FULL-LENGTH ORIGINAL RESEARCH

TRPC3 channels play a critical role in the theta component of pilocarpine-induced status epilepticus in mice

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Summary

Objective: **Canonical transient receptor potential (TRPC) channels constitute a family of cation channels that exhibit a regional and cell-specific expression pattern throughout the brain. It has been reported previously that TRPC3 channels are effectors of the brain-derived neurotrophic factor (BDNF)/trkB signaling pathway. Given the long postulated role of BDNF in epileptogenesis, TRPC3 channels may be a critical component in the underlying pathophysiology of seizure and epilepsy. In this study, we investigated the precise role of TRPC3 channels in pilocarpine-induced status epilepticus (SE).**

Methods: **The role of TRPC3 channels was investigated using TRPC3 knockout (KO) mice and TRPC3-selective inhibitor Pyr3. Video and electroencephalography (EEG) recording of pilocarpine-induced seizures were performed.**

Results: **We found that genetic ablation of TRPC3 channels reduces behavioral manifestations of seizures and the root-mean-square (RMS) power of SE, indicating a significant contribution of TRPC3 channels to pilocarpine-induced SE. Furthermore, the reduction in SE in TRPC3KO mice is caused by a selective attenuation of pilocarpine-induced theta activity, which dominates both the preictal phase and SE phase. Pyr3 also caused a reduction in the overall RMS power of pilocarpine-induced SE and a selective reduction in the theta activity during SE.**

Significance: **Our results demonstrate that TRPC3 channels unequivocally contribute to pilocarpine-induced SE and could be a novel molecular target for new anticonvulsive drugs.**

KEY WORDS: **Beta rhythm, Electroencephalography, Gamma rhythm, Seizures, Spectral analysis, Transient receptor potential channels.**

Canonical transient receptor potential channel (TRPC)3 is a member of the large *trp* family of cation channels.[1] Although all TRPC channels can be activated by G protein–coupled receptors and are widely expressed in multiple types of tissues and organs, distinct functional roles for each specific TRPC are emerging.[2] TRPC3 channels are expressed both in the central nervous system and peripheral tissues, and messenger RNA (mRNA) for TRPC3 is detected widely in the hippocampus and neocortex.[3] However, our knowledge of the specific functional role of TRPC3 in the brain is still limited.

Although exhibiting high sequence homology to TRPC6 and 7, TRPC3 is unique in its gating by tyrosine kinase.[4] Phosphorylation of the tyrosine residue Y226 is required for the activation of TRPC3 channels. This property may confer a unique role to TRPC3 channels in signaling cascades that involve both nonreceptor tyrosine kinases and receptor tyrosine kinases. Recent studies have suggested that TRPC3 channels are critical for the signaling cascade of brain-

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Key Points

- Genetic ablation of TRPC3 reduces seizure scores of pilocarpine-induced SE
- Genetic ablation of TRPC3 reduces RMS of pilocarpine-induced SE
- The reduction of RMS is caused by a selective reduction of theta waves
- A selective TRPC3 channel inhibitor causes similar effects

derived neurotrophic factor (BDNF).[3,5,6] BDNF plays a critical role in modulating synaptic plasticity in the hippocampus and contributes to learning and memory.[7] In addition, it has been postulated as a critical contributor to many neurologic disorders such as epilepsy, Huntington's disease, and Alzheimer's disease.[8,9]

Although the upregulation of BDNF in seizures has been demonstrated repeatedly,[10–12] it remains uncertain whether this change is the underlying cause of epilepsy or is merely a consequence of seizure activity. The absence of BDNF has limited effect in most seizure models, whereas *trkB* knockout mice generally exhibit a more pronounced attenuation of seizures.[13–16] Previous studies have shown upregulation of TRPC3 channel expression post status epilepticus (SE).[17,18] However, it remains unknown whether TRPC3 channels are directly involved in SE. In this study, we investigated how genetic ablation of TRPC3 channels affected pilocarpine-induced seizures in vivo. Our results show that the genetic ablation of TRPC3 channels selectively attenuates pilocarpine-generated theta activity but promotes a pilocarpine-induced beta wave. A selective TRPC3 channel inhibitor, Pyr3, causes a similar reduction of seizure intensity and a selective reduction of the theta component of SE. Collectively, our data suggest that TRPC3 channels are involved in the pathophysiologic processes of SE.

Materials and Methods

TRPC3 knockout mice and wild-type control

All animal experiments were performed under protocols approved by the Institutional Animal Use and Care Committee at the University of Arkansas for Medical Sciences (UAMS). TRPC3 knockout (KO) mice were generated on a mixed 129SvEv:C57BL/6J background as described previously,[19] and obtained from the Comparative Medicine Branch of the National Institute of Environmental Health Sciences as homozygous TRPC3−/− mice. These TRPC3−/− mice were crossed with C57B6/129J mice (Stock#101043) from Jackson Laboratory at UAMS. TRPC3−/− and TRPC3+/+ littermates were identified through genotyping as described previously,[19] which were then used to breed experimental wild-type (WT) and TRPC3 KO mice used in this study. Mice were housed under a 12 h light–dark cycle with food and water ad libitum.

Surgery and electroencephalography recording

For electroencephalography (EEG) surgery, age-matched WT or TRPC3 KO male mice (2.8 to 3-months-old) were anesthetized with isoflurane. Stainless steel screws were used as electrodes, placed on top of the dura through five small holes drilled through the skull, and then fixed to the skull using dental cement as described previously.[20] A screw was placed over motor cortex and sensory cortex on each brain hemisphere, and the fifth screw was placed over the cerebellum as the ground electrode. After 5–7 days of recovery from the surgery, EEG signals and synchronized video were recorded using the Pinnacle 8200 system (Pinnacle Technology, Lawrence, KS, U.S.A.). The head mount was connected to a preamplifier tethered to the analog-digital converter box. The voltage differential between the pair of electrodes from each brain hemisphere was amplified with a high-pass filter (1 Hz), and recorded. EEG signals were sampled at 400 Hz and videos were recorded at 30 frames/s. To minimize the possibility of signal saturation of the analog-digital conversion system during the peak of SE, preamplifier gain was reduced in comparison to our earlier work.[20,21]

Pilocarpine-induced seizures

Age-matched WT male mice or TRPC3 KO male mice were administered a single dose of methyl scopolamine nitrate (1 mg/kg, i.p.) to block the peripheral effects of pilocarpine, followed 30 min later with a single dose of pilocarpine at 175, 222, or 280 mg/kg, i.p. Seizures induced by pilocarpine were recorded either using an integrated video-EEG recording system (Pinnacle Technology) or by a digital camcorder and scored using a modified Racine scale for each 5-min period by two investigators as described previously to avoid investigator bias.[22,23] SE was considered as occurred when a mouse exhibited multiple stage 5 or above behavioral seizures.

Electroencephalographic spectral analysis

Fast Fourier power spectral analysis of EEG signal was performed using Sirenia Seizure Pro software with a Hanning window applied to reduce spectral leakage. The band widths for the full, delta, theta, alpha, beta, and gamma frequency bands were set as 0–1,000, 0.5–4, 4.5–7.5, 8–13, 13–30, and 35–45 Hz, respectively. Only root-mean-square (RMS) values from the left hemisphere were used for analysis because RMS values from the left and right hemisphere exhibited significant correlation ($R = 0.98$)[20].

Statistical analysis

One-way or two-way analysis of variance (ANOVA) with post hoc analysis were used unless stated otherwise. Post

hoc power analysis was performed for the EEG RMS analysis. Our experiment had an 80% power to detect a 19% difference between means (WT vs. TRPC3 KO), or a 36% difference between means (SE vs. SE + Pyr3) with a significance level (alpha) of 0.05 (two-tailed).

Results

TRPC3 KO mice exhibit significantly reduced pilocarpine-induced seizure scores

To determine whether TRPC3 channels play a role in pilocarpine-induced acute seizures, we compared the TRPC3 knockout mice (3.76 ± 0.14 months old) to WT mice (3.68 ± 0.15 months old) in a matching genetic background as described previously (Fig. 1)[22,23] A threshold dose (175 mg/kg), an intermediate dose (222 mg/kg) or a high dose (280 mg/kg) of pilocarpine was administered to either WT or TRPC3 KO mice, and the resulting seizures were videotaped and graded using a modified Racine scale.[22,23] In both WT mice and TRPC3 KO mice, pilocarpine-induced seizure scores increased in a dose-dependent manner (Fig. 1A,B). Furthermore, contingency analysis indicated that there was no significant difference between the percentage of SE occurrence between WT and TRPC3 KO mice at two of three tested pilocarpine doses (32 vs. 0% at 175 mg/kg, $p = 0.14$; 60 vs. 39% at 222 mg/kg, $p = 0.08$; 83 vs. 50% at 280 mg/kg, $p = 0.04$). Therefore, the sensitivity to pilocarpine appears to be minimally altered in TRPC3 KO mice. However, there was a significant genotypic effect in seizure scores between WT and TRPC3 KO mice at higher doses of pilocarpine (Fig. 1A). This difference was significant at multiple time points at 280 mg/kg pilocarpine (Fig. 1A) and more prominent on average (Fig. 1B), suggesting that there may be a clear difference in SE intensity between WT and TRPC3 KO mice. Consistent with a reduced SE intensity, acute mortality was also reduced in TRPC3 KO mice (Fig. 1C). Collectively, our data indicate that TRPC3 channels contribute significantly to pilocarpine-induced SE in vivo.

TRPC3 KO mice exhibit significantly altered early EEG responses to pilocarpine

A recent study has revealed limitations of the Racine scale as a primary index of seizure intensity in status

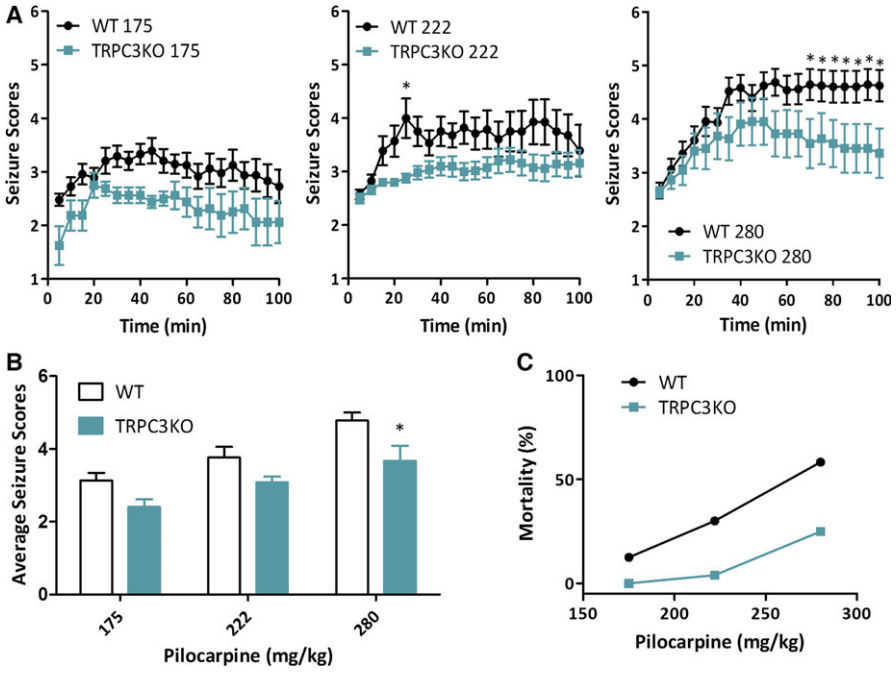

Figure 1.

Pilocarpine-induced seizures were significantly reduced in TRPC3 KO mice. (**A**) The time course of pilocarpine-induced seizures in WT and TRPC3 KO mice after administration of a single dose of pilocarpine (175, 222, or 280 mg/kg, i.p.). A modified Racine scale was used as described previously.[23] Average seizure scores (mean ± standard error of the mean [SEM]) was plotted ($n = 24$, 14, and 24 for WT and 8, 25, and 12 for TRPC3 KO). Note significantly reduced seizure scores in TRPC3 KO mice at the higher doses of pilocarpine (280 mg/kg: $p < 0.001$ for genotypic effect, two-way ANOVA; 222 mg/kg: $p < 0.05$ for genotypic effect, two-way ANOVA; *: $p < 0.05$, Fisher's least significant difference test). (**B**) Average seizure scores (25–90 min post-pilocarpine administration) were lower in TRPC3 KO compared to WT mice ($p < 0.001$, two-way ANOVA; *: $p < 0.05$, Holm-Sidek post hoc tests against WT). Average seizure scores (mean ± SEM) was plotted ($n = 24$, 14, and 24 for WT at 175, 222, and 280 mg/kg pilocarpine; $n = 8$, 25, and 12 for TRPC3 KO at 175, 222, and 280 mg/kg pilocarpine). (**C**) Mortality following pilocarpine injections was reduced in TRPC3 KO mice ($n = 8$, 25, and 11) compared to WT mice ($n = 24$, 14, and 24).

epilepticus, and the correlation between the Racine scale and the RMS power of EEG signals during the SE phase is poor.[20] Thus, direct measurement of RMS power during SE is required to determine whether genetic ablation of TRPC3 indeed significantly reduces the intensity of SE. We compared the EEG signals of five WT and four TRPC3 KO mice exhibiting SE after administration of pilocarpine (280 mg/kg). Pilocarpine-induced changes in EEG activity were then visualized using spectral density heat maps that were generated by the Sirenia Seizure Pro software package (Pinnacle Technology). As reported previously,[24] administration of pilocarpine elicited a rapid suppression of EEG activity in WT mice (Fig. 2A). However, it is evident from the power density heat map that asynchronous baseline EEG activity converted into synchronized theta and delta waves within minutes after the administration of pilocarpine (Fig. 2A). These EEG waves persisted until the onset of the first cortical ictal activity, which marked the beginning of the transition period toward SE. In TRPC3 KO mice, the overall latencies of the first cortical seizure and SE were comparable to those in WT mice (Table 1). However, in TRPC3 KO mice, the theta wave quickly disappeared and a prominent beta wave developed afterward (Fig. 2B). Thus, pilocarpine-induced changes in EEG activity prior to the appearance of cortical ictal activities were drastically altered in TRPC3 KO mice.

To better characterize and quantify the differences in early EEG activity changes after the administration of pilocarpine, spectral plots and cumulative distribution curves at two different time points during the latent period (i.e., the time period after the administration of pilocarpine but prior to cortical ictal activity) were generated (Fig. 3) and compared to the baseline (i.e., 5 min before the administration of pilocarpine). As shown in the spectral plot (Fig. 3A) and the cumulative distribution curve (Fig. 3B), the spectral distribution of baseline EEG activity (Fig. 3A) is comparable

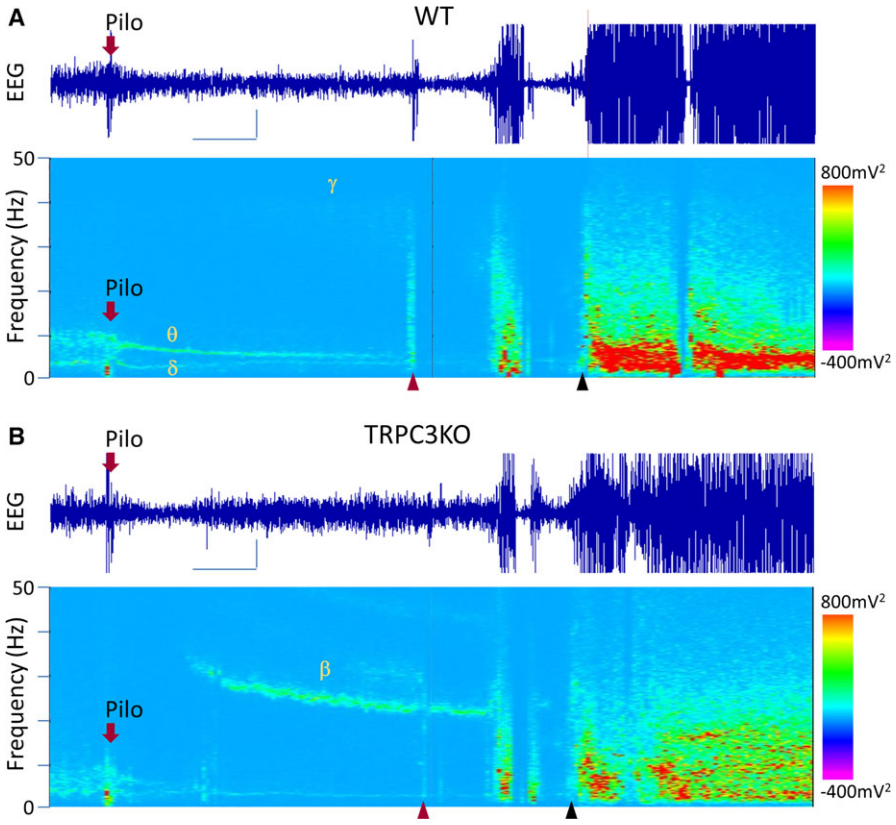

Figure 2.
Comparison of spectral characteristics of pilocarpine-induced SE in WT and TRPC3 KO mice. EEG signals and spectral heat map from a representative WT mouse (**A**) and TRPC3 KO mouse (**B**) were shown (scale bars for the EEG plot: 0.2 mV, 5 min). Note that in WT mice, within minutes after the administration of pilocarpine, the EEG activity synchronized into prominent delta and theta waves. A slow build-up of gamma waves accompanied by a gradual decrease of delta and theta waves preceded the appearance of first cortical ictal activity (maroon triangle). In TRPC3 KO mice, there was a more prominent increase of EEG activity leading to the first cortical ictal activity, and this increase in EEG activity was caused by a specific increase in beta waves in the corresponding power density heat map. On the other hand, the prominent synchronization around delta and theta bands immediately after pilocarpine administration was attenuated. The start of the SE is marked by a black triangle.

Table 1. Latencies of first cortical seizures and SE induced by pilocarpine (280 mg/kg, i.p.)

Genotypes	WT (n = 6) (mean ± SE)	TRPC3 KO (n = 4) (mean ± SE)
Latency of first cortical seizures (s)	1,787 ± 122	2,001 ± 110
Latency of SE (s)	2,310 ± 87	2,583 ± 73

between WT and TRPC3 KO mice ($p > 0.05$, Kruskal-Wallis H test), suggesting that genetic ablation of TRPC3 channels did not fundamentally alter the neural networks responsible for generating the low frequency waves (delta, theta, and alpha waves) that account for approximately 80% of total EEG activity. However, the spectral distribution curves after pilocarpine administration were significantly altered in TRPC3 KO mice (Fig. 3C, E; $p < 0.001$, Kruskal-Wallis H test). Shortly (5 min) after pilocarpine administration, the spectral plot revealed a sharp peak in the theta band centered around 6.5 Hz in WT mice (Fig. 3C), which accounted for >50% of the total EEG activity (Fig. 3D). Such a peak was absent in TRPC3 KO mice (Fig. 3C,D). As reported previously,[21] the progression toward the onset of cortical ictal activity was accompanied by an increase in gamma wave activity (Fig. 3E), which had increased from approximately 10% of total EEG activity to approximately 30% in WT mice (Fig. 3F). In TRPC3 KO mice, a large peak in the beta band appeared during this time period, which accounted for approximately 50% of total EEG activity (Fig. 3E,F). The low frequency activities (delta and theta waves) accounted for <10% total EEG activity at this point in TRPC3 KO mice. Collectively, these observations indicate that genetic ablation of TRPC3 channels significantly attenuates pilocarpine-induced theta wave activities during the latent phase of pilocarpine-induced SE.

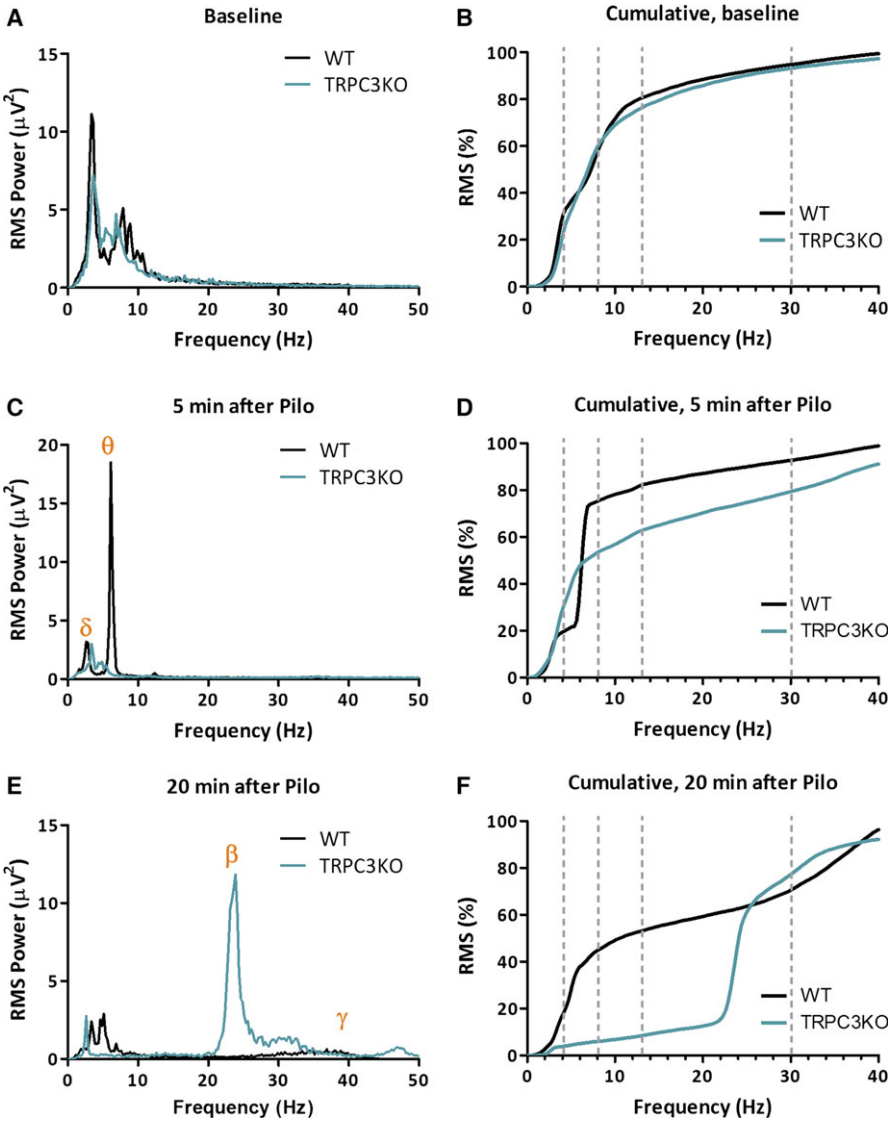

Figure 3.
Genetic ablation of TRPC3 channels selectively attenuates pilocarpine-induced theta activity during the latent period. Spectral plots and cumulative distribution curves 5 min before (**A**, **B**), 5 min after (**C**, **D**) and 20 min after (**E**, **F**) administration of pilocarpine (280 mg/kg) from WT and TRPC3 KO mice are shown. The spectral data from a 4 min window centered at a given time point were analyzed with Sirenia Seizure Pro to derive the spectral plot. Note that the spectral plot and cumulative distribution curve in WT and TRPC3 KO mice were comparable before the administration of pilocarpine (**A**, **B**). However, pilocarpine induced a rapid synchronization of EEG activities dominated by a large peak in the theta band in WT mice, which is absent in TRPC3 KO mice (**C**, **D**). Later on, a prominent gamma component developed in WT mice, whereas a beta component dominated in TRPC3 KO mice (**E**, **F**).

Genetic ablation or pharmacologic blockade of TRPC3 channels reduces SE intensity by selectively attenuating theta activity

To determine whether the intensity of SE was reduced in TRPC3 KO mice, we aligned the onset of SE and plotted the averaged RMS power in WT and TRPC3 KO mice (Fig. 4A). The intensity of SE as indexed by the RMS power in the full spectrum over a 30 min period was consistently less in TRPC3 KO mice compared to WT mice (Fig. 4A). Spectral plots (Fig. 4B) of SE (at 60 min after pilocarpine administration) revealed a sharp and large peak in the theta band in WT mice, whereas this peak was absent in TRPC3 KO mice, suggesting that the reduction in SE RMS power in TRPC3 KO mice is caused by a selective attenuation of pilocarpine-induced theta wave during the SE. This was confirmed by detailed analysis of RMS power across delta, theta, alpha, beta, and gamma frequency bands (Fig. 4C). Consistent with power density heat maps (Fig. 2) and spectral plots (Fig. 4B), the composition of SE in the TRPC3 KO is distinct from the composition of SE in WT mice. SE in TRPC3 KO mice appeared to distribute evenly across delta, theta, alpha, and beta frequency bands, whereas in WT mice, SE was dominated by the theta waves (Fig. 4C). Furthermore, RMS power was only significantly reduced in the theta frequency band (Fig. 4C); therefore, the reduction of the full RMS power in TRPC3 KO mice is due to a selective reduction of theta activity.

Pyr3 has been reported to be a selective TRPC3 channel blocker and was used to block TRPC3 channels in vivo.[25] To determine whether pharmacologic blockade of TRPC3 channels can recapitulate the reduction of SE observed in TRPC3 KO mice, we administered Pyr3 (3 mg/kg, i.p.) approximately 25 min after the onset of cortical ictal activities. This time point was chosen to satisfy the operational definition of SE. As shown in the spectral plots (Fig. 5A), the effect of Pyr3 on SE was similar to the effect of genetic ablation of TRPC3 channels. The theta peak of pilocarpine-induced SE was selectively attenuated, whereas SE activity in other spectral bands was unaltered. The full spectral power of SE was significantly reduced after administration of Pyr3 (Fig. 5B). Furthermore, this reduction of RMS power by Pyr3 was also the result of a selective attenuation of theta activity (Fig. 5B).

Collectively, genetic and pharmacologic manipulation of TRPC3 channels resulted in parallel changes in pilocarpine-induced SE, particularly in the theta component, which was selectively attenuated. Thus, we have demonstrated convincingly that TRPC3 channels play a critical role in the theta activity, the dominant component of pilocarpine-induced SE.

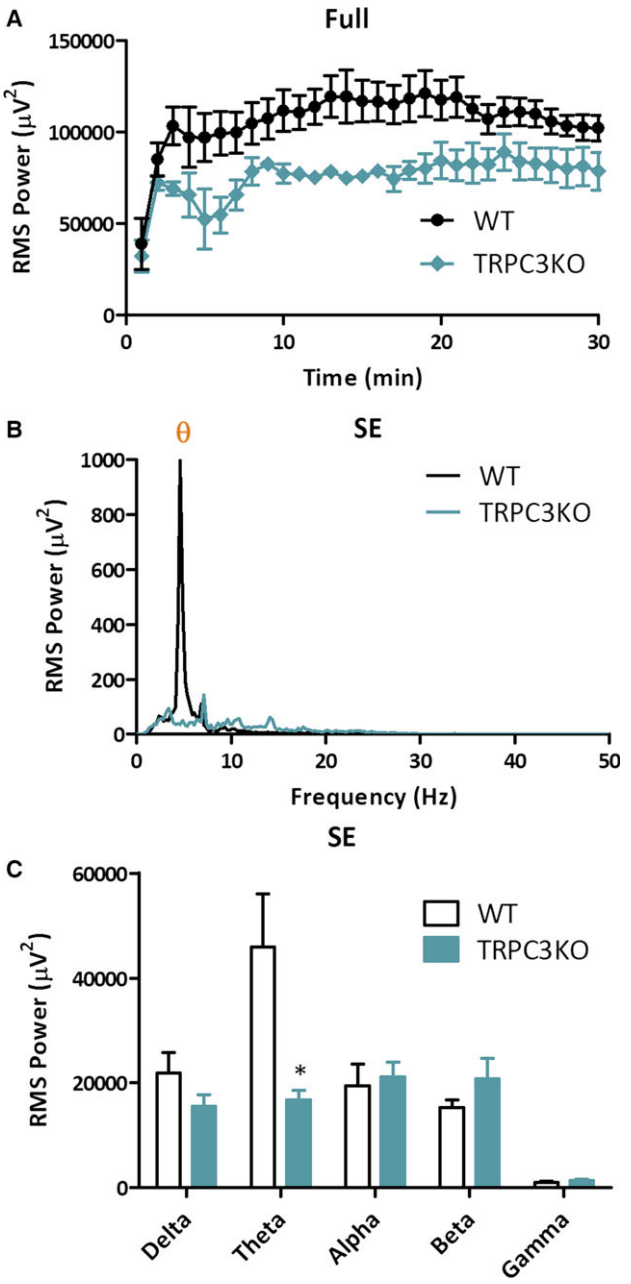

Figure 4.
Reduction of seizure intensity in TRPC3 KO mice is caused by selective attenuation of theta wave. (**A**) Pooled data plotting the RMS power during the SE state in WT mice (n = 5) and TRPC3 KO mice (n = 4). Note that the mean RMS power was significantly reduced in TRPC3 KO mice ($p < 0.05$, two-way ANOVA). (**B**) Spectral plots of EEG activity during the SE phase (60 min after administration of pilocarpine) from WT and TRPC3 KO mice were plotted. Note the large peak in the theta band in WT mice, which is absent in TRPC3 KO mice. (**C**) Spectral analysis of RMS power during the SE state in WT mice (n = 5) and TRPC3 KO mice (n = 4). Note that the theta waves were selectively reduced in TRPC3 KO mice (*: $p < 0.05$, unpaired *t*-test), whereas there was no significant difference in delta, alpha, beta, and gamma waves between WT and TRPC3 KO mice.

Discussion

Previous studies in humans and rats have demonstrated potential involvement of TRPC3 in temporal lobe

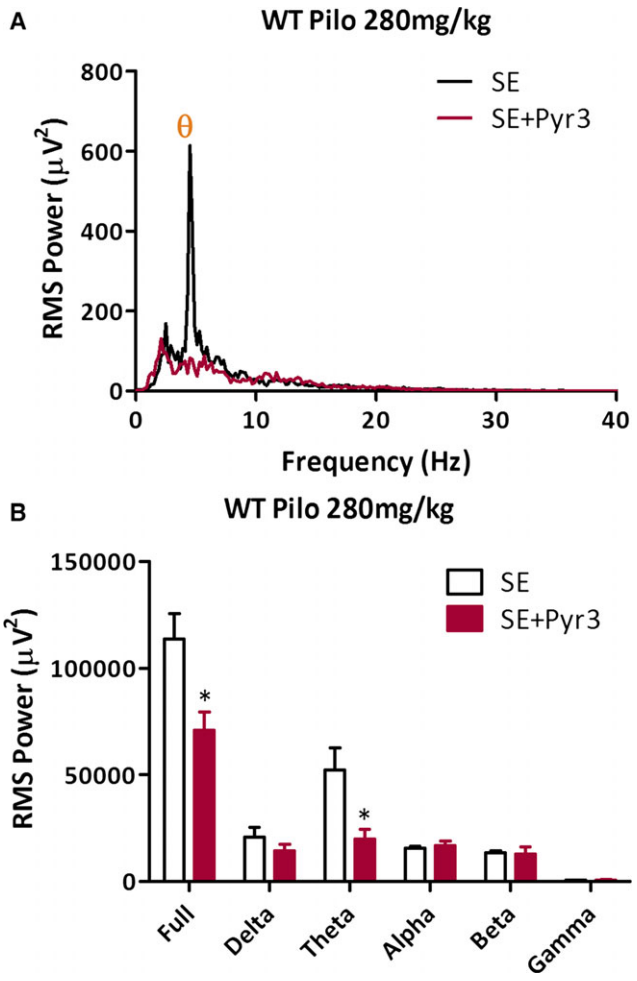

Figure 5.
Pyr3 reduces full RMS power of SE by selectively attenuating pilocarpine-induced theta waves. (**A**) Spectral plots of EEG activity before and 15 min after administration of Pyr3 in WT mice were plotted. SE was elicited by pilocarpine (280 mg/kg, i.p.). Pyr3 (3 mg/kg, i.p.) was administered approximately 25 min after the onset of cortical ictal activities. Note that the large peak in the theta band was greatly attenuated by Pyr3. (**B**) The effect of Pyr3 on RMS power during the SE state in WT mice (n = 4). Note that the full RMS power was significantly reduced after administration of Pyr3 (3 mg/kg, i.p.). Furthermore, theta waves were selectively reduced (*: $p < 0.05$, unpaired *t*-test), whereas there was no significant reduction in delta, alpha, beta, and gamma waves after administration of Pyr3.

epilepsy.[17,18] TRPC3-channel expression was upregulated in patients with epilepsy and in rats days after SE, and this upregulation persisted for weeks. In this study, using TRPC3 global KO mice, we have shown that TRPC3 channels play crucial roles in acute seizure generation. Genetic ablation of TRPC3 channels resulted in a reduction in behavioral scores based on a modified Racine scale, and a reduction in full-spectrum RMS power during SE in EEG recordings. Blocking TRPC3 channels pharmacologically with the selective blocker Pyr3 produced similar reduction in SE intensity. This finding alleviates concerns about the potential genetic drift between controls and TRPC3 KO mice, or potential developmental or compensatory changes caused by TRPC3 global knockout. The consistent changes in both convulsive behaviors and EEG seizure intensity indicate that TRPC3 channels contribute to pilocarpine-induced SE, and provide further support that the BDNF-signaling pathway, for which TRPC3 channels are an essential downstream effector, is involved in the pathophysiology of seizure and epilepsy.

One intriguing finding of our study is a specific role of TRPC3 channels in pilocarpine-induced theta activity. It should be noted that the baseline theta activity is not significantly altered in TRPC3 KO mice, suggesting that the neural circuitry for the generation of theta rhythm is not disturbed. Our present data show that pilocarpine causes an immediate synchronization of EEG activity, with the largest peak in the theta frequency band. This effect of pilocarpine on EEG activity is comparable to the effect of optogenetic stimulation of cholinergic neurons in the medial septum.[26] This synchronization was masked by the overall inhibitory effects on EEG activity by pilocarpine shown in our previous studies,[20,21] but became evident in the present power density heat maps. In TPRC3 KO mice, this synchronization around theta band frequency occurs initially, but the theta peak disappears within a few minutes. The functional implications of this pilocarpine-induced theta wave and its attenuation in TRPC3 KO mice remain uncertain. The pilocarpine-induced theta wave is unlikely to be directly involved in the induction of SE because the attenuation of this theta wave in TRPC3 KO mice does not lead to a delay in the onset of SE. However, the involvement of TRPC3 channels in theta waves generated by activation of muscarinic receptors is likely functionally important because such theta waves are often linked to learning tasks.[27] Indeed, a recent study has revealed a role of TRPC3 channels in contextual fear conditioning.[28]

An unexpected finding of our study is the appearance of pilocarpine-induced beta wave in TRPC3 KO mice during the latent period, that is, before the appearance of ictal activities in the cortical EEG. This beta wave follows the same time course as the pilocarpine-induced gamma wave.[21] However, the source of the beta wave is probably not the same as the gamma wave. The appearance of pilocarpine-induced beta wave during the latent period suggests that there is a distinct possibility that the neural circuits activated by pilocarpine in TRPC3 KO mice are different from the circuits activated by pilocarpine in WT mice.

Our present data show that pilocarpine-induced SE is dominated by theta activity. Furthermore, the reduction of SE intensity by either genetic ablation or pharmacologic blockade of TPRC3 channels is mediated by a selective reduction of pilocarpine-induced theta activity during SE. This selective reduction in theta activity during SE is likely

responsible for the reduced seizure score in TRPC3 KO mice, because the RMS power of theta activity during SE shows a weak but significant correlation with behavioral manifestation of seizures.[20] The relevance of this finding to human epilepsy remains to be determined. It is possible that this theta dominance of SE is model specific, that is, unique for pilocarpine-induced SE. Further studies are needed to determine whether it is also applicable to other animal models of seizures.

The long-term consequences of this selective reduction of SE intensity by genetic ablation or pharmacologic blockade of TRPC3 channels remain to be determined. A previous study reported that interventricular infusion of Pyr3 in rats reduces SE-induced neuronal cell death in all regions of the hippocampus.[17] This would be consistent with our finding of the reduced SE intensity in TRPC3 KO mice. However, we were unable to detect significant reduction of SE-induced neuronal cell death in either CA1 or CA3 region in TRPC3 KO mice using stereology (n = 9). In our preliminary experiments, a single treatment with Pyr3 (3 mg/kg, i.p.) also failed to reduce neuronal degeneration after SE (n = 4). Further studies are needed to reconcile these conflicting observations, and determine whether TRPC3 channels are required for pathologic changes underlying epileptogenesis.

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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