Published in final edited form as:
Epilepsy Res. 2012 November ; 102(1-2): 126–130. doi:10.1016/j.eplepsyres.2012.07.019.

Conditional deletion of TrkC does not modify limbic epileptogenesis

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Abstract

The neurotrophin receptor, tropomyosin-related kinase B (TrkB), is required for epileptogenesis in the kindling model. The role of a closely related neurotrophin receptor, TrkC, in limbic epileptogenesis is unknown. We examined limbic epileptogenesis in the kindling model in TrkC conditional null mice, using a strategy that previously established a critical role of TrkB. Despite elimination of TrkC mRNA, no differences in development of kindling were detected between TrkC conditional null and wild type control mice. These findings reinforce the central role of TrkB as the principal neurotrophin receptor involved in limbic epileptogenesis.

Keywords

Tropomyosin-related kinase C; TrkC; Kindling model; Epileptogenesis; Cre recombinase

INTRODUCTION

Although simultaneous overexpression of exogenous BDNF and FGF2 can limit epileptogenesis in the pilocarpine model (Paradiso et al., 2009), converging lines of evidence demonstrate that excessive activation of the receptor tyrosine kinase, TrkB, by endogenous ligands is critical for induction of limbic epileptogenesis (reviewed by McNamara et al., 2006; Brooks-Kayal et al., 2009). Specifically, the genetic perturbation of brain-derived neurotrophic factor (BDNF) (Kokaia et al., 1995; Croll et al., 1999; He et al., 2004; Barton and Shannon, 2005) or its receptor, tropomyosin-related kinase B (TrkB) (Lähteinen et al., 2002; He et al., 2004, 2010; Kotloski and McNamara, 2010; Heinrich et al., 2011), have convincingly demonstrated that this particular neurotrophin pathway is required for limbic epileptogenesis. In contrast to TrkB, whether TrkC is activated by seizures or is required for epileptogenesis has not been investigated. Nevertheless, this

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

remains a relevant question because the elimination of just one allele of neurotrophin-3 (NT-3), an endogenous agonist of TrkC, has been shown to delay epileptogenesis in the kindling model (Elmer et al., 1997).

We examined the role of TrkC in limbic epileptogenesis using a genetic strategy identical to that which established a critical role for TrkB in the kindling model (He et al., 2004). Because deletion of TrkC from the germline is lethal shortly after birth (Klein et al., 1994), we used a conditional approach in which mice with floxed alleles of TrkC were mated with mice expressing Cre recombinase driven by a synapsin-1 promoter (Syn-Cre), resulting in mice in which both TrkC alleles were eliminated from a subset of central nervous system neurons. We assessed epileptogenesis by quantifying development of kindling and used both RT-PCR and *in situ* hybridization to verify deletion of TrkC mRNA.

METHODS

All animal experiments were carried out in accordance with current IACUC guidelines under animal protocol A298-09-03. Detail of the animals used, genotyping, qRT-PCR, *in-situ* hybridization, electrode implantation and kindling procedure are described in the Supplemental Methods section.

Electrode implantation and kindling procedure

Surgical and kindling procedures followed that of He et al., 2010 (See supplemental methods). In brief, the electrographic seizure threshold (EST) was determined by administering a 1 sec train at 50 μA with additional stimulations increasing by 25 μA (at 1 min intervals) until an electrographic seizure was detected. Stimulations were subsequently administered twice per day at an intensity of the EST until the animals exhibited 3 consecutive seizures of class 4 or greater. Seizures were classified according to a modified Racine (1972) scale. Kindling data are presented as the mean ± SEM for each group.

RESULTS

Neuron-specific TrkC conditional knockout mice

To selectively eliminate TrkC expression from CNS neurons, mice in which exon 14 of the TrkC gene was flanked by loxP sites (Chen et al., 2005) were crossed to Syn-Cre transgenic mice. Reduction of TrkC mRNA was evidenced by qRT PCR study of hippocampal homogenates which revealed levels in *TrkC*$^{-/-}$ mutant mice approximating 57.2 ± 0.2% of *WT* mice ($p<0.05$, Figure 1D, right and middle). Importantly, the floxed TrkC mice in the absence of Syn-Cre exhibited no decrease in TrkC mRNA levels (Figure 1D, right) relative to non-floxed wildtype controls (Figure 1D, left). *In situ* hybridization revealed striking reductions of TrkC mRNA in the dentate granule and CA3 pyramidal cells with lesser reductions in CA1 pyramidal cells of *TrkC*$^{-/-}$ mutant compared to *WT* mice (Figure 1A, B, C), a pattern identical to that found with TrkB mRNA using the same Cre driver line (He et al., 2004). Together these findings demonstrate the efficacy of Cre recombinase in reducing TrkC mRNA expression.

Development and persistence of kindling is equivalent in WT and TrkC$^{-/-}$ mice

The development of kindling as measured by electrophysiological and behavioral responses to stimulation of amygdala proceeded similarly in *WT* and *TrkC*$^{-/-}$ mice. No differences were found between *WT* (n=7) and *TrkC*$^{-/-}$ (n=7) mice with respect to the following measures: First, the current required to evoke the initial electrographic seizure was similar in *WT* and *TrkC*$^{-/-}$ mice (279 ± 42 μA and 271 ± 50 μA for *WT* and *TrkC*$^{-/-}$, respectively). Second, the duration of the initial electrographic seizure and the progressive lengthening of

electrographic seizure duration were similar in *WT* and *TrkC*$^{-/-}$ mice (Fig. 2A). Third, the development of behavioral seizure intensity progressed similarly in mice of both genotypes (Fig. 2B). No significant differences were found in the number of stimulations required to evoke the first clonic motor seizure (class 4 or greater) or the 3rd consecutive clonic or tonic motor seizure lasting at least 10 sec (Fig 2C).

To determine whether a null mutation of TrkC influenced the persistence of the hyperexcitability following the completion of kindling, *WT* (n=7) and *TrkC*$^{-/-}$ mutants (n=7) were stimulated following a stimulation-free period of 2 weeks after the 3rd consecutive class 4 or 5 seizure had been evoked. No significant differences were detected in EST assessed after a 2 week stimulation-free period (average ESTs of 338 ± 132 μA and 343 ± 144 μA of *WT* and *TrkC*$^{-/-}$ mutants). Moreover, the number of additional stimulations required to evoke a class 4 or 5 seizure did not differ between the *WT* and *TrkC*$^{-/-}$ mice (1.9 ± 0.4 and 1.2 ± 0.2 stimulations, respectively; Fig 2C, far right column). In addition, there was no significant difference in the duration of electrographic seizure (23.1 ± 1.2 and 26.0 ± 1.7 sec) or seizure class evoked in the *WT* and *TrkC*$^{-/-}$ mutants. Thus, the persistence of the hyperexcitable state established by kindling was unaffected in the *TrkC*$^{-/-}$ mutant mice.

DISCUSSION

The objective of this study was to test the hypothesis that a conditional deletion of TrkC inhibits epileptogenesis in the kindling model. Two principal findings emerged: 1) crossing Syn-Cre transgenic mice to floxed-TrkC mutant mice reduced TrkC mRNA content as assessed by two independent methods; 2) this reduction of TrkC content did not affect epileptogenesis as revealed by the development of kindling or persistence of hyperexcitability. We conclude that the partial reduction of TrkC expression in this conditional mutant mouse does not modify limbic epileptogenesis in the kindling model.

To compare the effects of TrkC with TrkB on epileptogenesis in the kindling model, the identical genetic strategy using the same Cre driver line was used. Despite reductions of TrkC in a pattern similar to that observed for TrkB in earlier studies (He et al., 2004), no differences were detected in the development of kindling in *TrkC*$^{-/-}$ compared to *WT* control mice. The contrast is striking because the development of kindling was eliminated altogether in the conditional *TrkB*$^{-/-}$ mice (He et al., 2004). Even more modest reductions of TrkB content in the conditional *TrkB*$^{+/-}$ heterozygous mice resulted in a 50% increase in the number of stimulations required to induce the development of kindling (He et al., 2004).

The absence of detectable inhibition of the development of kindling in the conditional *TrkC*$^{-/-}$ null mutant mice is particularly surprising in light of previous studies of NT-3 heterozygotes in the kindling model. That is, NT-3, a neurotrophin with high affinity and efficacy for TrkC (Lamballe et al., 1991), is thought to function as the principal neurotrophin agonist of TrkC *in vivo*. Mutant mice carrying just one allele of NT-3 exhibit a 50% increase in the number of stimulations required to induce kindling (Elmer et al., 1997). While the present results were surprising in light of the studies of NT-3$^{+/-}$ mice, our findings are consistent with earlier studies of Binder et al. (1999) that examined the effects on kindling development of intraventricular (ICV) infusion of recombinant proteins in which the ligand recognition domain of TrkA or B or C was fused in frame with the Fc portion of human IgG1, proteins that bind to and scavenge the endogenous neurotrophins. Whereas ICV infusion of TrkB-Fc markedly inhibited the development of kindling, infusion of either TrkA- or TrkC-Fc had no effect (Binder et al., 1999). The failure of ICV infused TrkC-Fc to inhibit kindling development is consistent with the similarity of kindling development in *WT* and *TrkC*$^{-/-}$ conditional mutant mice in the present study. The possibility that

endogenous NT-3 promotes the development of kindling by activation of TrkB seems unlikely because ICV TrkC-Fc would be expected to scavenge NT-3 and partially inhibit development of kindling, yet it did not (Binder et al., 1999).

The present findings underscore several unanswered questions arising with respect to NT-3 in epileptogenesis. What is the cellular and molecular mechanism by which the development of kindling is inhibited in NT-$3^{+/-}$ mice? What is the functional consequence of the seizure-induced reduction of NT-3 mRNA described in multiple animal models (Gall, 1993; Kokaia et al., 1996; Elmer et al., 1997; Ferencz et al., 1997)? Does TrkC undergo activation following seizures, a possibility suggested by the small increases of pTrk immunoreactivity detected in Western blot analyses of TrkB mutant mice (He et al., 2010)? Addressing this last question is hampered by the lack of antibodies that selectively detect TrkC in western blot or by immunoprecipitation. The answers to these questions notwithstanding, the present results demonstrate that TrkC exerts neither a detectable pro- nor anti-epileptogeneic action in the kindling model. These findings underscore the specificity of TrkB among the neurotrophin tyrosine kinase receptors regulating epileptogenesis in animal models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. David Ginty for the TrkC$^{-/-}$ mice, and Ms. Claudia Clarke for technical support. This work was supported by a grant from the National Institutes of Health (5R01NS056217) to J.O.M. and by a Ruth L. Kirschstein National Research Service Award (F32 NS051064-01) to A.S.L.

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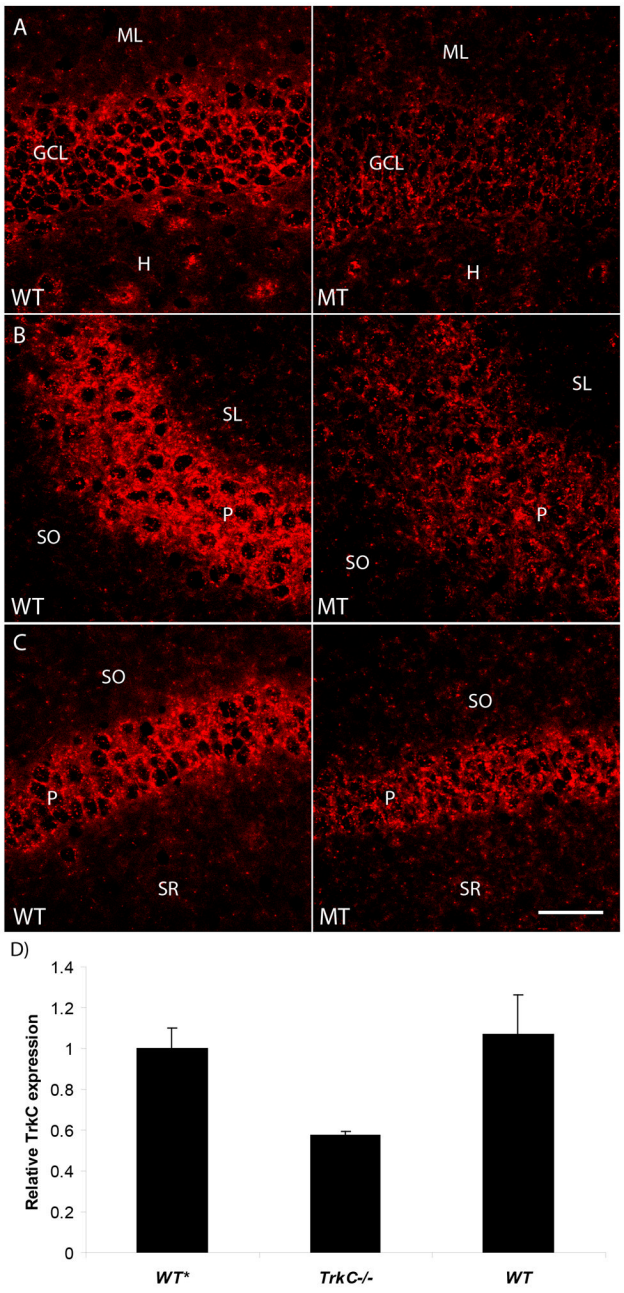

Figure 1.

In situ hybridization and qRT PCR of TrkC mRNA in the hippocampus of *WT* and $TrkC^{-/-}$ mutant mice. **A–C)** The granule cell layer (GCL) of the dentate gyrus adjacent to the hilus (H) and molecular layer (ML) exhibited a clear decrease in TrkC mRNA in the $TrkC^{-/-}$ mutant mice (A, right panel) relative to *WT* controls (A, left panel). The CA3 pyramidal layer (P) adjacent to the stratum oriens (SO) and stratum lucidem (SL) exhibited a clear decrease in TrkC mRNA in the $TrkC^{-/-}$ mutant mice (B, right panel) relative to *WT* controls (B, left panel). The TrkC mRNA levels in the CA1 pyramidal layer (P) adjacent to the SO and stratum radiatum (SR) exhibited a modest reduction in the $TrkC^{-/-}$ mutant mice (C, right panel) compared to *WT* controls (C, left panel). Scale bar = 50 μm. **D)** qRT PCR analysis demonstrating $TrkC^{-/-}$ (n=7; Cre^{+}, floxed/floxed) mutant mice exhibit reduced

hippocampal mRNA content (†, $p<0.05$) relative to *WT* (n=3; Cre^{-}, floxed/floxed) and non-floxed *WT** (n=4) controls.

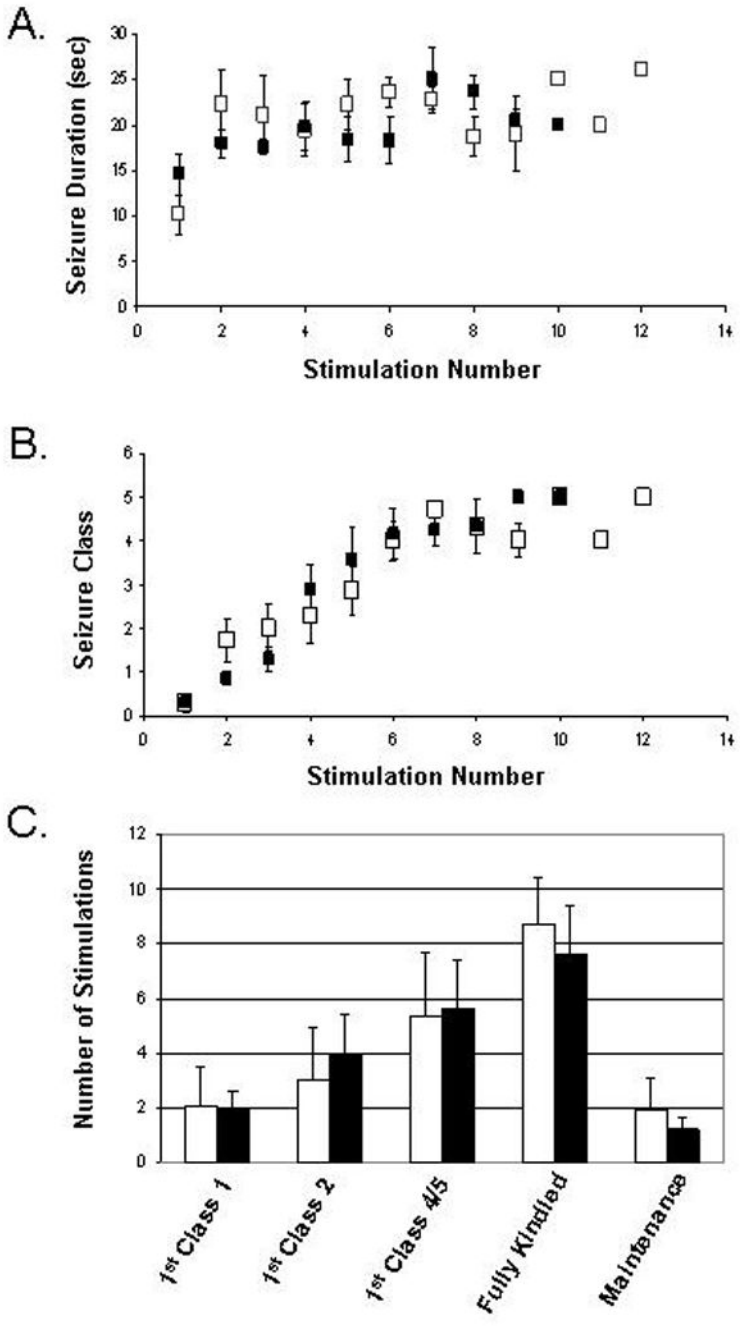

Figure 2.

Kindling development is equivalent in *WT* (n=7; open squares) and *TrkC*$^{-/-}$ (n=7; closed squares) mutant mice. **A&B)** Kindling development is presented as electrographic seizure duration (A) and behavioral seizure class (B). Stimulation number (x axis) refers to the number of stimulations that evoked an electrographic seizure with duration of least 5 sec. **C)** Number of stimulations to reach different seizure classes (y axis) in *WT* and *TrkC*$^{-/-}$ mice. Left to right: First class 1, class 2 and class 4/5 behaviors; Fully kindled refers to the third consecutive class 4/5 behavioral seizures. The maintenance stimulation was to assess persistence of the kindled state.