Inhibition of Neuron Restrictive Silencing Factor (REST/NRSF) Chromatin Binding Attenuates Epileptogenesis

https://doi.org/10.1523/NEURO.0006-24.2024

Received: 26 December 2023
Revised: 8 April 2024
Accepted: 15 April 2024

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Inhibition of Neuron Restrictive Silencing Factor (REST/NRSF) Chromatin Binding Attenuates Epileptogenesis

Transcription factors and epilepsy development

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AMH and TZB conceived the paper; AMH conducted experiments, analyzed data and wrote paper draft. NK performed experiments and analyzed them, MS, HSM KC analyzed data. TZB rewrote and YC TZB edited manuscript and figures.

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6. Number of Figures: 4
7. Number of Tables: 0
8. Number of Multimedia: 0
9. Number of words for Abstract: 250
10. Number of words for Significance Statement: 85
11. Number of words for Introduction: 466
12. Number of words for Discussion: 798

13. Acknowledgements
The authors thank Jennifer Daglian, Maggie Roa Tiffany (My-Tien) Nguyen, and Gissell A. Sanchez for technical support.

14. Conflict of Interest

A. Authors report no conflict of interest

15. Funding sources: NIH grants NS108296, NS35439
Inhibition of Neuron Restrictive Silencing Factor (REST/NRSF) Chromatin Binding Attenuates Epileptogenesis

Abstract
The mechanisms by which brain insults lead to subsequent epilepsy remain unclear. Insults including trauma, stroke, infections and long seizures (status epilepticus; SE) increase the nuclear expression and chromatin binding of the neuronal restrictive silencing factor / RE-1 silencing transcription factor (NRSF/REST). REST/NRSF orchestrates major disruption of the expression of key neuronal genes, including ion channels and neurotransmitter receptors, potentially contributing to epileptogenesis. Accordingly, transient interference with REST/NRSF chromatin binding after an epilepsy-provoking SE suppressed spontaneous seizures for the 12-day duration of a prior study. However, whether the onset of epileptogenesis was suppressed or only delayed has remained unresolved. The current experiments determined if transient interference with REST/NRSF chromatin binding prevented epileptogenesis enduringly, or, alternatively, slowed epilepsy onset.

Epileptogenesis was elicited in adult male rats via systemic kainic acid–induced SE (KA-SE). We then determined if decoy, NRSF-binding-motif oligodeoxynucleotides (NRSE-ODNs), given twice following KA-SE (a) prevented REST/NRSF binding to chromatin, using chromatin immunoprecipitation; (b) prevented the onset of spontaneous seizures, measured with chronic digital video–EEG.

Blocking NRSF function transiently after KA-SE significantly lengthened the latent period to a first spontaneous seizure. Whereas this intervention did not influence the duration and severity of spontaneous seizures, total seizure number and seizure burden were lower in the NRSE-ODN compared with scrambled-ODN cohorts.

Transient interference with REST/NRSF function after KA-SE delays and moderately attenuates insult-related hippocampal epilepsy, but does not abolish it. Thus, the anticonvulsant and
antiepileptogenic actions of NRSF are but one of the multifactorial mechanisms generating epilepsy in the adult brain.
Significance Statement

The mechanisms by which brain insults can lead to subsequent epilepsy remain unclear. Insults may influence neuronal functions by enduringly changing their gene expression programs, often via changes in master regulators such as transcription factors (TFs). The TF REST/NRSF is activated by insults, alters gene expression selectively, and thus promotes aberrant neuronal function and connectivity. Previously, blocking REST/NRSF function transiently in developing brain prevented cognitive problems that accompany SE-induced epilepsy. Here, blocking REST/NRSF DNA binding transiently following SE in adult rats delayed and attenuated epileptogenesis, but did not abolish it.
INTRODUCTION

Epilepsy, the generation of apparently spontaneous seizures, is the third most common chronic brain disorder and exerts an enormous toll on human potential (Committee, 2012). The epilepsies may derive from genetic factors, including increasingly recognized gene mutations (EpiPM, 2016; Heilig et al., 2016, 2018), or arise in the context of prior insults such as traumatic brain injury (Pitkanen, 2014), brain infection (Patel et al., 2017) or stroke (Chan et al., 2022; Phan et al., 2022). Because of the clear and causal association of insults and epileptogenesis, uncovering the mechanisms leading from an insult to the onset of epilepsy and the commonly associated cognitive and emotional problems, is critical for the identification of preventative and therapeutic targets (Committee, 2012).

Experimental models of insult-related epilepsy, including those involving the generation of inciting prolonged seizures (status epilepticus, SE) have contributed greatly to the discovery of epileptogenic mechanisms (Loscher, 2012). These mechanisms include triggering of innate immune / inflammatory processes which disrupt neuronal and circuit activity (Vezzani et al., 2011), as well as enduring changes in neuronal and circuit functions triggered by persistent changes in the repertoire of genes they express (Roopra et al., 2012; Hauser et al., 2018; Kobow et al., 2020). Several key transcriptional factors and pathways have been directly implicated in epileptogenesis, including TGFβ (Friedman et al., 2014), JAK/STAT (Raible et al., 2015), and the neuronal restrictive silencing factor REST/NRSF (Garriga-Canut et al., 2006; Bassuk et al., 2008; McClelland et al., 2011, 2014; Brennan et al., 2016; Hwang and Zukin, 2018; Navarette-Modesto 2019; Prestigio et al., 2021).
In adult rats that developed epilepsy following SE provoked by the convulsant kainic acid (KA), repression of about three dozen key neuronal genes was identified, including the ion channel HCN1 and glutamate and GABA receptor subunits (McClelland et al., 2011, 2014).
REST/NRSF was identified as a key orchestrator of these gene changes: REST/NRSF nuclear expression was augmented after SE for about a week (McClelland et al., 2014), and transiently blocking REST/NRSF function (i.e., its binding to the chromatin) prevented the onset of spontaneous seizures during the first 10 days following SE (McClelland et al., 2011). In addition, in immature rats experiencing SE provoked by experimental fever, a subsequent transient block of REST/NRSF prevented SE-induced cognitive problems in adulthood (Patterson et al., 2017).

Together, these studies suggested that NRSF is a key contributor to transcriptional changes induced by SE, and these changes may underlie epileptogenesis and / or cognitive comorbidities of epilepsy. Further, transient interference with the binding of REST/NRSF to the chromatin suffices to prevent memory problems associated with epileptogenesis in immature animals. However, because of the short duration of recording in adult rats following KA-SE, it remained unclear whether blocking REST/NRSF suppressed the onset of spontaneous seizures transiently or, instead, attenuated epileptogenesis long-term. The current studies address this question.

MATERIALS AND METHODS

Animals and experimental design

Adult (~2-month old) male Sprague-Dawley rats (Harlan; RRID:RGD_5508397) were used. They were housed under a 12-hour light-dark cycle in quiet rooms with ad libitum access to food and water. The Experimental Design is schematized in Figure 1. We included four groups: controls receiving scrambled (SCR) oligodeoxynucleotide (ODN); controls receiving NRSE-ODN, KA-SE receiving either SCR-ODNs and KA-SE receiving NRSE-ODNs. The primary outcome measures were the presence or absence of spontaneous seizures, the latency to their
onset, and their frequency and durations. We calculated group sizes using power analysis (https://select-statistics.co.uk/calculators/sample-size-calculator-two-means/) and aimed for \( n = 11 \) group for the KA-SE groups and \( n = 5 \) for controls.
To assess the chromatin binding of NRSF/REST, a separate group of male rats (n = 4 per group, based on effect sizes in McClelland et al., 2011 and 2014) served as controls or underwent KA-SE with administration of either SCR- or NRSE-ODNs. These rats were killed 48 hours (control and KA-SE) or 72h (all groups) after the end of the SE (receiving 2 doses of the ODN), times shown to be associated with high levels of SE-induced REST/NRSF hippocampal levels and binding to the chromatin. The 48h and 72h groups were combined, and we lost one of the KA-SE-NRSE-ODN rats resulting in sample sizes of n = 3-8.

None of the controls developed any measurable seizures; thus, the results reported here are primarily from the KA-SE-SCR and KA-SE-NRSE cohorts, except for control rats used for the chromatin immunoprecipitation. Two SCR and one NRSE rat were excluded for technical reasons including the loss of EEG headcap. All experiments were approved by the University Institutional Animal Care and Use Committee and conformed to NIH guidelines. We made all efforts to minimize the number and suffering of animals.

Surgery

We anaesthetized adult rats weighing ~250 g, two control groups n = 5/group and two KA-SE groups n = 11-12/group) with 4% isoflurane. Skulls were shaved and placed into a stereotaxic frame, isoflurane levels were maintained using 2-3%, and eyes were hydrated using GenTeal. We treated the skin with iodine and ethanol, then made a mid-line scalp incision to expose the skull. We positioned bilateral infusion cannula on the cortical surface (−1.0 mm posterior, ± 1.5 mm lateral from Bregma) directly above the lateral ventricles, using the coordinates of Paxinos and Watson (Paxinos et al. 1985). We implanted bipolar electrodes (Plastics One) bilaterally
into the hippocampus (AP: 3.3 mm; ML: 2.3 mm; DV: –2.8 mm from Bregma). For the tethered system, we secured 2 reference skull screws above the frontal cortex and 2 more skull screws
to anchor the head-cap. We encased the electrodes, cannula and screws in dental cement. For
the remote system, we created a subcutaneous pocket using large scissors and a blunt
dissection technique to make a pocket from the scalp to the left shoulder. Then, we inserted the
implantable small animal CNS telemetry probe (Data Science International (DSI); Model F40-
EET) within the pocket and sutured the incision. Rats received 5 ml of 0.9% saline via
subcutaneous injection to rehydrate and aid in recovery from surgery. We monitored the rats
and allowed them 5–7 days for postsurgical recovery.

**Induction of status epilepticus**

We induced status epilepticus (SE) using a published protocol (Brennan et al. 2016; McClelland
et al. 2011), following the procedure devised by Heller and Dudek (Hellier & Dudek 2005).
Briefly, intraperitoneal injections of 5 mg/kg of kainic acid (Abcam; Cat# ab120100) were
repeated to reach and maintain SE for 3 hours (two rats received half doses, 2.5 mg/kg). We
continuously monitored and scored seizures using the Racine scale (Racine 1972). After 3
hours of SE, KA-SE rats received a 8 mg/kg dose of diazepam (Patterson Veterinary, Inc.) to
terminate SE. We monitored the rats’ behavior for overt seizure activity and gave another
diazepam dose if necessary. We gave rats a subcutaneous injection of 5 ml of 0.9% saline for
rehydration and provided a soft food diet for 3 days.

**Intracerebroventricular (ICV) infusions**

To eliminate the potential anticonvulsant / antiepileptic effects of the NRSE intervention, we
administered the intervention after the pro-epileptogenic insult was over. Specifically, as
described above, we generated a 3 hours SE in all animals, then terminated the SE in all rats
using diazepam, ascertaining an equal insult in all groups. Only then did we infuse the NRSE intervention as described below.
Thus, Immediately following the termination of the SE using diazepam, we anesthetized the rats with 4% isoflurane and maintained anesthesia with 2.5%. We then infused either a scrambled oligodeoxynucleotide (ODN; AGGTCGTACGTTAATCGTCGC) or an active probe consisting of the DNA sequence of the DNA REST/NRSF binding site (NRSE), into the lateral ventricle of each hemisphere of the brain (McClelland et al., 2011). The structure of the NRSE-ODN, was designed to mimic the DNA binding site for REST/NRSF and function as a decoy, i.e., bind cellular REST/NRSF and prevent its chromatin binding: GGAGCTGTCCACAGTTCTGAA. Both ODNs were protected from degradation by substituting phosphorothioates for the phosphate backbone (Sigma-Aldrich Co.). Specifically, the cannula needle was lowered so that the tip was within the lateral ventricle, and we infused 5 nmol/ventricle (5 µl at 1 nmol/µl) of NRSE-ODNs or SCR-ODNs at a rate of 0.5 µl/min once on the day of KA-SE and once two days later.

**Digital video–electroencephalogram (EEG) recording and analyses**

Following SE, we began video-EEG monitoring: EEG recording was synchronized to video and conducted continuously for up to 2 months. We used a tethered system (AD Instruments; PowerLab data acquisition hardware and BioAmplifiers, and Labchart7 software; Labchart 7 EEG recording required manual synchronization with Logitech DYNEX [DX-NW080] video webcam), or an implantable telemetry system (DSI) for EEG acquisition, and employed the same software for seizure detection and analysis (Neuroscore Version 3.0). In post hoc analyses there were no differences between the tethered and implantable systems in any parameter. In addition, two experienced investigators blind to group identity visually scanned the coded EEGs for seizures (Dube et al. 2010). Any suspicious activity led to the analysis of concurrent EEG and video recordings to identify behavioral manifestations of the apparent seizure. Only events with both EEG and behavioral changes and that lasted over 10 seconds
were classified as seizures. We evaluated typical behaviors associated with limbic-onset seizures, including sudden cessation of activity, facial automatisms, head bobbing and
prolonged immobility with staring. These progressed to alternating or bilateral clonus, rearing and falling (Racine 1972). Rats were considered epileptic if they had at least one documented seizure as defined above.

**Chromatin immunoprecipitation (ChIP)**

We anesthetized, and decapitated KA-SE rats 48 or 72 hours after SE termination and isolated hippocampi. The right hippocampus was cross-linked with 37% formalin for 10 min at room temperature in PBS and the cross linking was neutralized by adding 1.4 M glycine. Pelleted tissue was dissociated in homogenization buffer (50 mM HEPES pH 8.0, 140 mM NaCl, 1 mM EDTA, 0.4% Igepal CA-630, 0.2% Triton X-100, and a cocktail of protease inhibitors) and in a nuclear wash buffer (20 M mM Tris-Cl, pH 8.0, 0.15 M NaCl, and 1 mM EDTA). Nuclei collection was aided by centrifugation and the nuclei were then sonicated for 10 min using a Diagenode Bioruptor (Denville, NJ). After removing cellular debris, the supernatant was precleared overnight with Protein-A/G Beads (Santa Cruz Biotechnology, Dallas, TX; Cat# sc-2003) at 4°C, and incubated with 10 µg of either control non-immune serum (IgG) (Cell Signaling Technology; Cat# 2729S, RRID:AB_1031062), or anti-REST/NRSF (Santa Cruz Biotechnology, Dallas, TX) overnight at 4°C in a buffer containing 16.7 mM Tris-Cl, pH 8.0, 167 mM NaCl, 1.2 mM EDTA, 1.1% Triton X-100, and protease inhibitors. Protein A/G beads blocked with salmon sperm DNA (400 µg/mL) and BSA (400 µg/mL) were added to the lysate for 2 hours. Beads were washed twice with a low-salt wash buffer, high-salt wash buffer, LiCl wash buffer and TE to remove non-specifically bound protein, and then eluted using a buffer containing 2% SDS and 0.2 M sodium carbonate. Eluates were reverse cross-linked at 65°C overnight, and the bound DNA was purified using the QIAquick MinElute PCR purification kit (Qiagen; Cat# 28104). We performed quantitative PCR (qPCR) amplification using SYBR Green chemistry (Roche, Inc.) on a
Lightcycler 96 (Roche) with primers specific for the NRSE/REST region of the HCN1 target gene. \textit{HCN1}. Primer sequences were (5'-3'): forward: AGGGAGCTGTCACAGTTCTGAAT,
reverse: AAGTCCTCAGTGGGGTTTTC. We report antibody binding to the gene as percent input, calculated from average triplicate Ct (cycle threshold) input values for each sample and the REST/NRSF ChIP after subtracting non-specific binding assessed with IgG.

**Analyses and statistical considerations**

All analyses were conducted without knowledge of experimental group. Group sizes were determined *a priori* during the experimental design phase based on power analyses, and animals were assigned to groups randomly. Statistical analyses were performed using Graphpad Prism (RRID:SCR_002798) software and all data are expressed as mean plus or minus standard error, unless otherwise stated. Unpaired Student’s *t*-test was used to evaluate the difference between two groups and ANOVA was used for three groups. Error bars represent SEM. Outliers were excluded using the Graphpad Prism ROUT test for outliers.

**Statistical table:**

<table>
<thead>
<tr>
<th>Data Structure</th>
<th>Type of Test</th>
<th>Power</th>
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<td>Latency - <em>t</em>-test</td>
<td>See estimation plot, Figure 3D</td>
</tr>
<tr>
<td>Experiment 2: small group size</td>
<td>One-way ANOVA</td>
<td>$F_{2,9} = 6.033; \ p = 0.02$</td>
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**RESULTS**

SE augments REST/NRSF chromatin binding, and administration of ‘decoy’ ODNs consisting of the REST/NRSF binding motif attenuates REST/NRSF this binding.
SE, induced by KA or experimental febrile seizures, increases nuclear accumulation of REST/NRSF and the binding of this transcription factor to the chromatin (McClelland et al., 2011, 2014; Patterson et al., 2017). Administration of 10 nmol NRSE-ODN per hemisphere has been reported to block REST/NRSF chromatin binding (McClelland 2011, 2014). However, this
dose seemed to induce malaise in adult male rats, limiting our ability to record them long-term, as described by McClelland et al., 2011. Therefore, we opted to employ an NRSE-ODN at a lower dose, 5 nmol per hemisphere. To test for the efficacy of this approach we performed chromatin immunoprecipitation and measured the amount of REST/NRSF bound to chromatin, specifically to the REST/NRSF binding site (NRSE) of an established REST/NRSF target gene, *Hcn1*. As shown in Figure 2, compared with hippocampi from control rats, REST/NRSF binding at the HCN1-NRSE gene was increased eight-fold in KA-SE rats given SCR-ODN at 48 hours after the SE (CTL vs. KA-SE SCR-ODN, *p* = 0.017). In contrast, administration of NRSE-ODN to KA-SE rats significantly prevented the augmented REST/NRSF binding to the chromatin (*F*<sub>2,9</sub> = 5.19; *p* = 0.04; one-way ANOVA; CTL vs. KA-SE NRSE-ODN, *p* = 0.28). These results indicated that the lower dose of NRSE-ODN sufficed to diminish the binding of REST/NRSF to regulatory sites of an NRSF ‘target gene’ DNA. Notably, this dose was not associated with apparent ill effects on the rats, enabling prolonged continuous Video-EEG monitoring.

**Blocking REST/NRSF function transiently increases the latency to the emergence of spontaneous epileptic seizures and reduces seizure burden.**

There were no differences in the number of KA doses (average 3, a total of 15 mg/kg) required to maintain SE for three hours in the SCR vs. the NRSE groups (Figure 3A). We recorded KA-SE and control rats under continuous digital video–EEG for an average 48 days (Figure 3B), and compared rats given transient NRSE-ODN treatment (a dose on the SE day and a second two days later, *n* = 9), to the cohort given a scrambled-sequence oligodeoxynucleotide (SCR-ODNs; *n* = 12). The latency to the onset of the first spontaneous seizure in KA-SE rats receiving SCR-ODN averaged 5 days; in contrast, NRSE-ODN-receiving rats were seizure-free until the eighth day after the KA-SE on average (*p* = 0.0108; Figure 3C; estimation plot for this effect size is shown n 3D).
Aside from the latency to the onset of the first seizure, we quantified the average and median numbers of seizures per day, median seizure duration, and median seizure severity as determined by the Racine scale. Blocking REST/NRSF function transiently after KA-SE did not significantly influence these parameters, though there were weak trend for a reduction in both average seizure numbers (0.69/day in KA-SE NRSE and 1.028 in KA-SE SCR; p = 0.27) and median (0.86 and 0.49 respectively; p = 0.16, Mann Whitney test (Figure 4, A, B, C). The intervention did reduce the cumulative average number of seizures per rat in NRSE-ODN rats over the same recording period as SCR-ODN rats: A Kolomogorov-Smirnov frequency analysis suggests that the group differ: ks distance = 0.25 (p < 0.0001) in SCR-ODN; ks distance = -0.166 (p = 0.0037) in NRSE-ODN rats. In addition, the cumulative number (area under the curve) analysis showed total area was 777 and 545 in the SCR-ODN and NRSE-ODN groups, respectively, i.e., an attenuated cumulative seizure number of each rat.

Together, these experiments indicate that the augmented REST/NRSF chromatin binding induced by SE facilitates epileptogenesis. Accordingly, transient interference with this REST/NRSF function delays epilepsy development, and modestly attenuates seizure burden, at a minimum over seven weeks.

**DISCUSSION**

The principal findings of these experiments are (a) status epilepticus (SE) augments REST/NRSF chromatin binding, and it is possible to block this increase using NRSE-ODNs that are well tolerated; (b) transient reduction of REST/NRSF chromatin binding increases the latency to the first spontaneous seizure in the epileptogenic process triggered by SE; and (c) acute KA-SE-induced increase of REST/NRSF binding to the chromatin is not required for the
consequent epilepsy, although it may accelerate epileptogenesis and increase the overall seizure burden.
We identify here the augmented REST/NRSF chromatin binding instigated by KA-SE in adult rats, first shown in 2011 and documented by several groups (Navarette-Modesto 2019; Chmielewska et al., 2020; Ghanem et al., 2021; Prestigio et al., 2021). REST/NRSF is also upregulated by SE provoked by experimental fever in developing rats (Brennan et al., 2016; Patterson et al., 2017). Importantly, in developmental SE, blocking REST/NRSF transiently sufficed to mitigate hippocampus-dependent memory deficits enduringly, suggesting the REST/NRSF chromatin binding plays a key role in SE-related maladaptive plasticity within hippocampal circuits. In support of this notion, augmented REST/NRSF chromatin binding was found in the hippocampus following significant early-life adversity/stress that results in serious adult memory problems (Bolton et al., 2020), and in that paradigm, a transient interference with REST/NRSF chromatin binding rescued normal memory enduringly (ibid). Thus, in the developing brain, REST/NRSF may play important roles in the epigenetic processes resulting from a variety of insults, potentially in accord with the unique roles of this factor during development (Ballas & Mandel, 2005; Gao et al., 2011; McClelland et al., 2011b; Nechiporuk et al., 2016).

In the current experiments we demonstrate an increased latency and a modest attenuation of epileptogenesis after blocking REST/NRSF function subsequent to a defined pro-epileptogenic insult. These findings highlight the multifactorial nature and the complexity of epileptogenesis that follows insults (Committee, 2012; Loscher, 2012; EpiPM, 2015; Simonato et al., 2021). Numerous processes are clearly set in motion concurrently by an insult such as SE: neuronal death (although modest in the KA-SE paradigm used here; Dudek et al., 2002), as well as the SE itself trigger inflammatory mediators (Vezzani et al., 2011; Dedeurwaerdere et al., 2012;
Aronica et al., 2017; Klein et al., 2018), and blood brain barrier breaches further promote inflammatory processes (ibid). Metabolic changes may persist (Walker et al., 2016; Hall et al.,
2017; Rho & Boison, 2022), and several epigenetic pathways within neurons lead to enduring changes of gene expression repertoires and the firing and connectivity of neuronal ensembles. REST/NRSF is very likely a member of this SE-induced ‘orchestra’, because blocking its function prolonged latency to epilepsy and reduced overall seizure burden, in accord with recent work using selective deletion of the transcription factor in hippocampal excitatory cells (Natali et al., 2023). However, the current study indicates that the function of this transcription factor in itself during the acute phase of epileptogenesis that follows SE, is not fundamentally required for adult hippocampal epileptogenesis.

The current study examined whether a transient block of REST/NRSF binding to the chromatin aborts a pro-epileptogenic process, and did not assess the potential role of chronic attenuation of REST/NRSF function in epileptogenesis. The upregulation of REST/NRSF expression that follows SE lasts at least a week (McClelland et al., 2014), while the precise half-life and duration of ODN effects is not fully resolved yet appears to be a week or shorter (Patterson et al., 2017). Thus, it is conceivable that chronic treatment with NRSE-ODNs will have more lasting or profound effects on epileptogenesis. A second limitation of the current study is the inclusion of male rats only: although epileptogenesis takes place in both sexes, it is theoretically possible that the role of REST/NRSF in this process is modulated by sex.

What might be the evolutionary purpose of augmented function of REST/NRSF, a transcriptional repressor, after seizures or other neuronal insults? Analyses of the specific genes targeted and suppressed by REST/NRSF in the hippocampus demonstrate a preponderance of genes that specifically define neuronal function, such as ion channels and glutamate and GABA receptor subunits. Neuronal functions, as compared to the functions of many other cell types, are highly energy intensive: the maintenance of the resting membrane potential and the firing of neurons
require major energy expenditures (up to 50% of the total body energy utilization). Therefore, in
the face of major insults associated with ‘energy crises’ such as SE (massive demand) stroke
(plummeting supply) or chronic stress (increased demand and reduced supply), It is logical for a
neuron to repress expression of neuronal genes and aim to survive as a more quiescent cell. Augmented REST/NRSF function accomplishes this strategy.

In conclusion, the transcriptional repressor REST/NRSF contributes crucially to hippocampal plasticity that follows insults in the developing hippocampus. In the adult, interference with the augmented REST/NRSF function that follows pro-epileptogenic insults delays epileptogenesis and moderately reduces seizure burden.
REFERENCES


FIGURE LEGENDS

Figure 1. Experimental design and sample EEGs. Two rat cohorts were employed. A, The first cohort experienced kainic-acid induced status epilepticus (KA-SE; n = 21), or served as controls. KA-SE rats were then randomized to receive either a scrambled oligodeoxynucleotide (SCR-ODN), or an ODN corresponding to the REST/NRSF binding motif of DNA (NRSE-ODN) immediately following the SE as well as 2 days later. Rats were recorded chronically, and video-EEGs from these rats were analyzed without knowledge of group. B, A second cohort underwent the same KA-SE procedure or served as controls, then received the ODNs and were sacrificed 48 or 72 hours later for chromatin immunoprecipitation. C, Sample electroencephalogram (EEG) recorded from bipolar hippocampal electrodes showing typical hippocampal baseline rhythms. Note expanded scale: horizontal bar = 1 second; vertical bar = 200 microvolts. D, hippocampal EEG from a KA-SE rat that received SCR-ODN. A typical baseline is interrupted by spikes and then evolves into a seizure. The same pattern was observed in KA-SE rats receiving NRSE-ODNs. Scale: horizontal = 1 second; vertical = 500 microvolts.

Figure 2. Chromatin immunoprecipitation (ChIP) of hippocampi of control and two groups of kainic-acid induced status epilepticus (KA-SE) rats. The right hippocampus of control male rats (n = 8) and those experiencing KA-SA (n = 11) were obtained 48 or 72 h after termination of the insult. ChIP for the binding of REST/NRSF to the chromatin was performed as described in the Methods section. REST/NRSF chromatin binding was significantly different among groups: (F = 5.19; p = 0.042; one-way ANOVA). The REST/NRSF chromatin binding was augmented in KA-SE rats receiving scrambled oligodeoxynucleotide (SCR-ODN), compared with controls (p = 0.017), and to rats receiving ODN corresponding to the REST/NRSF binding motif on the DNA (NRSE-ODN) (p = 0.05). REST/NRSF binding in the KA-
SE NRSE-ODN group did not significantly differ from binding in the controls $p = 0.28$ (Student’s $t$-test).

**Figure 3.** Transient interference with REST/NRSF function following kainic-acid-induced status epilepticus (KA-SE) increases the latency to the emergence of spontaneous epileptic seizures and reduces seizure burden. **A,** The number of KA injections and the total doses required to maintain SE for three hours in the SCR vs. the NRSE groups did not differ. **B,** The overall duration of continuous video-EEG recording did not distinguish the groups (average 48 days). **C,** The latency to the onset of the first spontaneous seizure in KA-SE rats receiving SCR-ODN averaged 5 days; in contrast, NRSE-ODN- receiving rats were seizure free until the eighth day after the KA-SE ($p = 0.0108$). **D,** An estimation plot for the data shown in **C.** KA-SE NRSE-ODN: $n = 9$; KA-SE SCR-ODN: $n = 12$.

**Figure 4.** Transient interference with REST/NRSF function following kainic-acid-induced status epilepticus (KA-SE) reduces the total number of spontaneous seizures and attenuates seizure cumulation with little effect on seizure severity or duration. **A,** The median number of seizures per day was not significantly reduced in KA-SE rats receiving NRSE-ODNs (0.49; mean $= 0.69 \pm 0.18$) compared with those receiving SCR-ODNs (0.85; mean $= 1.028 \pm 0.23$; $p = 0.16$, Mann Whitney test) Similarly, median seizure duration, and median seizure severity as determined by the Racine scale, did not distinguish the groups (**B, C**). **D,** Blocking REST/NRSF function by two injections of a NRSE-ODN during the first 48 hours following KA-SE reduced the total number of seizures of NRSE-ODN rats, and attenuated the average cumulation of seizures per rat over the recording period. (**E**). A curve showing average
cumulative seizures in the KA-SE SCR-ODN group (green) and the KA-SE NRSE-ODN group.
A Kolomogorov-Smirnov frequency analysis suggests that the group differ: ks distance = 0.25 (p < 0.0001) in SCR-ODN; ks distance = -.166 (p = 0.0037) in NRSE-ODN rats. Area under the curve analysis: total area = 777 and 545 in the SCR-ODN and NRSE-ODN groups, respectively.
A

Median seizures per day

SCR | NRSE

B

Median Duration

SCR | NRSE

C

Median Maximal Racine Stage

SCR | NRSE

D

Total seizures per rat

SCR | NRSE

p = 0.012

E

Cumulative seizures per rat

- SCR per rat
- NRSE per rat

recording day