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Repeated exposure to low doses of kainic acid activates Nuclear Factor Kappa B (NF- κ B) prior to seizure in transgenic NF- κ B/EGFP reporter mice

James A. Miller¹, Kelly A. Kirkley¹, Rachel Padmanabhan¹, Li-Ping Liang³, Yogendra H. Rao², Manisha Patel³, Russell A. Bialecki⁴, and Ronald B. Tjalkens^{1,*}

¹Center for Environmental Medicine, Colorado State University, USA

²Department of Pediatrics, Division of Neurology, Translational Epilepsy Research Program, University of Colorado Anschutz Medical Campus, Aurora, CO USA

³Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO USA

⁴AstraZeneca, Department of Drug Safety and Metabolism, USA

Abstract

Predicting seizurogenic properties of pharmacologically active compounds is difficult due to the complex nature of the mechanisms involved and because of the low sensitivity and high variability associated with current behavioral-based methods. To identify early neuronal signaling events predictive of seizure, we exposed transgenic NF- κ B/EGFP reporter mice to multiple low doses of kainic acid (KA), postulating that activation of the stress-responsive NF- κ B pathway could be a sensitive indicator of seizurogenic potential. The sub-threshold dose level proximal to the induction of seizure was determined as 2.5 mg/Kg KA, using video EEG monitoring. Subsequent analysis of reporter expression demonstrated significant increases in NF- κ B activation in the CA3 and CA1 regions of the hippocampus 24 hrs after a single dose of 2.5 mg/Kg KA. This response was primarily observed in pyramidal neurons with little non-neuronal expression. Neuronal NF- κ B/EGFP expression was observed in the absence of glial activation, indicating a lack of neurodegeneration-induced neuroinflammation. Protein expression of the immediate-early gene, *Nurr1*, increased in neurons in parallel to NF- κ B activation, supporting that the sub-threshold doses of KA employed directly caused neuronal stress. Lastly, KA also stimulated NF- κ B activation in organotypic hippocampal slice cultures established from NF- κ B/EGFP reporter mice. Collectively, these data demonstrate the potential advantages of using genetically encoded stress pathway reporter models in the screening of seizurogenic properties of new pharmacologically active compounds.

*Corresponding Author: Ronald B. Tjalkens, Ph.D., Professor, Center for Environmental Medicine, Colorado State University, 1680 Campus Delivery, Fort Collins, CO 80523-1680, Tel: 970 491 2825, Fax: 970 491 7569, Ron.Tjalkens@colostate.edu.

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Keywords

NF- κ B; Kainic Acid; Safety Assessment; Seizure; neurotoxicity

1. Introduction

Drug-induced seizures have been documented for broad classes of pharmacologically active agents including CNS and Non-CNS targeted drugs. Consequently, seizure liability of new candidate drugs is of primary concern in safety assessment due to its high incidence and the potentially life-threatening consequences of such an adverse drug reaction. Historically, seizure liability has been assessed in late stage preclinical safety assessment during repeated high-dose toxicity studies, with overt behavioral signs of seizure as the only positive indicator of liability (Easter et al., 2009). The inherent limitation of a strictly behavioral method of detection is low sensitivity, where moderate seizurogenic activity may only be indicated by subtle behavioral changes that are difficult to distinguish from generalized stereotypic behavior. Additionally, observational studies are labor intensive due to the need for continuous observation to reduce the potential missing of a seizure episode and also require highly trained personnel to assess animal behavior changes. Moreover, compounds may not elicit overt seizurogenic effects but instead induce molecular changes that produce a cellular environment permissive to seizure that manifest as petit mal or absence seizure. Hence, seizure induction would only be identified during multiple subsequent challenges or in conjunction with additional seizure precipitating events. Early identification of chemical entities with the ability to elicit seizure or reduce seizure threshold requires development of new screening methodologies with higher sensitivity that can better predict these liabilities.

Seizure refers to a period of excessive and synchronous neuronal activity resulting from the disruption of the delicate balance between excitatory and inhibitory inputs. In rodents, behavioral symptoms of a seizure may range from periods of non-motion, excessive grooming, facial spasms, and head nodding to more severe indications such as rearing, loss of righting reflex and tonic-clonic convulsion (Racine, 1972). The difficulty in predicting pro-convulsant activity stems from the complex nature of the mechanisms involved in modulation of neuronal excitability and ultimately the lack of understanding about the early molecular signaling events and corresponding gene expression changes that coincide with or precipitate seizure. However, activation of a number of early cellular stress responsive pathways involved in regulating expression of early-immediate genes (IEG) have been observed during seizure induction (Curran and Morgan, 1995) and monitoring of these pathways may represent early and sensitive markers of potential seizurogenic properties of new pharmacologically active compounds in the absence of overt behavioral manifestations.

A key intracellular signaling pathway involved in early stress response is the nuclear factor kappa B (NF- κ B) pathway consisting of a family of highly conserved hetero and homodimeric Rel-protein transcription factors (P65/Rel A, P50, P52, Rel B, c-Rel). Evidence suggests that NF- κ B acts as a key point of convergence for multiple stress signals including intracellular Ca²⁺ changes, oxidative stress, pro-inflammatory cytokines, and neurotransmitters (Grilli and Memo, 1999). NF- κ B, in association with the inhibitory

subunit I κ B κ , is normally sequestered in the cytosol and upon I κ B kinase-dependent phosphorylation of I κ B κ and subsequent degradation, transcriptionally active NF- κ B translocates to the nucleus and initiates gene expression (Karin and Ben-Neriah, 2000). NF- κ B has been implicated in a number of cellular processes including neuronal plasticity, memory formation, inflammation, and cell death and survival (Meffert and Baltimore, 2005). There is strong evidence suggesting that NF- κ B may play a key role in modulating neuronal excitability and seizure susceptibility (Lubin et al., 2007). Additionally, downstream events activated by NF- κ B such as oxidative stress and inflammation are also known to be activated by seizure activity (Rowley and Patel, 2013; Vezzani et al., 2013).

In the present study, we evaluated the potential utility of monitoring early stress pathway activation as a sensitive indicator of possible seizurogenic activity. We employed the use of a transgenic NF- κ B fluorescent reporter mouse model in conjunction with sub-threshold dose of the prototypic seizurogenic compound, kainic acid (KA). Here we demonstrate that exposure to low levels of KA resulted in regionally selective expression of NF- κ B reporter in limbic structures consistent with the selective effect of the compound.

2. Materials and Methods

2.1. Animals and Treatments

To monitor activation of the NF- κ B signaling pathway a transgenic mouse harboring an EGFP reporter construct containing three high affinity *cis*-elements for NF- κ B was used in this study; *cis*-NF- κ BEGFP (Maggess et al., 2004), generously provided by Dr. Christian Jobin, University of North Carolina at Chapel Hill. Male and female mice, 12 weeks of age were randomly divided into the following groups: 1) sterilized saline ($n = 4$), 2) 2.5 mg/Kg KA dissolved in sterilized saline ($n = 3$), 3) 5 mg/Kg KA ($n = 3$), and 4) 10 mg/Kg KA ($n = 4$). KA was purchased from Ocean Products International (Shelburne, Nova Scotia, Canada). Animals were dosed by intraperitoneal injection once a day for up to 3 days and animals were terminated 24 hours after the last dose. All animal procedures were performed in accordance with NIH guidelines for the care and use of laboratory animals and were approved by the Colorado State University and the University of Colorado Anschutz Medical Campus Institutional Animal Care and Use Committees. Every effort was made to minimize pain and discomfort, and all terminal procedures were performed under deep isoflurane anesthesia.

2.2. Electroencephalography and Seizure Scoring

To monitor brain electrical activity, stainless steel electrodes were secured to the skull over the motor cortices using dental cement and video-electroencephalograph (EEG) activity was recorded using Stellate systems (Natus Medical, San Carlos, CA) in the University of Colorado Anschutz Medical Campus Rodent In Vivo Neurophysiology Core (EEG Core) Facility. Seizure severity was scored on a modified Racine scale (Racine, 1972).

2.3. Tissue Processing and Sectioning

Animals were sacrificed and preserved by transcardial perfusion under deep isoflurane anesthesia. Mice were perfused initially with phosphate-buffered saline containing 20 mM

sodium cacodylate (cacodylate-PBS) and 10 U/ml heparin, followed by 4% paraformaldehyde in cacodylate-PBS. After perfusion fixation the brains were carefully removed from the skull and immersion fixed in the same fixative at 4°C for an additional 3 hr. The tissue was then cryoprotected in 30% sucrose in PBS and stored at 4°C until sectioning. Representative sections were obtained at 25-micron thickness using a freezing sliding microtome (Microm HM450; ThermoScientific). Tissue sections were stored at -20°C, free floating in cryoprotectant (30% w/v sucrose, 30% v/v ethylene glycol; 0.5 M phosphate buffer, pH 7.2) until time of staining.

2.4. NF- κ B Reporter Expression

Representative free floating sections containing the hippocampus were rinsed with 0.05 M Tris-buffered saline (TBS; pH 7.2). The sections were then transferred to slides and incubated for 30 minutes with 0.5 mM CuSO₄ in ammonium acetate (50 mM; pH 5.0) to reduce autofluorescence (Schnell et al., 1999). The sections were then rinsed with TBS and mounted in medium containing 4',6-diamidino-2-phenylindole (DAPI) to visualize cell nuclei.

2.5. Immunofluorescence

Representative sections were selected and rinsed as described above. After being transferred to slides, the sections were incubated in blocking buffer consisting of 1% goat and 1% donkey serum in TBS for 1 hr. After blocking, the sections were incubated overnight at 4°C with primary antibodies specific to the following antigens: EGFP (Cell Signaling Technology, Beverly, MA), MAP2 to identify neurons (Abcam, Cambridge, MA), GFAP to label astrocytes (Cell Signaling Technology, Beverly, MA) and IBA-1 to identify microglia (Wako Chemical USA, Richmond, VA). After primary antibody incubation, sections were rinsed followed by incubation with Alexafluor-conjugated secondary antibodies (Life Technologies, Carlsbad, CA). The sections were then rinsed and mounted using DAPI containing mounting medium.

2.5. Organotypic Hippocampal Slice Culture

Hippocampal slice cultures were prepared utilizing the interface method, as previously described, with minor alterations (De Simoni and Yu, 2006). Briefly, *cis*-NF- κ B^{EGFP} transgenic reporter mice were terminated at day 14 postnatal by decapitation under heavy isofluorane anesthesia. The brain was rapidly removed and chilled in ice-cold slicing medium (EBSS containing 1 mM HEPES). The cerebellum was removed and the brain was bisected at the midline, with the cut face of each hemisphere affixed to the stage of the vibratome (VT1200; Leica) in order to obtain sagittal sections. An initial 2 mm brain slice was cut to rapidly reveal the hippocampus and subsequent 250-micron sections were cut with an average of 10–12 sections being recovered from a single brain. The hippocampus from each section was dissected away from the adjacent brain regions and the individual hippocampi were plated on wet membrane confetti (FHLIC membrane; Millipore) resting on culture plate inserts (Millicell; Millipore) in a 6 well plate. The slices were cultured for 14 days in culture medium (MEM containing Glutamax, Glucose, horse serum and 1× PSN).

Slices were exposed to 5 and 10 μ M KA for 3 hours followed by rinsing with PBS and incubation for 24 hours post exposure.

2.6. Statistical analysis

All results are presented as means $\pm$ SEM with n representing the number of biological replicates for each experiment. Comparative analysis of treatments were made by one-way or two-way ANOVA where appropriate followed by Bonferroni post hoc analysis. All statistical analyses were conducted using Prism software (Version 6.0, Graph Pad Software, Inc.).

3. Results

3.1. Establishment of the sub-threshold dosing model with kainic acid

In order to establish a maximal sub-threshold dose level of KA we employed video EEG to monitor dose-dependent induction of seizure activity. Multiple electrographic (Fig. 1) and behavioral (Table 1) seizures were observed within a one-hour observational period after the first dose of either 5 or 10 mg/Kg of KA in all animals tested. Subsequent exposures to KA at all levels resulted in a reduction in the number of seizure episodes (Fig. 1B, Table 1). In contrast to the 5 and 10 mg/Kg doses of KA, 2.5 mg/Kg did not elicit any electrographic or behavioral indications of seizure in 2 out of the 3 animals tested. One animal in the 2.5 mg/Kg group did present with mild seizurogenic symptoms including stage II behavioral changes consisting of facial clonus and headshakes associated with electrographic indicators of seizure and lasting for only 35 seconds. All other animals used for imaging studies showed no obvious signs of behavioral seizures at the 2.5 mg/Kg dose. These data indicate that the 2.5 mg/Kg dose of KA is proximal to the threshold level of KA required for induction of seizure where detectable behavioral and electrophysiological metrics are not likely to be observed. This sub-threshold dose was subsequently used to evaluate stress pathway activation prior to induction of seizure.

3.2. In vivo determination of NF- κ B pathway activation

To monitor activation of the NF- κ B stress pathway, transgenic reporter mice were systemically exposed to the sub-threshold dose of 2.5 mg/Kg KA and expression of the EGFP reporter was evaluated in the hippocampus 24 hours after dosing. Intrinsic GFP fluorescence signal was observed in the pyramidal cell layer of both the CA1 and CA3 regions of the hippocampus with little expression observed in the dentate and CA2 regions (Fig. 2 a–c). To rule out non-specific auto-fluorescent signal (Spitzer et al., 2011), we confirmed expression of the reporter using immunofluorescence for GFP in the CA3 region (Fig. 2 d–e). The majority of reporter expression appears to be localized in the pyramidal neurons in the CA3 and CA1 regions, determined by staining for the neuronal specific marker MAP2, although the presence of non-colocalized expression may suggest limited extra-neuronal expression of the reporter potentially in glial cells (Fig. 3). Additionally, all animals were co-treated with BRDU but sections co-stained for BRDU revealed an absence of cell turnover (data not shown), further supporting stress-induced, rather than cell death-induced, NF- κ B activation.

3.3. Gliosis

To confirm that the dose level of KA used did not induce significant neurodegeneration we examined neuroinflammatory responses using the glial markers, GFAP and IBA-1 as indicators of astrocyte and microglia activation, respectively. Exposure to a single dose of 2.5 mg/Kg KA did not result in significant changes to markers of gliosis in both astrocytes and microglia throughout the hippocampal formation (Fig. 4).

3.4. Additional stress response pathways

In addition to monitoring the activation of primary stress pathways, we assessed induction of the NF- κ B regulated, immediate early stress gene, Nurr1. Here we show that, comparable to previous studies, KA at the seizurogenic dose of 5 mg/Kg resulted in substantial increase in Nurr1 protein expression in the CA3 region of the hippocampus (Fig. 5). Moreover, the sub-threshold level of 2.5 mg/Kg KA also significantly increased Nurr1 protein expression in the CA3 region of the hippocampus 24 hours after exposure.

3.5. Activation of NF- κ B in hippocampal slice cultures

To test the feasibility of utilizing higher throughput screening platforms from genetically encoded reporter animals, organotypic hippocampal slice cultures were established. Exposure to 5 and 10 μ M KA resulted in significant increases in reporter expression (Fig. 6). Additionally, exposure to 1 mM pentylenetetrazole (PTZ), a GABA receptor antagonist caused reporter expression throughout all regions of cultured hippocampal slices. The pattern of NF- κ B/EGFP reporter expression was maintained in cultured slices exposed to KA and PTZ, relative to the region affected *in vivo*, with KA causing increased GFP expression primarily in CA1 and CA3, whereas PTZ caused a more general increase in GFP expression throughout the hippocampus.

4. Discussion

Alterations in immediate-early gene expression have been used to detect neuronal activation and to map specific brain regions involved in neurological responses to an array of physiological and pathophysiological stressors (Cullinan et al., 1995). A number of IEGs are rapidly and transiently upregulated during increased neuronal activity that occurs during seizure induction, including the widely studied proto-oncogenes, c-fos and c-jun (Curran and Morgan, 1995; Le Gal La Salle, 1988). This increased expression of IEGs, in turn, results in transcriptional activation of a number of target genes that may alter neuronal activity and susceptibility to neuronal damage. Rapid induction of IEGs results from activation of primary stress pathways including the NF- κ B pathway (Baeuerle, 1991). Indeed, activation of NF- κ B has been observed in response to a number of stimuli such as increased neuronal activity and chemically and electrically induced seizures (Prasad et al., 1994; Rong and Baudry, 1996). A significant amount of NF- κ B in neurons resides at the level of the synapse conferring a direct link from synapse to nucleus for rapid induction of gene expression upon synaptic stimulation (Kaltschmidt et al., 1993). Following excessive neuronal activity, NF- κ B stress pathway activation and subsequent gene regulation appears to render a neuroprotective response, as evidenced by the suppression of the NF- κ B pathway through genetic manipulation (p50 $-/-$) or κ B decoy DNA resulting in increased sensitivity to

seizure induction and neuronal degeneration following exposure to KA (Lubin et al., 2007; Yu et al., 2000). The strong evidence of a direct link between neuronal activity and NF- κ B activation supports the hypothesis that monitoring of this immediate-early stress pathway may offer a sensitive marker for seizure liability of new pharmacologic leads.

A majority of studies investigating molecular and cellular changes underlying seizure evaluate gene and protein expression profiles after prolonged or recurrent seizurogenic episodes to primarily elucidate mechanisms involved in epileptogenesis. Relatively unique to the present study, we set out to investigate the activation of stress pathways prior to induction of seizurogenic outcomes to determine the feasibility of predicting these events. In order to investigate the molecular changes that occur proximal to the induction of seizure we first established a subthreshold dosing level for the prototypic seizurogenic compound, kainic acid. Both behavioral and electrographic seizures were produced at the highest two doses of KA (Fig.1, Table 1). Conversely the lowest dose resulted in little indication of seizurogenic activity in the majority of mice tested. Interestingly, subsequent exposure to KA at all dose levels showed a reduction in seizure indicators. Although initially surprising due to the expected kindling effects of many seizurogenic compounds, this unanticipated observation is in line with a potential protective cellular response to the initial exposure resulting from activation of stress response pathways and corresponding induction of immediate-early genes (Blondeau et al., 2001; Mattson and Meffert, 2006). Activation of these neuroprotective stress pathways is a potential indicator for seizurogenic properties in spite of no observable evidence of seizure induction and may represent a sensitive marker.

Exposure to the sub-threshold dose of KA resulted in activation of the NF- κ B pathway as determined by expression of the reporter, EGFP, in the CA3 and CA1 region of the hippocampus with a notable absence of reporter expression in the CA2 and dentate (Fig. 2). The selective pattern of NF- κ B activation is consistent with the regional sensitivity to the effects of KA. Administration of KA is reported to induce epileptiform activity in the CA3 region of the hippocampus with subsequent propagation to the CA1 (Ben-Ari and Gho, 1988; Robinson and Deadwyler, 1981). Additionally, the CA3 followed by the CA1 region shows the earliest and greatest pyramidal cell degeneration after both systemic and intracerebral administration of KA, while the CA2 and Dentate subregions are relatively spared (Ben-Ari, 2006; Nadler et al., 1978). The regional patterning of both depolarization and degeneration by KA is believed to be due largely to the differential distribution of high affinity kainate receptors with the CA3 subregion exhibiting the highest receptor density (Ben-Ari, 1985; Werner et al., 1991). However, the CA3 region of the hippocampus appears to be inherently sensitive to increased synaptic transmission exemplified by the observations that a number of pro-convulsant compounds with various mechanisms of action result in selective seizurogenic activity in the CA3 region when isolated from the CA1 (Ben-Ari, 1985). These data support the conclusion that expression of the reporter in the CA1 signifies a response to increased neuronal activity and most likely not a direct effect of KA on the CA1 pyramidal neurons. Collectively, these observations suggest that activation of the NF- κ B pathway is a sensitive and selective marker for excessive neuronal activity congruent with vulnerability to seizure induction.

The role of NF- κ B signaling in activation of glia and subsequent neuroinflammation is well established. However, the temporal pattern and consequences of glial activation are still under debate but most likely constitute a spectrum of beneficial and detrimental outcomes depending on the stimulus and duration of the insult. Glia form intimate connections with neurons and the activation of glia results in substantial morphological and biochemical changes including hypertrophied processes and increased expression of inflammatory mediators. Activation of both microglia and astrocytes in the CA3 has previously been demonstrated 24 hours post-intracerebralventricular injection of KA in rat, in response to degenerating pyramidal neurons (Matsuoka et al., 1999). We demonstrate no significant changes in markers indicative of glial activation (Fig. 4). The absence of significant glial response in the current study further supports the sub-threshold level of KA used and illustrates the sensitivity of stress pathway activation. Furthermore, there appeared to be only a slight reduction in MAP-2 staining in CA1 in KA-treated animals (Fig. 2 a,d) but no obvious reduction in cellularity, supporting the conclusion that 2.5 mg/Kg is a sub-threshold dose of KA resulting in neuronal stress rather than neuronal death, which would elicit a strong glial response.

To further examine the activity-dependent induction of immediate-early response genes and activation of upstream pathways as sensitive indicators of seizurogenic properties we investigated expression of Nurr1, an IEG known to be regulated by NF- κ B (McEvoy et al., 2002). Nurr1 is a member of NR4A orphan nuclear receptor family, which includes the other members, Nur77 and Nor-1 (Maxwell and Muscat, 2006) but unlike the other members of the family, Nurr1 expression is relatively restricted to the brain. Nurr1 has primarily been studied for its essential role in the development and survival of midbrain dopaminergic neurons but it is also expressed in non-dopaminergic neurons and in a number of brain regions including the cerebral cortex and hippocampus (Zetterström et al., 1996). Studies on the expression of immediate-early gene response demonstrated that increased Nurr1 gene expression occurs rapidly and transiently after systemic exposure to seizurogenic levels of KA as well as induction of electroconvulsive seizure (Crispino et al., 1998; Xing et al., 1997). Additionally, Nurr1 shows selective upregulation in response to membrane depolarization in PC12 cells, support a direct role as an immediate-early stress response factor in neurons (Law et al., 1992). In the current study, exposure to the threshold seizurogenic dose of KA resulted in a significant increase in expression in Nurr1. Furthermore, the sub-threshold dose of KA likewise caused a significant increase in Nurr1. The ability of non-seizurogenic levels of KA to induce expression of Nurr1 demonstrates the sensitivity of IEG-dependent gene expression as a potential predictive marker for seizure induction and also confirms the utility of monitoring upstream activators of these genes including NF- κ B.

The advantage of using genetically encoded reporter constructs for the monitoring of stress pathway activation is the potential amenability to higher throughput platforms such as *in situ* slice culture or *in vitro* primary cell culture. Although simple cell culture is an attractive option for high-throughput screening, the complex nature of the mechanisms involved in regulating neuronal excitability as well as the network-driven processes of hypersynchronicity in seizure indicates that slice culture is a more appropriate model system

due to intact local neuronal circuits and surrounding non-neuronal components. Activation of the NF κ B reporter by KA exposure was observed in cultured hippocampal slices in a regionally consistent pattern as seen *in vivo*. Additionally, pentylenetetrazol, which represent a more broadly acting seizurogenic compound showed less regionally selective effects. Maintaining these circuit- and mechanism-based patterns of reporter expression is important to the capacity of a slice culture-based model system to accurately predict seizurogenic potential *in vivo*. These data support the potential utility of a slice culture based screening system to discriminate between pharmacological mechanisms underlying changes in NF- κ B-regulated stress responsive genes. In comparison to *in vivo* models, the system described can provide higher throughput detection of changes in IEG expression associated with seizurogenic compounds and offers the added advantage of monitoring multiple concurrent endpoints such as electrophysiological alterations corresponding to changes in gene expression (Easter et al., 2009).

Conclusion

Transgenic reporter models designed to monitor stress pathway activation, particularly, the NF- κ B pathway, show great potential for use in safety assessment of specific seizurogenic liability and possibly neurotoxic effects in general. We previously demonstrated that activation of the NF- κ B/EGFP reporter in glial cells was predictive of neuroinflammatory injury in the substantia nigra in a neurotoxic model of parkinsonism (Miller et al., 2011). The current data demonstrates that activation of the NF- κ B pathway in hippocampal neurons in response to sub-threshold levels of KA precedes both overt seizure and neuronal injury and may therefore be a predictive marker of seizurogenic potential of pharmacologic or environmental compounds. Additionally, monitoring of distinct immediate early genes in response to activation of early stress pathways may also add further selectivity and increase the predictive power of the reporter mouse model for seizure. Lastly, the regional pattern of expression of the NF- κ B/EGFP reporter corresponds to the selectivity of KA *in vivo* and *in vitro*, indicating that this genetically encoded reporter would be an appropriate model of seizure liability. However, further assessment with additional seizurogenic compounds is warranted to validate the utility of the hippocampal slice model for high-throughput screening.

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Abbreviations

NF-κB	nuclear factor kappa B
EGFP	enhanced green fluorescent protein
IEG	immediate-early gene
EEG	electroencephalogram

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Highlights

- Seizure liability of new candidate drugs is of primary concern in safety assessment.
- The NF-kappaB signaling pathway is an important stress-response pathway in seizure.
- We present a method for detecting seizurogenic potential in NF-kB reporter mice.
- Sub-seizure doses of kainic acid caused activation of neuronal NF-kB prior to gliosis.
- NF-kB-reporter activation can also be detected in cultured brain slice for screening.

Figure 1.

Dose-dependent seizure induction with sequential exposure to increasing concentrations of kainic acid. **(a)** Representative EEG traces show baseline and ictal activity associated with stage V behavioral seizure induced by 10mg/kg of KA in transgenic reporter mice. **(b)** Number of seizure episodes per animal at each of three sequential doses of KA.

Observations were collected over a 1 hr period after each dose. Data are expressed as mean $\pm$ SEM. Significant difference ($p < 0.05$) between groups denoted by different letters and significant difference between dose groups and saline control group denoted by ** $p < 0.01$.

Figure 2.

A single sub-threshold dose of kainic acid activates the NF- κ B pathway in the hippocampus of transgenic reporter mice. **(a–c)** Representative 10 $\times$ montage images of intrinsic GFP fluorescence **(a; Saline, b; 2.5 mg/Kg KA, c; 5 mg/Kg KA)**. Subset images are high magnification images (20 $\times$ and 40 $\times$) of the CA1 region of the hippocampus demonstrating EGFP expression in the pyramidal cell layer. **(d,e)** Representative 20 $\times$ images of EGFP (Green) expression by immunofluorescence, with MAP-2 (Red) and DAPI (Cyan) in the CA3 region of the hippocampus **(d; Saline, e; 2.5 mg/Kg KA)**.

Figure 3.

Kainic acid induced activation of NF- κ B occurs in pyramidal neurons of the hippocampus. (a,d) Representative 10 $\times$ montage images. (b,c,e,f) Representative 40 $\times$ images of the CA1 and CA3 regions of the hippocampus. Intrinsic GFP fluorescence (Green), MAP-2 (Red) and DAPI (Cyan). (a, b, c; Saline, d, e, f; 2.5 mg/Kg KA).

Figure 4.

A sub-threshold dose of 2.5 mg/Kg kainic acid did not induce gliosis in the hippocampus.

(a,d) Representative 10× montage images. (b,c,e,f) Representative 40× images of the CA1 and CA3 regions of the hippocampus. GFAP expression (White), IBA-1 (Red), DAPI (Cyan) (a, b, c; Saline, d, e, f; 2.5 mg/Kg KA)

Figure 5.

A single exposure to kainic acid dose-dependently induces the immediate early gene Nurr1. (a–c) Representative 10× images (a; Saline, b; 2.5 mg/Kg KA, c; 5 mg/Kg) of Nurr1 (Red) and DAPI (Cyan) in the CA3 region of the hippocampus. (d) Quantitation of Nurr1 expression. Data are expressed as mean fluorescence intensity in relative fluorescence units $\pm$ SEM (n=3). Significant difference between dose groups denoted by *** $p < 0.001$.

Figure 6.

Kainic acid exposure activates NF- κ B in hippocampal slice cultures. (a–b) Representative 10 $\times$ montage brightfield images of hippocampal slices (a; Saline, b; 5 μ M KA). (c–f) Representative 10 $\times$ montage images (c; Saline, d; 5 μ M KA, e; 10 μ M KA, f; 1 mM Pentylentetrazol, PTZ) of intrinsic GFP fluorescence (Green) and DAPI (Blue). (g) Quantitation of GFP expression. Data are expressed as mean fluorescence intensity in

relative fluorescence units $\pm$ SEM ($n=3$). Significant difference between dose groups denoted by * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 1

Behavioral scoring of seizure episodes.

Seizure Stage	2.5 mg/kg Kainic acid			5 mg/kg kainic acid			10 mg/kg kainic acid		
	1 st dose	2 nd dose	3 rd dose	1 st dose	2 nd dose	3 rd dose	1 st dose	2 nd dose	3 rd dose
Stage I	0 ± 0	0 ± 0	0 ± 0	4.3 ± 2.9	1.3 ± 0.9	0 ± 0	3 ± 1.1	1.2 ± 0.6	0.7 ± 0.5
Stage II	0.3 ± 0.3	0 ± 0	0 ± 0	2.7 ± 1.2	2 ± 0.6	0 ± 0	1.5 ± 0.5	0.7 ± 0.5	0.2 ± 0.2
Stage III	0 ± 0	0 ± 0	0 ± 0	2.3 ± 1.2	1 ± 0.6	0 ± 0	0.7 ± 0.2	0 ± 0	0 ± 0
Stage IV	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0	0.7 ± 0.5	0.5 ± 0.3	1.2 ± 0.6
Stage V	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.3	0.3 ± 0.3	0 ± 0	2 ± 1.2	1.25 ± 0.6	0.5 ± 0.3
Stage VI	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Values are mean ± S.E.M
Stage I: Freezing, facial clonus
Stage II: Facial clonus, head nodding
Stage III: Forelimb clonus
Stage IV: Forelimb clonus and rearing
Stage V: Rearing and falling
Stage VI: Status-epilepticus (continuous electroclinical seizures lasting 30 minutes or more)