clonazepam. We can also compare the results of this study to an earlier finding with mice lacking the $\beta 3$ subunit (15, 29). $\beta 3$ KO mice display a severe phenotype that includes prevalent neonatal lethality, seizures, and powerful, hypersynchronous thalamic oscillations. Synaptic recordings in the nRT of $\beta 3$ KO show a near absence of sIPSCs and a reduction in amplitude and efficacy of eIPSCs, demonstrating the involvement of nRT inhibition in suppressing thalamocortical function. The data in the present study provides complimentary, converse evidence: that while elimination of nRT inhibition produces powerful oscillations and spontaneous seizures, enhancement of nRT inhibition reduces oscillation length and abates induced seizures. We should note that the $\alpha 3$ subunit is also expressed by neurons in cortical layers V and VI (9), and therefore, we cannot exclude that the changes in seizure duration could be partially attributed to alterations in inhibitory synaptic transmission in the cortex. However, the isolated thalamic slice oscillation data shown here provides compelling evidence that implicates a thalamic origin.

An unresolved but interesting issue is the absolute subunit composition of the postsynaptic GABA_ARs in the nRT of $\alpha 3$ KO mice. Although our data suggest that these receptors likely contain $\beta 3$ and $\gamma 2$ subunits, pharmacology experiments here, along with immunohistochemical localization studies (13, 14) have failed to detect a complementary α subunit expressed in this nucleus. This result is somewhat confounding, because a preponderance of evidence supports the notion that phasic synaptic inhibition requires α , β and γ subunits to form a functional postsynaptic GABA_AR (30–33). There are 2 possible explanations to account for this discrepancy. First, deletion of the $\alpha 3$ subunit might produce up-regulation of an alternate α subunit that experimental methodology failed to detect. In early

development, nRT neurons display a transient expression of the $\alpha 5$ subunit at approximately postnatal day 5 (13). Through maturation, the $\alpha 5$ subunit in nRT is gradually replaced by $\alpha 3$, and it is therefore possible that engineered $\alpha 3$ subunit deletion causes $\alpha 5$ to persist postnatally in this nucleus and form synaptic receptor complexes comprised of $\alpha 5\beta 3\gamma 2$. Contrary to this, the specific $\alpha 5$ subunit inverse agonist L-655,708 has no effect on IPSC shape in $\alpha 3$ KO nRT neurons. Additionally, it has been shown that recombinant receptors comprised of $\alpha 5\beta 3\gamma 2$ display smaller peak amplitude currents (34), which is not consistent with the atypically large IPSCs observed in $\alpha 3$ KO nRT neurons.

A second possibility is that a modified GABA_AR subtype mediates inhibition in $\alpha 3$ KO. Ongoing activity in the thalamic circuit in the absence of intra-nRT inhibition might produce a strong compensatory mechanism that could drive expression and assembly of a receptor combination containing α subunits with alternatively spliced mRNA or posttranslational modifications to the mature protein, either of which might obscure identification through conventional immunological and pharmacological methods. Nonetheless, the failure to identify the subunit combination in $\alpha 3$ KO nRT neurons does not impact the major conclusion of this report — that intra-nRT connection strength is inversely related to strength and duration of pathological thalamocortical network oscillations. We propose further investigation of these inhibitory synapses in nRT, and postulate that $\alpha 3$ KO mice might be a promising model system for studying GABA_AR subunit modifications or accessory proteins involved in the formation of synaptic GABA_ARs.

Last, this study provides some insight regarding the relationship between synaptic current kinetics and higher order neural activity. One of the most striking observations was the profound changes in the shape of IPSCs in $\alpha 3$ KO mice. In wild-type rodents, nRT IPSCs display a characteristic long-lasting, slowly deactivating current that is attributed to the affinity of postsynaptic $\alpha 3\beta 3\gamma 2$ receptors (35). Because of this unique synaptic phenotype, we believed their role to be integral in the generation of rhythmic thalamocortical activity. However, it is apparent that this WT inhibition can be readily substituted with a higher-amplitude, fast-decaying kinetics with no deleterious consequences (36). On the contrary, the data here show that $\alpha 3$ KO mice are resistant to sustained thalamic activity and display of level of resistance to induced seizures. This would suggest that allosteric modulators that augment the amplitude of $\alpha 3$ containing receptors would be effective at terminating or controlling seizures of thalamic origin.

Materials and Methods (See *SI Text* for Detail)

Thalamic Oscillations. Slices were placed in an interface chamber at 34 °C and superfused with oxygenated ACSF. Electrical stimuli (20- to 100-V, 40- to 80- μ s) were delivered to the internal capsule through a pair of 50–100 K Ω tungsten electrodes (FHC) with a separation of \approx 100 μ m. Extracellular multiunit recordings were obtained with 50–100 K Ω tungsten electrodes placed in the VB and digitized with a Digidata 1200 and pClamp software (Molecular Devices) and band-pass filtered between 100 Hz and 3 kHz. To detect spikes in these recordings we used analysis methodology described (28). In brief, we detected spikes as steep slope deflections 3x greater than the background noise in each recording. We quantified the duration of oscillatory activity by the last instance of at least 5 spikes in a 50-ms sliding window.

Seizure Models. Male mice at least 25 days postnatal were anesthetized with ketamine/xylazine (100/10 mg/kg I.P.) and 4 stainless steel electrode screws were surgically implanted on the dural surfaces of the right and left sides of both frontal and parietal cerebral cortex and attached to a connection pedestal glued to the surface of the skull. After at least 7 days of postsurgery recovery, EEG recordings were performed. Mice were placed in the recording area, habituated, and 30 min of baseline EEG activity was monitored on an XLTek acquisition system. Absence seizures were induced by s.c. injection of the drugs γ -butyrolactone (GBL, 100 mg/kg, Sigma) or pentylenetetrazol (PTZ, 25 mg/kg, Tocris). EEG activity was recorded simultaneously with video monitoring for 60 min postinjection. Recordings were band passed filtered off-line and wavelet analysis was performed with MatLab.

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