Effect of Spontaneous seizures on GABA_A α4 receptor subunit expression in an animal model of Temporal Lobe Epilepsy

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Summary

Objective—Temporal lobe epilepsy (TLE) is frequently medically intractable and often progressive. Compromised inhibitory neurotransmission due to altered GABA_A receptor α4 (GABARα4) subunit expression has been emphasized as a potential contributor to the initial development of epilepsy following a brain insult (primary epileptogenesis), but the regulation of GABARα4 during chronic epilepsy, specifically, how expression is altered following spontaneous seizures, is less well understood.

Methods—Continuous video-EEG recordings from rats with pilocarpine-induced TLE were used to capture epileptic animals within 3 hours of a spontaneous seizure (SS) or greater than 24 hours after the last SS to determine whether recent occurrence of a seizure was associated with altered levels of GABARα4 subunit expression. We further evaluated whether this GABARα4 subunit plasticity is regulated by signaling mechanisms active in primary epileptogenesis, specifically, increases in brain-derived neurotrophic factor (BDNF) and early growth response factor 3 (Egr3).

Results—Elevated levels of GABARα4 subunit mRNA and protein were observed following spontaneous seizures, and were associated with higher levels of BDNF and Egr3 mRNA

Significance—These data suggest that spontaneous, recurrent seizures that define chronic epilepsy may influence changes in GABARα4 subunit expression, and that signaling pathways known to regulate GABARα4 expression after SE may also be activated after spontaneous seizures in chronically epileptic animals.

Keywords

spontaneous seizure; GABA(A) receptor; pilocarpine

Introduction

Fast synaptic inhibition is primarily mediated by post-synaptic $GABA_A$ receptors (GABARs). The most abundant receptor subtype, $\alpha1\beta2\gamma2$, is localized at the synapse, and mediates phasic inhibition[1,2]. Alterations in subunit expression and composition that effect the localization, function, and pharmacology of GABARs have been demonstrated during primary epileptogenesis and following the onset of spontaneous seizures in animal models of epilepsy[3-9], as well as in tissue resected from patients with intractable TLE[10,11]. Status epilepticus (SE) results in changes in the expression and membrane localization of several GABAR subunits (e.g., $\alpha1$, $\alpha4$, $\gamma2$, and δ) in hippocampal dentate granule cell neurons; specifically, $\alpha1$ and δ subunits decrease and $\alpha4$ and $\gamma2$ subunit expression increases, resulting in a change in receptor subunit composition and localization in these neurons[7,12,13] that is predicted to impair inhibitory function[14]. Preventing these changes using viral gene transfer can inhibit development of epilepsy in the pilocarpine model of TLE[15].

The signaling mechanisms that mediate GABARα4 subunit plasticity after SE (in primary epileptogenesis) have been defined. Increases in GABARα4 subunits are transcriptionally regulated by BDNF activation of the TrkB receptor, its downstream signaling cascades (protein kinase C (PKC) and mitogen activated protein kinase (MAPK)), as well as upregulation of its target (early growth response factor 3 (Egr3))[16]. Moreover, levels of both BDNF and Egr3 increase[12,16,17], and binding of Egr3 to the Egr response element (ERE) in the endogenous GABARα4 subunit gene increases (*Gabra4*)[17] following SE. Whether similar mechanisms mediate changes in GABAR α4 during chronic epilepsy, and the role, if any, spontaneous seizures may play in this regulation remains unknown.

The current studies investigate whether GABARα4 protein expression in the dentate gyrus of chronically epileptic rats is altered following recent spontaneous seizure (SS), and if this is regulated by activation of signal transduction pathways similar to those that are activated during primary epileptogenesis. We now report for the first time increases in levels of BDNF and GABARα4 within 3h after SS that parallel those seen following SE, and that increases in GABARα4 following SS are transient, but associated with overall higher mean seizure frequencies.

Materials and Methods

Pilocarpine injections—SE was induced in adult Sprague-Dawley rats (Charles-River Labs, Kingston, PA) as previously described[12] using 385 mg/kg i.p. pilocarpine after pre-treatment with 1mg/kg i.p. scopolamine and followed by diazepam (6 mg/kg, i.p.) 1h after onset of SE, with additional 3 mg/kg every 2h as needed to ablate persistent motor seizures. Age-matched control rats received subconvulsive doses of pilocarpine (38.5 mg/kg i.p.), did not develop SE nor spontaneous seizures, and were handled and housed identically to the epileptic animals. All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Colorado Anschutz Medical Campus approved and conducted in accordance with the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals.

Surgical implantation of electrodes—Chronically epileptic rats (4 weeks post-SE) and controls were implanted with two stainless steel screws placed 4mm caudal to bregma and 2.5mm lateral from midline bilaterally with reference and ground electrodes placed bilaterally behind lambda, as previously described[18]. Animals were allowed to recover from surgery for 1 week before proceeding with any further experimentation.

EEG Acquisition and Analysis—Rats subjected to pilocarpine-induced SE and control rats were video-EEG recorded 24h/day using a Pinnacle digital video-EEG system with flexible cables to monitor seizure frequency as previously described[18]. EEG signals were sampled at 1kHz, amplified by 100x, and band-pass filtered between 0.3 Hz and 600 Hz. Prior to inclusion in the study, all pilocarpine treated SE animals were confirmed to have at least 2 EEG-documented spontaneous seizures (SS) and thus meet criteria for epilepsy. EEG records of these chronically epileptic animals were then reviewed daily, and animals were sacrificed if they had (1) experienced a seizure within the past 3 h (recent SS or <3h group) or (2) had been seizure-free for > 24 h (no recent SS or >24h group). After sacrifice, and tissue collection, electrographic recordings spanning the 7 days prior to sacrifice were visually examined by a trained technician blinded to all experimental parameters to determine the number of electrographic seizures that had occurred for each animal. Electroencephalographic seizures were differentiated from background noise by the appearance of large-amplitude (> 3 times baseline), high-frequency (> 5 Hz) activity, with progression of the spike frequency lasting > 10 sec. Seizure quantification was conducted based on seizure number and type (convulsive or non-convulsive). Motor seizures were scored by standard behavioral classes[19], with class 3 or greater seizures categorized as convulsive seizures and class 2 or below considered non-convulsive.

RT-PCR and Western Blots—Epileptic animals (56.21 ± 13.99 days after pilocarpine-induced SE) confirmed to have had at least 2 SS were sacrificed <3h from the time of a SS or >24h after the last SS. Tissue from age-matched controls was also analyzed to assess changes in protein and mRNA potentially induced by spontaneous seizure activity. RNA was extracted from microdissected hippocampal dentate gyrus tissue using RNeasy Mini RNA extraction kit (Qiagen). Primers and probe for BDNF (Rn02531967), *Gabra4* (Rn00589846_m1), and cyclophilin (Rn00690933_m1) were purchased from Applied Biosystems. Primers and probe for Egr3 were designed using primer express software (PE Biosystems). Primer and probe sequences for Egr3 were: Egr3 forward 5′-GAGATCCCCAGCGCGC-3′, Egr3 reverse 5′-CATCTGAGTGTAATGGGCTACCG-3′, Egr3 Taqman 5′-CAACCTCTTCTCCGGCAGCAGTGAC-3′. Samples were repeated in duplicate with each reaction split into two wells in a total volume of 20 μl containing 16ng of RNA using an ABI Prism 7900HT machine. PCR cycling parameters were 50°C for 30 minutes, 95°C for 10 minutes, 50 cycles of 95°C for 15 sec, and 60°C for 1 minute. All values were normalized to cyclophilin expression to control for loading variability and expressed as fold change with respect to the mean control values (defined as 1).

Western blot was performed on protein (25 μg for GABARα4, 30 μg for Egr3) extracted from microdissected DG as previously published[15]. Membranes were incubated with rabbit polyclonal antibodies raised against GABARα4 (anti-GABARα4, Millipore AB5457;

RRID: AB_177479; 1:2,000) in 1% milk/TBS-T or Egr3 (anti-Egr-3 (H-180) N –terminus, Santa Cruz, sc22801; RRID: AB_2097199; 1:400) in 2% milk/TBST overnight in 4°C. Membranes were then washed and incubated with anti-rabbit secondary antibody (GE health, 1:10,000 in 1% milk/TBST for GABAR) or (Veryblot, Abcam AB131366; 1:500 in 2% milk/TBST for Egr3) for 1h at room temperature. Bands were detected using chemiluminescent solution (Pierce), membranes were stripped and reprobed with rabbit polyclonal antibody raised against β-actin (1:40,000, Sigma) in 1% milk/TBS-T overnight. GABARα4 and Egr3 values were normalized to β-actin expression in the same samples and expressed as percent change relative to mean control values in the same run (defined as 1). Densitometry was performed with NIH Image J version 1.42q (RRID:nif-0000-30467). Statistical significance was was calculated with Prism software (RRID:rid_000081) using a One-way ANOVA with a Tukey's test for multiple comparisons or an unpaired student's t-test as indicated.

Chromatin Immunoprecipitation—Rats were perfused with cold 1X PBS (with 1:1000 phosphatase inhibitors) then 4% formaldehyde. Whole brains were immersed overnight in 4% formaldehyde and sectioned at 600 μm. Microdissected DG was sonicated to produce 300-500 bp fragments of crosslinked protein-DNA complexes and precipitated using an Egr3 specific antibody (10μgs; Santa Cruz Biotechnology) or normal rabbit IgG (2μgs; Santa Cruz Biotechnology) to account for non-specific DNA pulldown. Immunoprecipitated protein-DNA complexes were reverse-crosslinked and purified for PCR analysis using the Protein A-conjugated magnetic bead ChIP kit (Millipore). Quantitative real-time RT-PCR was performed using primers that flank the Egr3 binding site in the GABRA4 promoter and a Taqman probe: forward, 5′- GAACAAACTTGCCTAGCTTCGCGT-3′; reverse, 5′- TCCTCCAGCCTAGCCGC-3′; Taqman probe, 5′- AAGTTCACCGGCGAGCAGCGCTTTCA-3′. Data were normalized to input signal [Egr3 / Input] and expressed as fold change with respect to control (defined as 1). Input represents the DNA signal from sample preparation prior to immunoprecipitation.

Results

Regulation of GABARα4 protein by spontaneous seizure activity

Previous experiments have demonstrated that increased transcription of *Gabra4* following prolonged pilocarpine-induced seizures (SE) is mediated by binding of Egr3 to the *Gabra4* ERE site in the core promoter region 24h following SE[17]. Here for the first time, we evaluated whether increased GABARα4 expression may also occur during secondary epileptogenesis in response to acute SS. Animals sacrificed within 3h of a recent SS had significantly higher GABARα4 protein levels than control rats (Fig 1A and 1C), yet epileptic rats that had not had a seizure for >24h have GABARα4 protein levels similar to control rats (Fig 1B and 1C). Most importantly, epileptic rats having a SS within the last 3h also had significantly higher GABARα4 protein levels when compared to epileptic rats without a seizure for >24h (Fig 1C) suggesting recent SS activity induces increased GABARα4 protein expression in chronically epileptic animals. Continuous video-EEG collected from the same animals for 7-days prior to sacrifice was analyzed to determine whether there was a difference in overall seizure frequency between the two groups. Video-

EEG data demonstrated that rats sacrificed <3h after SS which had higher levels of GABARα4 protein expression, also had significantly higher daily seizure frequency than the rats sacrificed >24h after the last seizure (Fig 1D). These results suggest that spontaneous seizures may induce a cascade of events that increase GABARα4 on a relatively short timescale, and that animals with frequent SS (ie., more severe epilepsy) may have higher overall GABARα4 levels.

Spontaneous seizures increase GABARα4, BDNF and Egr3 mRNA levels

Evaluation of the mechanisms leading to increased GABARα4 protein levels demonstrated that rats with a recent SS had significantly higher levels of GABARα4 mRNAs in the DG than controls (Fig 2A), while epileptic rats with no seizures for >24h had GABARα4 mRNA levels similar to controls. Levels of BDNF mRNAs in the DG were increased 5-fold within 3h of SS in chronically epileptic rats relative to control rats (Fig 2B). BDNF mRNA levels in epileptic rats with no SS within 24h of sacrifice were similar to controls, suggesting changes in BDNF expression may be driven by recent seizure activity rather than being a residual effect of SE, or inherent to the epileptic state. As BDNF has previously been shown to regulate GABARα4 expression via its control over Egr3 levels, and the binding of Egr3 to the *Gabra4* core promoter after SE[16], we examined whether Egr3 mRNA levels also change following recent SS. Levels of Egr3 mRNAs increase by greater than eight-fold following a recent (<3h) spontaneous seizure and were significantly higher than epileptic rats with no seizures for >24h (Fig 2C). However, the 14.37% increase in mean Egr3 protein expression following recent spontaneous seizures (i.e., in the <3h SS group) in the dentate gyrus was not significantly different from controls. Chromatin immunoprecipitation was used to examine whether increased binding of Egr3 protein to the promoter of the *Gabra4* subunit gene occurred within 3h of a spontaneous seizure, and might account for the increased GABARα4 mRNA levels. Although Egr3 protein binding to the ERE promoter of the *Gabra4* subunit gene was observed in the dentate gyrus following spontaneous seizures, the levels were not significantly different than that seen in controls (Fig 2D).

Changes in seizure semiology related to GABAR differences

Continuous video-EEG monitoring was used to characterize seizure frequency, seizure type, and temporal distribution for 7 days prior to sacrifice (Fig 3). Rats sacrificed within 3h of a recent seizure had significantly higher seizure frequencies overall than rats sacrificed >24h after the last spontaneous seizure (Fig 3A), suggesting they had more severe epilepsy in general. However, despite the difference in seizure frequency between the <3h SS and >24h SS groups, each group had similar seizure durations and severity (i.e. convulsive vs. nonconvulsive [3B and 3C]). Interestingly, rats sacrificed >24h after SS, had significantly more prolonged interictal intervals (defined as >300 min or ~6h) than animals sacrificed <3h after SS (3D). Increases in GABARα4 mRNA and protein levels occurred following high frequency seizure clusters with or without prolonged inter-cluster intervals (fig 3A, Fig 3E-F) suggesting that spontaneous seizure clusters can induce GABARα4 subunit plasticity even when they occur infrequently. High frequency seizure clusters were more common in <3h SS animals, but were present in some of the >24h SS group, however, the group consistently had GABARα4 mRNA and protein levels similar to control rats when sacrificed following long periods of seizure freedom (i.e., long interictal intervals (G-H)).

This suggests that GABARα4 levels are quite plastic, increasing transiently following a spontaneous seizure and then falling again after a long period of seizure freedom.

Discussion

The results of these studies are the first demonstration that spontaneous seizures may acutely regulate GABARα4 RNA and protein expression, suggesting that spontaneous seizures (regardless of whether convulsive or non-convulsive in nature) result in acute transcriptional up-regulation of the *Gabra4* gene in the DG of epileptic rats.

Spontaneous seizures, GABARα4 increases, and chronic epilepsy severity

Increased levels of GABARα4 RNAs and protein were associated with higher overall seizure frequencies in the epileptic rats sacrificed within 3h of a SS. These data could support two different, but not mutually exclusive, interpretations. One interpretation is that in chronic epilepsy the spontaneous, recurrent seizures drive GABARα4 increases via acute activation of cellular signaling pathways after each epileptic event (i.e., reactive plasticity of GABAR subunits). Long periods of seizure freedom allow the system to "reset" and GABAR subunit levels return to control levels until a SS again induces GABARα4 increases. In addition, it is possible that the increased seizure frequency observed in these animals may, in part, be a consequence of long-term changes in GABA$_A$-receptor subunit expression. The most parsimonious explanation of our data is that these scenarios exist together, with spontaneous seizures themselves stimulating the change in GABARα4 subunit expression, and that these alterations, in turn, may contribute to higher overall seizure frequencies that then perpetuate and/or exacerbate the GABAR alterations.

Changes in GABAR function in chronically epileptic rats with spontaneous seizures

Increases in GABA$_A$ receptor α4 subunit expression have been demonstrated in epileptic animals in several TLE models[3,5,7,20]. The functional consequences of these increases in GABARα4 subunit levels depends in large part on what other subunits it pairs with to compose a receptor. Immuno-precipitation and immunogold labeling studies suggest that α4 subunits can assemble with either δ or γ2 subunits[12,21,22], with α4δ-containing receptors predominating in normal dentate gyrus and α4γ-containing receptors becoming more abundant in epileptic tissue[5,7,12]. Delta-containing GABA$_A$ receptors are located primarily at extrasynaptic sites and have traditionally been thought to mediate tonic inhibition, while phasic inhibition is governed by γ-containing-GABA$_A$ receptors located at synaptic or perisynaptic regions[23-26]. Despite a diminished expression of δ subunit mRNA[7,20] and a reduction in functional α4δ-containing GABARs in the hippocampus of epileptic animals[13], tonic GABAergic inhibition is maintained or increased in epileptic animals[27]. Some studies in chronically epileptic animals suggest that the novel α4γ2-containing receptors are responsible for maintaining tonic inhibition[13], while others find that an increase in such receptors in synaptic and/or perisynaptic locations are responsible for altered phasic GABA currents in epileptic hippocampus[7]. Thus, alterations in GABARα4 subunit composition may be associated with changes in both tonic and phasic GABAR-mediated inhibition.

Alterations in α-subunit subtype can result in differences in $GABA_AR$ modulation by benzodiazepines, neurosteroids, and zinc[28-31]. Pharmacological experiments suggest that increased levels of α4γ-containing receptors in epileptic brain may contribute to an altered response of GABARs to GABA and GABAergic drugs. Lagrange et al., 2007 reported that α4γ-containing receptors are more rapidly desensitized and recover more slowly from desensitization. They further found that exposure to prolonged low levels of GABA greatly suppressed the response of α4γ-containing receptor currents to higher concentrations of GABA. Overall, these receptors appeared less efficacious when exposed to prolonged low levels of GABA or during repetitive stimulation, as may occur during seizures. These results are consistent with the observaton that, in epileptic animals, GABARs become largely insensitive to higher GABA concentrations[32]. Thus, drug treatments that increase the levels of extrasynaptic GABA may negatively modulate the ability of extrasynaptic α4γ-containing receptors to respond to variations in ambient GABA that may occur following SSs, as well as during status epilepticus[33]. Additionally, as α4-containing $GABA_ARs$ are more sensitive to zinc blockade, a shift to more α4-containing $GABA_ARs$ may be associated with the enhanced blockade of the $GABA_A$ response by zinc in the dentate gyrus seen in both acutely after SE and in chronic epilepsy in both animals[28-30,34] and in humans with TLE[35]. Zinc is distributed by aberrant mossy fiber axons of dentate granule cells that innervate the inner molecular layer of the DG in late stages of epileptogenesis (i.e., after the onset of SSs). The α4-containing $GABA_A$ receptors present on dendrites of epileptic DG neurons are exquisitely sensitive to blockade by zinc, and thus may contribute to loss of inhibitory function and increased excitability[29,35]. The transcriptional upregulation of $GABA_AR$α4 subunit gene expression seen in association with spontaneous seizures may thus result in changes in both tonic and phasic GABAR-mediated inhibition that could impact seizure susceptibility in chronically epileptic rats.

Caveats related to assessing Egr3 regulation of *Gabra4* changes

The increases in BDNF levels following recent spontaneous seizure activity (i.e., within 3h) suggest that SSs may recurrently "reactivate" signaling pathways that could contribute to the perpetuation and progression of epilepsy. However, although there was a marked and signficant increase in levels of Egr3 mRNA, as well as BDNF, following recent SS, we did not detect an associated increase in Egr3 binding to the *Gabra4* promoter. Without this evidence we cannot definitively conclude that BDNF-induced increases in Egr3 are driving the induction of GABARα4 transcription following SS. Our evidence does allow us to conclude, however, that Egr3 is present at the promoter region and is part of the transcriptional complex that most likely underlies increased rates of transcription in our studies. Little is known about the mechanism of Egr3 directed gene regulation in neurons and research in this area has been hampered by the lack of antibodies with which to probe the expression of its many variants, an active area of investigation in our laboratories

Conclusions

These studies provide the first evidence of acute molecular regulation of GABAR subunit expression in association with spontaneous seizures during chronic epilepsy. The current study specifically examines changes in GABARα4, and as it is known that expression of multiple GABAR subunits may be alerted in chronic epilepsy, the role of spontaneous

seizures in regulating other subunits, and the subsequent effects on receptor composition and function, merits additional study. Better understanding of the molecular changes induced by spontaneous seizures and their functional consequences could contribute to the development of disease modifying therapies that do not just symptomatically treat seizures but potentially inhibit their progression and long-term adverse consequences.

Acknowledgments

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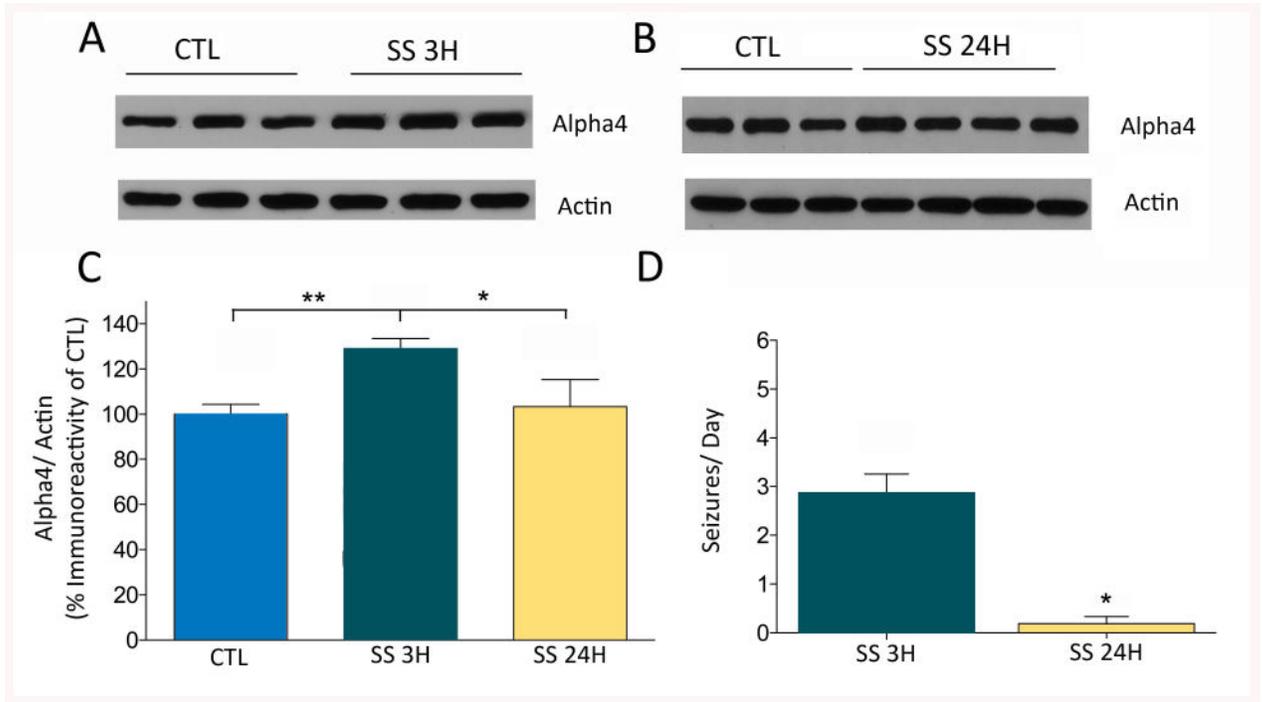
Fig. 1. Spontaneous seizures increase GABARα4 protein expression in DG

Western blots of DG protein homogenates of control rats or epileptic rats sacrificed within **(A)** 3h of a spontaneous seizure (n=6 controls, n=6 SS) or **(B)** > 24h after last spontaneous seizure (n=3 controls, n=4 No SS) reacted with anti-Alpha4 and anti-Actin antibodies. **(C)** Densitometry analysis demonstrates that rats with recent seizure activity show ∼23% increase in GABARα4 expression relative to controls (p<0.01) and ∼20% increase relative to epileptic rats without recent spontaneous seizures (p<0.05) using a one-way ANOVA with Tukey's test for multiple comparisons. Normalized data are presented as mean ± SEM and expressed as percent change. **(D)** The same rats that express increased GABARα4 protein levels in DG following a recent SS (i.e., <3h SS (SS 3H,n=6)) demonstrate a significantly higher seizure frequency (i.e., seizures/day) during the 7-day monitoring period prior to sacrifice relative to the >24h SS group (SS 24H, n=4; *p=0.0139). Significant differences in seizure frequency were detected using a Mann-Whitney test. For all graphs, Error bars, mean±S.E.M.

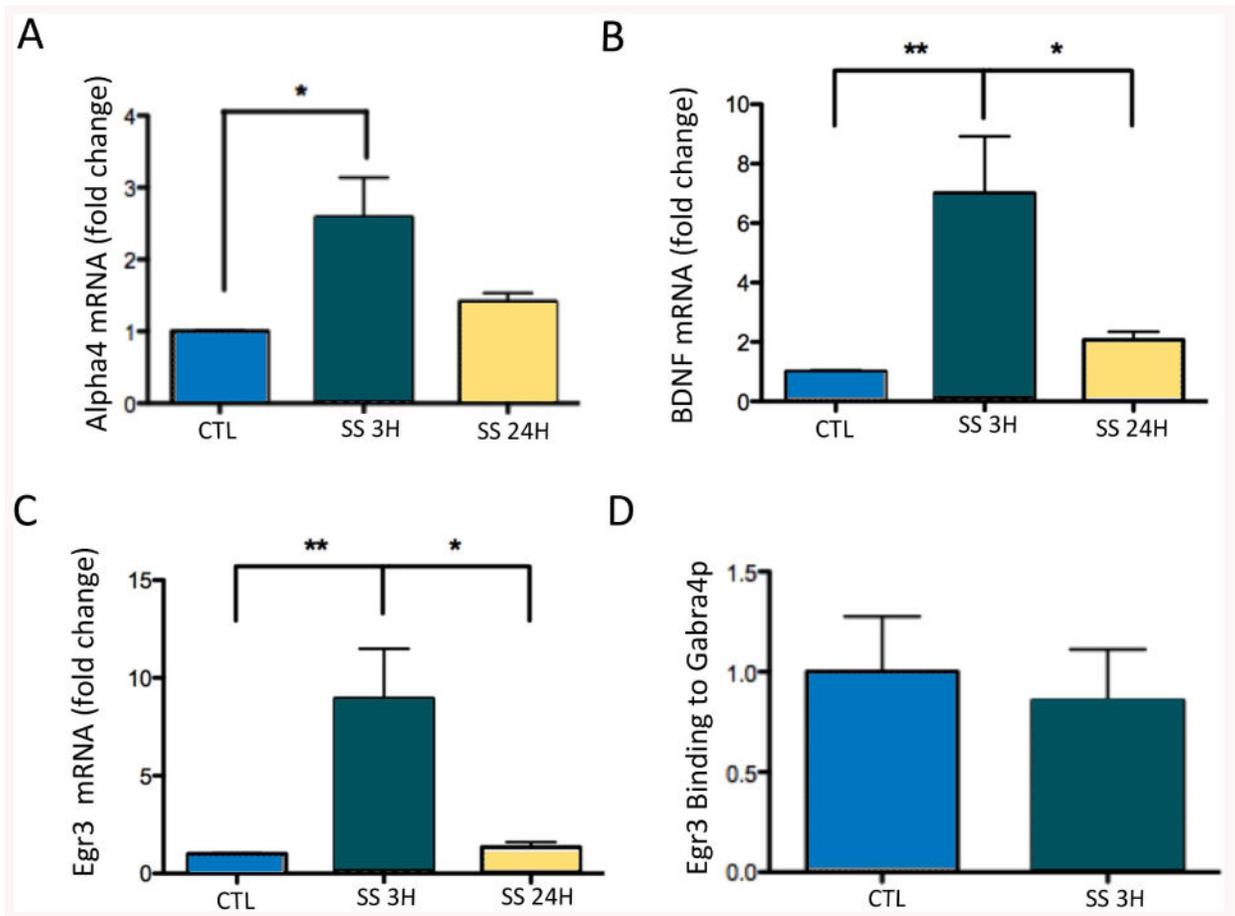**Fig 2. Spontaneous seizures increase GABARα4, BDNF and Egr3 mRNA expression in DG**
(A) Quantification of RT-PCR analysis of GABARα4 mRNA expression in DG <3 and
>24h after SS (controls, n=4; SS 3H, n=5; SS 24H, n=4) demonstrates a significant (p<0.05)
increase in GABARα4 mRNA levels following recent spontaneous seizure activity (within
3h) relative to control rats. Epileptic rats that had not had a seizure for >24h have
GABARα4 mRNA levels similar to controls. **(B)** DG from epileptic rats with recent SS
(within 3h, n=8) showed significant (p<0.05) increases in BDNF mRNA expression
compared to control (n=7), and compared to rats without SS for >24h (n=6). **(C)** DG from
epileptic rats with recent SS (within 3h) showed significant (p<0.05) increases in Egr3
mRNA expression compared to rats that had not had a SS for >24h and controls (controls,
n=4; SS 3H, n=8; SS 24H, n=4). Expression of all mRNAs was normalized to cyclophilin
and expressed as fold change compared to controls (defined as 1). **(D)** Quantification of
protein-DNA binding in DG <3h and >24h after SS (controls, n=3; SS 24H, n=4)
demonstrates no difference in Egr3 protein binding to the ERE promoter of *Gabra4*
following recent spontaneous seizure activity (within 3h) relative to control rats. Data were
normalized to input signal [Egr3/Input] and expressed as fold change with respect to control
(defined as 1). Input represents the DNA signal from sample preparation prior to
immunoprecipitation. Statistically significant differences in mRNA levels and in protein-

DNA binding were determined using a One-way ANOVA with Tukey's test for multiple comparisons. For all graphs, Error bars, mean±S.E.M.
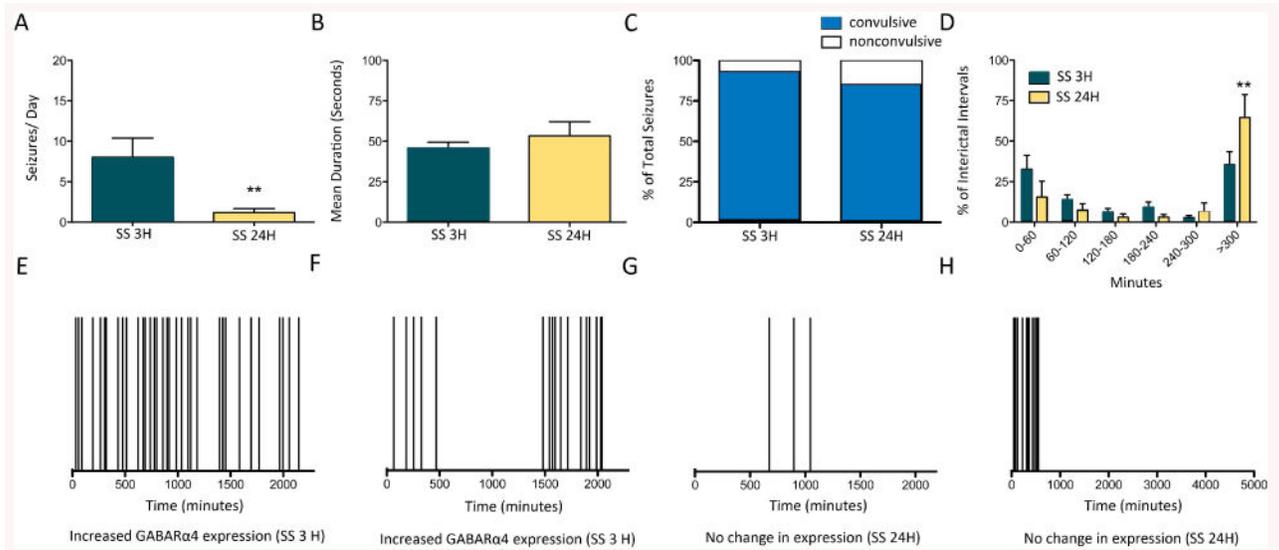
Fig. 3. Characterization of seizures in epileptic rats

(A) EEG data across all rats used for above experiments for <3h SS group (n=16) demonstrate a significantly higher seizure frequency (i.e., seizures/day) during the 7-day monitoring period prior to sacrifice relative to the >24h SS group (n=6; **p=0.0019). Statistically significant differences were determined using a Mann-Whitney test. During the same 7-day period, continuous EEG monitoring demonstrated no significant difference in seizure duration **(B)** or in the proportion of convulsive seizures to non-convulsive seizures present in the <3h SS group or the >24h SS group **(C).** Statistically significant differences were determined using an unpaired t-test and a Fischer's exact test, respectively. **(D)** Frequency histograms of the interseizure intervals for the group of epileptic rats sacrificed within 3h of last spontaneous seizure and >24h after last spontaneous seizure. The first bin (<60 min or >1 seizure/h) had a high number of interseizure intervals for both groups suggesting the presence of seizure clusters. A significant difference between the two groups was found in <300 (or ~6 h) bin demonstrating the SS 24 H group had more long interseizure intervals (or inter-cluster intervals). Statistically significant differences were determined using a One-way ANOVA with a Newman-Keul's test for multiple comparisons. Representative raster plots depicting the time of previous seizure occurrence under the conditions of increased GABARα4 mRNA and protein expression within 3h of a spontaneous seizure **(E and F)** or unchanged GABARα4 mRNA and protein expression **(G and H)** in DG of rats without seizures for >24h.