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Subunit composition of glutamate and gamma-aminobutyric acid receptors in status epilepticus

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Abstract

Purpose: To describe the subunit composition of glutamate and gamma-aminobutyric acid (GABA) receptors in brain tissue from patients with different types of status epilepticus.

Patients and methods: The subunit composition of glutamate and GABA receptors was analyzed in: 1) surgical brain samples from three patients with refractory convulsive status epilepticus, three patients with electrical status epilepticus in sleep, and six patients with refractory epilepsy, and 2) brain autopsy samples from four controls who died without neurological disorders. Subunit expression was quantified with Western blotting and messenger ribonucleic acid (mRNA) expression was quantified with reverse polymerase chain reaction.

Results: Western blot analysis demonstrated the following patterns (as compared to controls): 1) Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors: elevated GluA1/GluA2 ratio in electrical status epilepticus in sleep ($465\% \pm 119$) and refractory epilepsy ($329\% \pm 125$) ($p < 0.01$); 2) N-methyl-D-aspartate (NMDA) receptors: increased GluN2B/GluN2A ratio in electrical status epilepticus in sleep ($3682\% \pm 1000$) and refractory convulsive status epilepticus ($3520\% \pm 751$) ($p < 0.05$); 3) GABA receptors: elevated $\alpha 2/\alpha 1$ ratio in refractory epilepsy ($321\% \pm 138$, $p < 0.05$) and refractory convulsive status epilepticus ($346\% \pm 74$; $p < 0.05$); and 4) patients with

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underlying malformation of cortical development had increased ratios in GluA1/GluA2 (382% $\pm$ 149; $p<0.01$), GluN2B/GluN2A (3321% $\pm$ 1581; $p<0.05$) and $\alpha 2/\alpha 1$ (303% $\pm$ 86; $p<0.01$). Quantification of mRNA demonstrated an elevated *GABRA2/GABRA1* ratio in refractory epilepsy (712; $p<0.05$) as compared to controls.

Conclusions: The subunit composition of glutamate and GABA receptors in patients with status epilepticus mirrors that found in animal models of refractory status epilepticus and may promote self-sustaining seizures. Receptor subunit changes may provide additional targets for improved treatment.

Keywords

Children; Epilepsy; Refractory status epilepticus; Receptor subunit composition; Therapy

1. INTRODUCTION

Status epilepticus is a life-threatening condition that can occur following any seizure type and in the course of any epilepsy syndrome. Mortality in patients with status epilepticus is estimated at 17–22% (Claassen et al., 2002; DeLorenzo et al., 1996) and, among those who survive, cognitive impairment is seen in up to 23% of cases (Claassen et al., 2002). A significant proportion of the morbidity and mortality occur in the setting of refractory status epilepticus because the longer the seizures persist, the less they respond to antiepileptic drug treatments and the more severe the outcome (Mazarati et al., 1998). Epilepsy surgery has emerged as a useful treatment option for selected patients with refractory status epilepticus (Alexopoulos et al., 2005; Loddenkemper et al., 2009). In addition, epilepsy surgery has opened a unique opportunity for the study of the subunit composition of neurotransmitter receptors in human brain tissue from these patients.

Brain tissue studies in animal models have demonstrated that the patterns of subunit expression of glutamate and GABA receptors in the immature brain promotes an enhanced susceptibility to seizures during the first stages of brain development (Rakhade and Jensen, 2009). Similarly, the subunit composition patterns of glutamate and GABA receptors in animal models of refractory status epilepticus resemble those of the immature and developing brain, potentially promoting hyperexcitability and self-sustained seizures (Brooks-Kayal et al., 1998; Galanopoulou, 2007; Mathern et al., 1998). However, studies on the subunit composition of glutamate and GABA receptors in refractory status epilepticus in human brain tissue are not available.

This study aims to address this gap in knowledge. Our objective is to describe the subunit composition of glutamate and GABA receptors in human brain samples collected during epilepsy surgery for refractory status epilepticus. Our working hypotheses were that the subunit composition of glutamate and GABA receptors: 1) is altered in patients with status epilepticus as compared to controls, and 2) is similar to the subunit composition of receptors in animal models of refractory status epilepticus. Our results indicate that the subunit composition of glutamate and GABA receptors is altered in patients with status epilepticus mirroring that of animal models of refractory status epilepticus, which validate the findings from these animal models in humans.

2. PATIENTS AND METHODS

This study was approved by the Institutional Review Board at Boston Children's Hospital and at Cleveland Clinic. Informed consent to participate in this study was obtained from all the patients according to the Declaration of Helsinki.

2.1 Patients.

Brain tissue from patients was obtained during epilepsy surgery for refractory convulsive status epilepticus (SE), refractory electrical status epilepticus in sleep (ESES) or refractory epilepsy (EPI) (Table 1). Prior to surgery, more than two weeks of aggressive pharmacotherapy in the pediatric intensive care unit failed to control SE in all patients. Prior to surgery patients with ESES had severe epilepsy and marked neuropsychological regression that did not respond to antiepileptic drugs. Patients with EPI had severe epilepsy prior to surgery that did not respond to antiepileptic drugs. All cases underwent a thorough pre-surgical evaluation including continuous Video-EEG monitoring and high-resolution magnetic resonance imaging (MRI). The diagnosis of refractory SE, ESES or EPI was confirmed by Video-EEG in all cases. The postsurgical outcome in some of our SE and ESES patients has been reported as part of two previous clinical outcome series (Alexopoulos et al., 2005; Loddenkemper et al., 2009).

2.2 Controls.

Brain tissue from autopsy controls (CO) was obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD. Autopsy controls were selected based on a combination of clinical criteria (cause of death and post-mortem interval) and neuro-pathological diagnosis that ensured the lack of brain pathology in these subjects (Table 2).

2.3 Tissue collection and storage.

Immediately after the surgical resection the tissue was fresh frozen and also fixated in 4% paraformaldehyde and then cryoprotected in 30% sucrose and stored at -80°F (-62.2°C). Between uses, tissue blocks were kept frozen at -80°F to avoid tissue degradation and to optimize staining quality.

2.4 Immunoblotting.

Western blot quantification for glutamate and GABA receptor subunit expression (GluA1, GluA2, GluN2A, GluN2B, $\alpha 1$, $\alpha 2$) was performed on all patients and all values were normalized to actin. Tissue lysates from frozen tissue samples were prepared using the tissue protein extraction reagent (T-Per) as described by the manufacturer (Pierce Biotechnology, Rockford, IL, USA). Quantitative estimation of the protein extracted was measured using the Biorad Protein Assay Kit (Biorad, Hercules, CA, USA). Equal amounts of protein were solubilized in 200 mM dithiothreitol (DTT) sample buffer for varying time periods and temperature and resolved on 10% reducing SDS-polyacrylamide gels to visualize antibodies by western blotting (Supplementary Table 1). Quantification was performed by image analysis of X-ray film (Eastman Kodak Company, Rochester, NY, USA) on non-saturated images after scanning with an Epson 1680 flatbed scanner with transparency adapter

followed by quantitation using Metamorph Imaging software (Molecular Devices Corporation, Downingtown, PA, USA).

2.5 RNA extraction and reverse transcription.

Total RNA was isolated from 70 mg brain tissue using the Qiagen RNeasy Mini Kit (Qiagen, CA). In order to not bias the study for any anatomical area and to avoid artifacts in the gene expression studies that could result from small amounts of contamination, we prepared total RNA from samples with equal proportions, visually approximated, of gray and white matter. Areas with any obvious structural lesions were excluded from the samples for isolating RNA. Isolated RNA was then diluted four-fold in 10 mM Tris-HCl, and a NanoDrop™1000 Spectrophotometer (Thermo Scientific, MA) was used to quantify the RNA. To assess the purity of each RNA sample, we checked that the OD 260/280 was greater than 1.8. Total RNA extracted from each sample was equalized, then reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA). Undiluted samples were again quantified using the NanoDrop™ 1000, then equalized to 50 ng/μl for use in qPCR analyses.

2.6 qPCR analysis.

An Eppendorf Mastercycler ep *realplex*² (silver block) thermocycler was used to collect the data. The TaqMan gene expression assays used were: Hs00168058_m1 (*GABRA1*), Hs00168069_m1 (*GABRA2*), Hs00181348_m1 (*GRIA1*), Hs00181331_m1 (*GRIA2*), Hs00168219_m1 (*GRIN2A*), Hs00168230_m1 (*GRIN2B*), and Hs00362795_g1 (*PES1*). *PES1* was used as an endogenous control to normalize data. As the effects of ESES, SE, and epilepsy on normal housekeeping gene expression patterns were unknown, we evaluated a panel of housekeeping genes as potential candidates for endogenous controls. Samples from each group were run on a TaqMan human endogenous control plate (Applied Biosystems) containing 32 genes. *PES1* showed the least variance in cycle threshold (C_t) across the samples (30.7 ± 0.3), and as such was selected to serve as our endogenous control. The expression of each gene was normalized by subtracting the C_t for *PES1* from the C_t for that gene. Since the number of gene copies doubles every PCR cycle, the normalized C_t (C_{tn}) as a power of 2 represents the abundance of a particular mRNA relative to *PES1*. This value was calculated for each sample, normalized to average control, and then averaged for each group (by etiology or pathology). In addition, ratios of *GABRA2/GABRA1*, *GRIN2B/GRIN2A*, and *GRIA1/GRIA2* were calculated and averaged for each group.

2.7 Statistical analysis.

The results from the three patient groups were compared to the results of post-mortem controls. Individual values for each receptor subunit were expressed as percentage of the mean expression of control samples run on the same blot (100%). Wilcoxon signed rank test was used for all analyses. The α level for statistical significance was set at 0.05. All statistical analyses were performed using SPSS 19 (SPSS Inc., Chicago, IL) and Graphpad Prism.

3. RESULTS

3.1 Immunoblotting.

Western blot analysis demonstrated in all patients and for all receptors a subunit composition that mirrored (whether these differences reached statistical significance or not) the subunit composition of these receptors in the immature brain (Figures 1–3). All numeric results are presented as mean $\pm$ standard deviation of the mean.

3.1.1 AMPA receptors: GluA1 was higher in ESES (ESES $1306 \pm 386\%$, $p < 0.01$) as compared to CO. GluA2 was not significantly elevated in any epilepsy group compared to CO. The GluA1/GluA2 ratio was elevated in ESES ($465\% \pm 119$) and EPI ($329\% \pm 125$) groups compared to CO ($p < 0.01$), but not significantly changed in SE (Figure 1).

3.1.2 NMDA receptors: GluN2B expression was increased in SE ($2543\% \pm 1363$; $p < 0.05$) but not significantly changed in the EPI or ESES compared to CO. No changes in GluN2A expression were seen in any of the epilepsy groups. However, the GluN2B/GluN2A ratio was elevated in ESES ($3682\% \pm 1000$) and SE ($3520\% \pm 751$) compared to CO ($p < 0.05$) (Figure 2).

3.1.3 GABA_A receptors: $\alpha 1$ and $\alpha 2$ subunit expression were not significantly changed in any epilepsy group compared to CO, but the $\alpha 2/\alpha 1$ ratio showed a significant increase in both the SE ($346\% \pm 74$; $p < 0.05$) and EPI ($321\% \pm 138$; $p < 0.05$), but not in the ESES group as compared to CO (Figure 3).

3.1.4 Pathology.—Independent of the electro-clinical syndrome, cases with underlying malformation of cortical development (MCD) showed a significant increase in GluA1/GluA2 ratio ($382\% \pm 149$; $p < 0.01$), an increase in GluN2B/GluN2A ratio ($3321\% \pm 1581$; $p < 0.05$) and elevated $\alpha 2/\alpha 1$ ratios ($303\% \pm 86$; $p < 0.01$) as compared to CO. There was a trend towards a more immature receptor composition in patients with early vascular lesion (EVL), but it did not reach statistical significance (Figure 4).

3.2 RNA expression.

Quantification of the RNA demonstrated the following patterns of subunit expression. *GABRA2* expression was higher in SE and EPI (SE 803% , $p < 0.01$; EPI 601% , $p < 0.05$) as compared to CO. When compared to ESES (241%), *GABRA2* expression was significantly higher in SE ($p < 0.05$) and trending towards higher levels in EPI. *GABRA1* was not elevated in any epilepsy group compared to CO. The *GABRA2/GABRA1* ratio was elevated in EPI compared to CO (EPI 712 , $p < 0.05$). Additionally, cases with EVL pathology showed a significant increase in *GABRA2* expression (735% , $p < 0.01$) as compared to CO, independent of EEG signature. *GRIN2B*, *GRIN2A*, *GRIA1*, *GRIA2* and their ratios (*GRIN2B/GRIN2A*, *GRIA1/GRIA2*) did not show any significant changes as compared to CO (Figure 5).

4. DISCUSSION

Our data demonstrates that the subunit composition of glutamate and GABA receptors is abnormal in patients with status epilepticus. Subunit composition mirrors the patterns seen in the immature brain and in animal models of refractory status epilepticus.

4.1 Increased seizure and epileptogenesis susceptibility in the immature brain.

Because neuronal activity is critical for synaptogenesis and brain development, excitation predominates over inhibition in neuronal networks during the first years of life and this hyperexcitability tends to progressively disappear as the brain matures (Rakhade and Jensen, 2009). The particular expression pattern of glutamate and GABA receptors in the immature brain largely contributes to the age-related brain hyperexcitability (Rakhade and Jensen, 2009). Not only certain patterns of neurotransmitter receptor expression predispose to seizures in the immature brain, but seizures further modify the expression pattern of glutamate and GABA receptors promoting further hyperexcitability (Rakhade and Jensen, 2009).

Similar changes in subunit expression are thought to occur in status epilepticus. Previous literature on animal models of status epilepticus and on human brain tissue of other epileptic conditions supports this hypothesis (Table 3). The present study adds to this field by providing information on receptor subunit composition during status epilepticus in human brain tissue.

4.2 Subunit composition of the AMPA receptors (Table 3).

The reduced expression of GluA2 subunit in animal models of the immature brain alters the permeability of AMPA receptors to Ca^{2+} probably contributing to a lower threshold for seizures (Rakhade and Jensen, 2009). AMPA receptors without the GluA2 subunit are typically expressed in the immature brain and their presence corresponds to an increased risk of excitotoxic cellular injury due to hypoxic-ischemic injury and possible subsequent epileptogenesis both in animals and humans (Talós et al., 2006a; Talós et al., 2006b). In animal models, unilateral hippocampal knockdown of GluA2 subunit provokes seizure-like behavior (Friedman and Koudinov, 1999) and hypoxia-induced seizures promote the inactivation of GluA2 receptors (Rakhade et al., 2008). Brain tissue from our patients demonstrated a similar elevation in GluA1/GluA2 ratio (Figure 1).

4.3 Subunit composition of the NMDA receptors (Table 3).

NMDA receptors with a high proportion of GluN2B subunits appear up-regulated in dysplastic epileptogenic tissue (Talós et al., 2008) and specific inhibitors of the GluN2B NMDA receptor suppressed epileptiform potentials in human brain slices of epileptogenic dysplastic tissue (Moddel et al., 2005). In rat models of the immature brain GluN2B, NR2C, NR2D and NR3A subunits are overexpressed and promote hyperexcitability (Rakhade and Jensen, 2009) and fluorothyl-induced early-life seizures lead to a lack of maturation in the subunit composition (Swann et al., 2007). These effects were not seen in adult rats (Swann et al., 2007). The up-regulation of receptor subunits follows an activity-dependent pattern (Williams et al., 2003) but the non-dysplastic, mature brain is less likely to up-regulate

GluN2B, as also suggested by the lack of elevation of NRB2 in three patients with chronic refractory epilepsy without malformation of cortical development (Finardi et al., 2006). Our patients with chronic epilepsy showed a trend towards higher GluN2B subunit expression as compared to controls but this did not reach statistical significance. However, the GluN2B/GluN2A ratio was elevated in ESES and SE mirroring the subunit composition in the immature brain (Figure 2).

4.4 Subunit composition of the GABA_A receptors (Table 3).

GABAergic neurotransmission is incompletely developed in the immature rat brain, and can be excitatory and depolarizing, instead of inhibitory and hyperpolarizing (LoTurco et al., 1995). The concentration of chloride is higher inside the immature neuron and, therefore, when the GABA_A receptor-associated Cl⁻ channel opens, the concentration gradient promotes an efflux of Cl⁻ that leads to cell depolarization (Dzhala et al., 2005). KCC2, the co-transporter that changes the Cl⁻ cell gradients during the development of the immature brain, is the primary determinant of the maturation of GABAergic neurotransmission (Lu et al., 1999; Rivera et al., 1999). However, the subunit composition of GABA_A receptors also plays a role in the shift from excitatory to inhibitory neurotransmission: the $\alpha 1$ subunit of the GABA_A receptor is developmentally regulated and expressed at low levels in the immature brain, when seizures can be refractory to conventional antiepileptic drugs (Brooks-Kayal and Pritchett, 1993; Brooks-Kayal et al., 1998). The receptor subunit composition in our patients mirrored the immature brain with an elevated $\alpha 2/\alpha 1$ ratio (Figure 3).

4.5 Subunit ratios in different etiologies.

Subunit composition has been previously found to mirror that of the immature brain in MCD (Crino et al., 2001; Finardi et al., 2006; Talos et al., 2012). In our series, receptor expression was immature in patients with MCD, and there was a tendency towards more immature receptors in patients with EVL that did not reach statistical significance (Figure 4). The expression of more immature receptors in patients with ESES secondary to EVL has been previously suggested (Sánchez Fernández et al., 2012).

4.6 RNA expression.

The overexpression of *GABRA2* is in accordance with the results by previous studies that found a reduced *GABRA1*/non*GABRA1* ratio. The series by Crino et al found a reduced expression of both *GABRA1* and *GABRA2*, but this study differed from other series in that control brain tissue included epilepsy surgical specimens with cortex that was in the epileptogenic focus but that did not present signs of MCD (Crino et al., 2001).

4.7 Clinical relevance of our findings.

Status epilepticus responds to most agents early in its course but tends to be quite refractory once it is established (Goodkin et al., 2003; Goodkin et al., 2005). During status epilepticus benzodiazepine sensitive GABA_A receptors move from the synaptic membrane to the cytoplasm where they are functionally inactive while NMDA and AMPA receptors move from subsynaptic sites to the synaptic membrane (Goodkin et al., 2008; Goodkin et al., 2005). These underlying molecular mechanisms are consistent with findings in experimental

models that GABAergic antiepileptic drugs lose their efficacy early in the course of status epilepticus (Mazarati et al., 1998) but NMDA receptor blockers suppress seizures even late in the evolution of status epilepticus (Mazarati and Wasterlain, 1999). Therefore, NMDA receptor blockers were proposed as the optimal therapy for refractory status epilepticus (Mazarati and Wasterlain, 1999). Our findings expand these results and provide a solid rationale for targeting specific subunits that are specifically up-regulated during status epilepticus.

4.8 Challenges.

Our data must be interpreted in the context of data acquisition. The number of patients in this study was small, their ages range was broad at the time of surgery and their treatment regimen was heterogeneous (Table 1). In spite of the wide age range, we did not find major differences in the ratios of subunit composition of the different receptors between the different age groups. Brain tissue was collected from different lobes and this can potentially confound results if subunit composition varies by lobe. Other potential confounders are the numerous and varied antiepileptic drugs that patients with refractory seizures received prior to epilepsy surgery. It is known that certain toxins such as alcohol and drugs such as sevoflurane can modify the subunit composition of glutamate and GABA receptors (Brady et al., 2013; Good and Lupica, 2010; Piehl et al., 2010), but the specific influence of antiepileptic drugs on the subunit composition has not been studied in detail. Additionally, our control group was not ideal because it was obtained from a post-mortem brain tissue bank and this tissue may be flawed by post-mortem changes (Table 2). However, obtaining fresh brain tissue from controls without indications for brain surgery is not ethically feasible and the use of autopsy controls is the best approximation to that ideal standard in human research. These challenges are common in all previous similar studies and only a prospective multicenter study, probably in the context of an international collaborative research effort, will provide the large number of samples that will allow to control for possible confounders such as underlying etiology, cerebral lobe location and antiepileptic drugs (Finardi et al., 2006; Talos et al., 2008; Talos et al., 2012). The current results therefore present the best we could do with the current specimens and provides a glimpse into human receptor unit changes.

Changes in subunit composition of the neurotransmitter receptors have also been described in animal models of acute status epilepticus. Our results are derived from a pediatric population and need to be replicated in adults. Due to limitations in the size of tissue samples we were not able to specifically study the concentration of other proteins that may significantly alter synaptic excitability and development of seizures such as the ion channels NKCC1 and KCC2 that have been shown to be altered in epileptogenic tissue from tuberous sclerosis patients (Talos et al., 2012), and the protein PSD-95 that was found to be altered in human epileptogenic tissue (Ying et al., 2004).

Multiple different pathologies are present in epileptic tissue and these can differentially contribute to changes in neurotransmitter receptor expression. Tissue samples are composed of different cellular types and, therefore, the measured receptor expression represents an average of the receptor expression of the individual cells. In addition, our study was not

specific enough to provide information on the specific cortical layer or cell type, which can vary in subunit composition (Loup et al., 2006; Loup et al., 2009) and we did not perform an analysis of the proportion of white matter in every sample. However, different studies on brain tissue from animal models and human tissue are concordant with our results (Table 3). Additionally, the few studies that have analyzed epileptogenic cells individually have found that, albeit variations between different cell types do occur, their subunit composition is similar to that of the abovementioned averaged expression in tissue (Talós et al., 2008; White et al., 2001). No other human data on receptor subunit changes during status epilepticus exist.

Another potential limitation of studying subunit composition in tissue samples is that the specific distribution of the receptor in the cellular membranes is lost: that is, a particular subunit could appear at normal levels in the studied tissue but its level of expression in the synaptic cellular membrane may be low because the subunit is kept in the extrasynaptic compartment (Granger et al., 2013) or in the membranes of the internal organelles (Cornejo et al., 2007). Curiously, after a single episode of kainate-induced seizures in immature rats, there is a long-term shift of GluA1 receptor from the membrane to the intracellular compartment and a loss of total GluN2A in the rat hippocampus (Cornejo et al., 2007) and a study to replicate those findings in human tissue is in preparation.

A major strength of the present study is that all the results point in the same direction: independently of the epileptic syndrome that led to refractory seizures, all presented a receptor subunit composition that mirrored that of the immature brain compared to controls.

4.9 Outlook.

The analysis of brain samples with homogeneous pathology from a large number of patients is limited by the rarity of epilepsy surgery in patients with status epilepticus. The present results may fuel the development of multicenter tissue banks that will overcome this limitation and we are in the process of setting a similar tissue depository up within the pediatric status epilepticus research group to replicate findings in a larger series. Alternative approaches consist of studying the pattern of expression of neurotransmitter receptors from autopsy brain samples of patients that passed away during status epilepticus. However, these tissue samples are also rare, currently not readily available and may also be influenced by post-mortem changes. Other approaches in animal models of status epilepticus have been studied in the past.

5. CONCLUSION

Our data demonstrate that the expression pattern of receptors is altered in status epilepticus and validates the findings from animal models of status epilepticus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Subunit composition of glutamate and GABA receptors changes in status epilepticus
- AMPA receptors: elevated GluA1/GluA2 ratio
- NMDA receptors: elevated GluN2B/GluN2A ratio
- GABA receptors: elevated $\alpha 2/\alpha 1$ ratio
- These changes promote hyperexcitability and self-sustaining seizures

Figure 1. Expression of the different subunits in the AMPA receptor.

Patients with repeated seizures had a tendency towards a more immature subunit composition with relative overexpression of GluA1. Arrowheads show the band of interest for each specific blot.

Figure 2. Expression of the different subunits in the NMDA receptor.

Patients with repeated seizures had a tendency towards a more immature subunit composition with relative overexpression of GluN2B. Arrowheads show the band of interest for each specific blot.

Figure 3. Expression of the different subunits in the GABA receptor.

Patients with repeated seizures had a tendency towards a more immature subunit composition with relative overexpression of $\alpha 2$. Arrowheads show the band of interest for each specific blot.

Figure 4. Subunit ratios in different underlying etiologies.

Patients with malformation of cortical development (MCD) and with early vascular lesion (EVL) had a tendency towards a more immature subunit composition that only reached statistical significance in patients with MCD.

Figure 5. mRNA expression in different electro-clinical conditions.

A. RNA receptor subunit expression in different conditions. B. Ratios of RNA receptor subunit expression in different conditions. C. RNA receptor subunit expression in different underlying etiologies. Only the expression of mRNA for *GABRA2* differed from controls.

Table 1.

Clinical and neuropathologic features of our patients

Patient	Sex	Age at epilepsy onset (years)	Antiepileptic medications at the time of surgery	Age at surgery (years)	Resected tissue	Causal lesion (Neuroimaging/Pathology)
ESES1	F	1.5	FB VPA MTX CLP PD VNS	8.17	R FH	MCD type IA
ESES2	M	4	LEV VPA CLP DZP	8.33	R FH	MCD polymicrogyria
ESES3	M	1.75	LTG VPA	4.75	R FH	EVL (right frontotemporal hemorrhage)
SE1	M	2	PHT TPM CLZ MDZ	7.17	R FR	MCD TSC
SE2	M	3	TPM VPA CBZ PB	13.92	R FR	MCD type IIB
SE3	M	4.75	VPA OXC DZP PB PHT PT	9.92	R T	EVL (perinatal right temporal hemorrhage)
EPI1	M	1.67	VPA LTG LEV LZP	6.17	L FR	MCD type IIA
EPI2	F	7.75	LEV VPA LZP	8.92	L FR	EVL & MCD. Left perisylvian and fronto-parietal polymicrogyria probably secondary to a remote in-utero infarct
EPI3	F	5	LEV TPM LZP	16.5	R T	EVL & MCD. Right periventricular leukomalacia
EPI4	F	2.67	PHT OXC	2.83	L FR	MCD type IIB
EPI5	M	3.5	LTG OXC	5.83	R T	MCD type IA
EPI6	M	0.08	PB	1.25	FR	MCD type IIB

Legend: EVL: early vascular lesions. F: female. FCD: focal cortical dysplasia. FH: functional hemispherectomy. FR: frontal. H: hemispherectomy. L: left. M: male. MCD: malformation of cortical development. R: right. T: temporal. TSC: tuberous sclerosis complex.

Abbreviations for antiepileptic medications: CLP: clonazepam. CLZ: clorazepate. DZP: diazepam. FB: felbamate. LEV: levetiracetam. LTG: lamotrigine. LZP: lorazepam. MDZ: midazolam. MTX: methosuximide. OXC: oxcarbazepine. PB: Phenobarbital. PD: pyridoxine. PHT: phenytoin. PT: pentobarbital. TPM: topiramate. VNS: vagus nerve stimulator. VPA: valproate.

Table 2.

Clinical and neuropathological characteristics of control subjects

Control	Sex	Age at death	Post-mortem interval (hours)	Cause of death	Sampled brain region
CO1	F	4.5	21	Lymphocytic myocarditis	Frontal
CO2	M	7.75	12	Drowning	Frontal
CO3	M	20.42	8	Gunshot to the chest	Frontal
CO4	F	20.58	9	Motor vehicle accident	Temporal

Legend: CO: control. F: female. M: male

Table 3.

Subunit receptor changes in our series as compared to similar previous literature

AMPA RECEPTORS	NMDA RECEPTORS	GABA _A RECEPTORS
ANIMAL MODELS OF THE IMMATURE BRAIN WITHOUT SEIZURES		
GLUA2 ↓ nonGLUA2/GLUA2 ↑ GLUA4/GLUA2 ↑ (Kumar et al., 2002; Talos et al., 2006a)	GluN2A ↓ GluN2B ↓ GluN3A/GluN1 ↑ (Monyer et al., 1994; Wong et al., 2002)	
ANIMAL MODELS OF SEIZURES AND STATUS EPILEPTICUS		
	GluN2A ↓ GluN2B ↓ (Swann et al., 2007)	<i>GABRA1</i> ↓ <i>GABRA1</i> /non <i>GABRA1</i> ↓ (Brooks-Kayal et al., 1998)
DATA FROM NEONATAL BRAIN SAMPLES		
GLUA1 ↑ GLUA2 ↓ GLUA1/GLUA2 ↑ (Talos et al., 2006b)		
DATA FROM EPILEPSY SURGERY PERFORMED FOR REFRACTORY EPILEPSY		
GLUA1 ↑ GLUA2 ↓ (Talos et al., 2008)	<i>GRIN2A</i> ↓ <i>GRIN2B</i> ↑ (Crino et al., 2001; Finardi et al., 2006; Talos et al., 2008)	<i>GABRA1</i> ↓ <i>GABRA2</i> ↓ (Crino et al., 2001; Talos et al., 2012)
DATA FROM EPILEPSY SURGERY PERFORMED FOR REFRACTORY STATUS EPILEPTICUS		
GLUA1 ↑ GLUA1/GLUA2 ↑ (Present study)	GluN2B ↑ GluN2B / GluN2A ↑ (Present study)	α2/α1 ↑ <i>GABRA2</i> ↑ <i>GABRA2</i> / <i>GABRA1</i> ↑ (Present study)

Legend: ↑ elevated, ↓ decreased (relative to controls in every study)

Direct comparisons between the present data and the existing literature needs to be done cautiously, because different study populations, technical approaches and control tissues have been used.