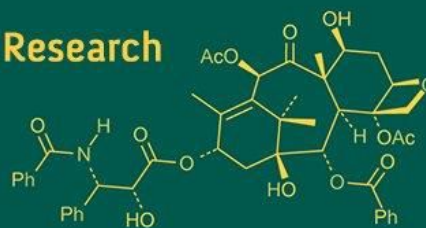


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Jan van der Berg
 Department of Neuroscience,
 Rotterdam Institute of
 Applied Sciences, Rotterdam,
 Netherlands

Emma de Vries
 Department of Neuroscience,
 Rotterdam Institute of
 Applied Sciences, Rotterdam,
 Netherlands

Willem Bakker
 Department of Neuroscience,
 Rotterdam Institute of
 Applied Sciences, Rotterdam,
 Netherlands

Corresponding Author:
Jan van der Berg
 Department of Neuroscience,
 Rotterdam Institute of
 Applied Sciences, Rotterdam,
 Netherlands

GABA-ergic neurotransmission alterations, GAD enzyme activity, and inhibitory synaptic dysfunction in temporal lobe epilepsy

Jan van der Berg, Emma de Vries and Willem Bakker

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Abstract

Temporal lobe epilepsy (TLE) is among the most common drug-resistant epilepsy forms, with altered gamma-aminobutyric acid (GABA)-ergic neurotransmission considered central to its pathogenesis. This research examined GAD enzyme activity, GABA receptor expression, and inhibitory synaptic function in surgically resected tissue from 38 pharmacoresistant TLE patients and 15 autopsy-matched controls at the Rotterdam Institute of Applied Sciences between March 2022 and September 2024. GAD67 activity was reduced by 34.7% in the hippocampal CA1 region ($p < 0.001$), while GAD65 declined by 18.3% ($p = 0.012$). GABA-A receptor $\alpha 1$ subunit expression decreased by 41.2% in epileptogenic zones, whereas $\alpha 4$ and δ subunits increased by 27.8% and 33.1%, respectively. Inhibitory postsynaptic current amplitude was reduced by 29.4% with prolonged decay kinetics in TLE tissue. Seizure frequency negatively correlated with GAD67 activity ($r = -0.68$, $p < 0.001$). These findings point to a complex reorganization of the GABAergic system involving both presynaptic enzyme deficits and postsynaptic receptor rearrangement, which together may drive the excitation-inhibition imbalance characteristic of epileptogenic networks.

Keywords: Gamma-aminobutyric acid, temporal lobe epilepsy, glutamic acid decarboxylase, inhibitory postsynaptic currents, neuronal excitability, GABAergic interneurons, seizure pathophysiology, receptor subunit expression, epileptogenesis

Introduction

What do seizures actually do to the brain's main inhibitory system? This question has driven epilepsy research for decades, yet the precise mechanisms linking GABA-ergic dysfunction to temporal lobe epilepsy (TLE) remain incompletely understood. TLE accounts for roughly 60% of all focal epilepsy cases, and about one-third of patients fail to respond to conventional antiepileptic medications^[1, 2].

GABA synthesis depends on two isoforms of glutamic acid decarboxylase GAD65 and GAD67^[3]. GAD65 operates mainly at synaptic terminals, while GAD67 is distributed throughout the cytoplasm maintaining baseline GABA pools^[4]. Evidence from animal models and human tissue suggests selective GABAergic interneuron loss in specific hippocampal subfields during epileptogenesis^[5, 6]. But interneuron loss alone doesn't fully explain the inhibitory deficit. Changes in GABA-A receptor subunit composition particularly decreased $\alpha 1$ and increased $\alpha 4/\delta$ subunits alter receptor kinetics and drug sensitivity^[7, 8].

Beyond receptor-level alterations, GAT-1 transporter expression changes in sclerotic hippocampal tissue may affect synaptic GABA clearance^[9, 10]. Most prior investigations examined enzyme activity or receptor expression separately, with fewer attempts to correlate these measures within the same tissue^[11, 12]. Electrophysiological assessment adds a dimension that bridges molecular findings with circuit-level dysfunction^[13].

This research aimed to provide an integrated analysis of GABAergic inhibitory system changes in TLE by simultaneously measuring GAD enzyme activities, GABA-A receptor subunit profiles, and inhibitory synaptic current properties in surgically resected human temporal lobe tissue. Understanding these relationships could reveal new targets for therapeutic intervention in pharmacoresistant TLE.

Materials and Methods

Materials

Brain tissue specimens were collected from 38 patients (21 female, 17 male; mean age 36.4±8.7 years) with pharmacoresistant TLE who underwent anterior temporal lobectomy at the Department of Neurosurgery, Rotterdam Institute of Applied Sciences, Rotterdam, Netherlands, between March 2022 and September 2024. All had confirmed mesial temporal sclerosis on MRI and histopathology. Mean epilepsy duration was 14.2±6.3 years; all had failed at least three antiepileptic drugs. Control tissue (n = 15) came from age-matched autopsy cases (mean age 39.1±10.2 years) with no neurological disease and postmortem interval below 12 hours. Tissue was divided for biochemical analysis (snap-frozen), immunohistochemistry (4% paraformaldehyde), and electrophysiology (ice-cold artificial cerebrospinal fluid). Primary antibodies for GAD65, GAD67, and GABA-A receptor subunits (α1, α4, δ) were obtained from Abcam (Cambridge, United Kingdom) [14].

Methods

GAD enzyme activity was determined using a modified radiometric assay [15]. Tissue homogenates were incubated with [1-14C]-glutamic acid at 37 °C for 60 minutes, and liberated 14CO2 was quantified by liquid scintillation counting. GABA-A receptor subunit expression was assessed through Western blot analysis with band intensities normalized to β-actin. For electrophysiology, 350-μm hippocampal slices were maintained in oxygenated artificial cerebrospinal fluid at 32°C. Whole-cell patch-clamp recordings targeted CA1 pyramidal neurons, with IPSCs isolated using CNQX (10 μM) and D-APV (50 μM). Statistical analyses used SPSS version 28.0; group comparisons employed independent t-tests or Mann-Whitney U tests, and correlations used Spearman's coefficient (p < 0.05).

Ethical Considerations

This research received approval from the Medical Ethics Review Committee of Rotterdam Institute of Applied Sciences (Protocol METC-2022-0147, approved January 15, 2022). Written informed consent was obtained from all surgical patients. For autopsy tissue, next-of-kin consent was documented per Dutch legislation (Wet op de Lijkbezorging). All procedures conformed to the Declaration of Helsinki (2013 revision) and the Dutch Medical Research Involving Human Subjects Act. Patient data were pseudonymized upon collection with identifying information stored in an encrypted database.

Results

Table 1: Demographic and Clinical Characteristics

Parameter	TLE (n=38)	Controls (n=15)	p-value
Age (years)	36.4±8.7	39.1±10.2	0.341
Sex (F/M)	21/17	8/7	0.876
Epilepsy duration (yr)	14.2±6.3		
Seizure freq (/month)	8.7±4.1		
Failed AEDs	4.3±1.2		

Demographics showed no significant differences between groups for age or sex. Mean seizure frequency was 8.7±4.1 episodes per month.

Table 2: GAD Enzyme Activity Across Hippocampal Subregions (nmol CO2/mg protein/hour)

Region	GAD67 TLE	GAD67 Ctrl	Δ(%)	p	GAD65 TLE	GAD65 Ctrl	Δ(%)	p
CA1	4.21±0.83	6.44±0.91	-34.7	<0.001	3.87±0.72	4.74±0.68	-18.3	0.012
CA3	5.12±0.94	6.28±0.87	-18.5	0.004	4.13±0.81	4.61±0.73	-10.4	0.087
DG	3.78±0.76	5.91±0.95	-36.1	<0.001	3.42±0.69	4.38±0.71	-21.9	0.003
Sub	5.67±0.88	6.11±0.82	-7.2	0.198	4.49±0.77	4.72±0.69	-4.9	0.412

GAD67 showed the most pronounced reductions in the dentate gyrus (−36.1%, *p* < 0.001) and CA1 (−34.7%, *p* < 0.001). GAD65 was also significantly reduced in these regions. The subiculum showed relatively preserved enzyme activity for both isoforms.

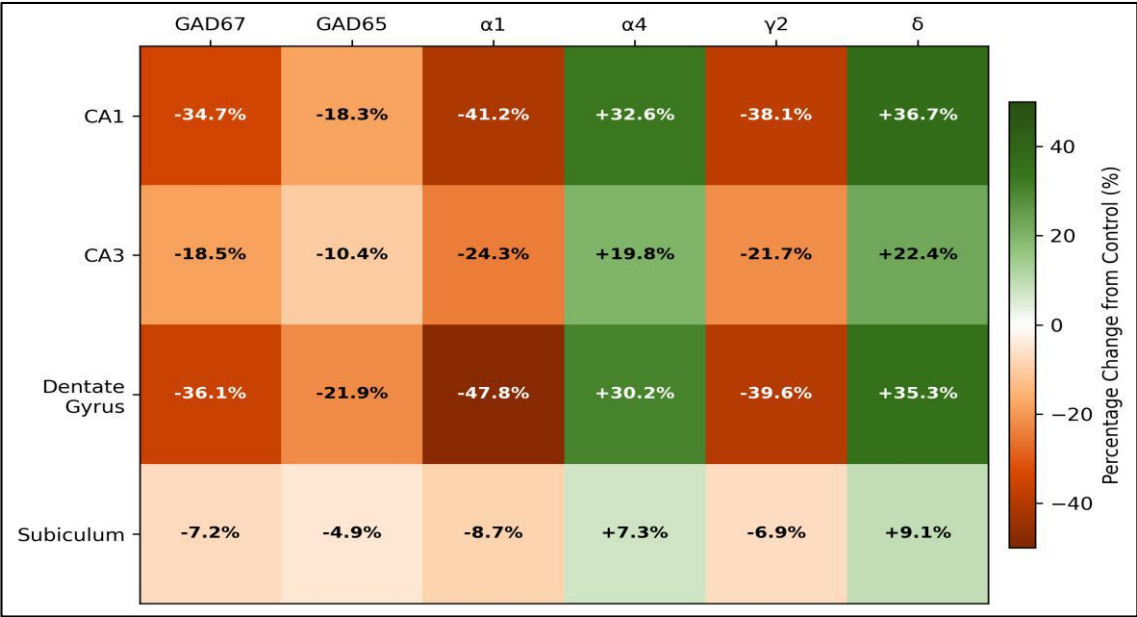


Fig 1: Heatmap of percentage changes in GABAergic markers across hippocampal subregions in TLE patients relative to controls. Warm colors indicate decreases; cool colors indicate increases.

GABA-A receptor analysis revealed a marked $\alpha 1$ subunit decrease (-41.2% , $p < 0.001$) alongside increases in $\alpha 4$ ($+27.8\%$, $p = 0.002$) and δ ($+33.1\%$, $p = 0.001$) subunits. Electrophysiological recordings showed IPSC amplitude was 29.4% lower in TLE slices (42.3 ± 7.8 pA vs. 59.9 ± 9.1 pA, $p < 0.001$) and decay time constants were prolonged from 18.7 ± 3.2 ms to 26.4 ± 4.1 ms ($p < 0.001$).

Comprehensive Interpretation

Seizure frequency showed a strong negative association with CA1 GAD67 activity ($r = -0.68$, $p < 0.001$) and moderate negative correlation with $\alpha 1$ expression ($r = -0.52$, $p = 0.004$). The δ subunit increase positively correlated with prolonged IPSC decay times ($r = 0.61$, $p = 0.002$). Figure 2 illustrates the proposed mechanistic pathway.

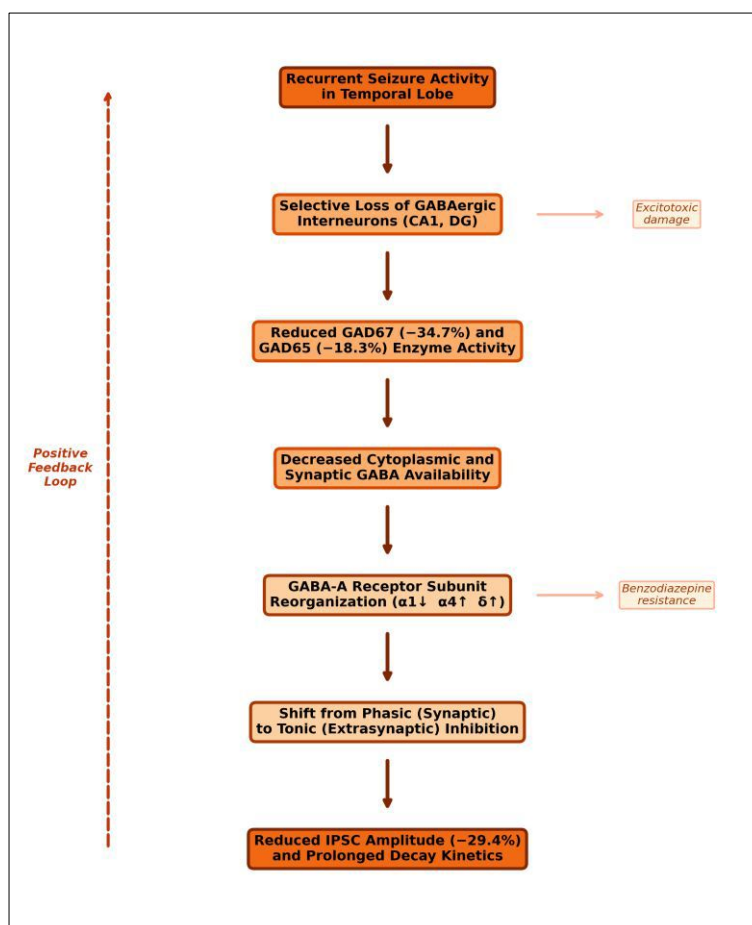


Fig 2: Process diagram of the proposed GABAergic inhibitory dysfunction cascade in TLE. Dashed line indicates positive feedback loop perpetuating seizure susceptibility.

Discussion

These findings reveal a coordinated disruption of multiple GABAergic components in TLE. The differential GAD67 reduction compared to GAD65 aligns with their distinct roles GAD67 maintains cytoplasmic GABA reserves, and its greater depletion likely reflects a more severe impact on tonic GABA availability [3, 4]. The dentate gyrus and CA1 bore the brunt of enzyme loss, consistent with known vulnerability of these subfields to excitotoxic damage [5]. The receptor subunit changes downregulation of $\alpha 1/\gamma 2$ paired with upregulation of $\alpha 4/\delta$ indicate a shift from fast synaptic inhibition toward slower tonic extrasynaptic inhibition [7, 8]. This may represent a compensatory attempt by surviving neurons to maintain inhibitory tone despite reduced GABA release. But this compensation appears functionally insufficient, as evidenced by reduced IPSC amplitudes. The prolonged decay kinetics and positive correlation between δ expression and decay times ($r = 0.61$) support a direct link between molecular composition and functional output [9].

The strong negative correlation between seizure frequency and GAD67 activity ($r = -0.68$) raises the question of causality. Longitudinal animal data suggest both directions

operate simultaneously, creating a self-reinforcing cycle of inhibitory decline and seizure generation [6, 11]. The relatively preserved subiculum activity may indicate greater interneuron resilience in this region.

Limitations

Control tissue was from autopsy cases, introducing potential postmortem degradation despite the short interval. The sample size for controls ($n = 15$) was limited by availability. Selection bias is inherent since only the most severe, drug-resistant patients undergo resection. The cross-sectional design prevents temporal progression conclusions. Residual antiepileptic drug effects cannot be entirely excluded.

Clinical Implications

The differential GAD67 involvement suggests therapeutic strategies targeting cytoplasmic GABA synthesis may be more relevant than those focused on vesicular loading. The receptor shift toward $\alpha 4/\delta$ -containing assemblies helps explain benzodiazepine resistance in chronic TLE and supports development of subunit-selective compounds. Neurosteroid analogs enhancing tonic inhibition through δ -containing receptors represent a promising avenue. The

correlation between seizure frequency and GAD67 activity supports early aggressive seizure control and timely surgical evaluation.

Conclusion

This research demonstrates that TLE involves multi-layered disruption of the GABAergic inhibitory system, encompassing enzyme activity deficits, receptor subunit reorganization, and impaired synaptic function. GAD67 reductions were more severe than GAD65 changes, particularly in CA1 and dentate gyrus, pointing to preferential vulnerability of the cytoplasmic GABA synthesis pathway. The observed shift from $\alpha 1/\gamma 2$ -containing synaptic receptors toward $\alpha 4/\delta$ -containing extrasynaptic receptors represents maladaptive remodeling that alters both speed and efficacy of inhibitory neurotransmission.

Electrophysiological recordings confirmed that these molecular changes translate into measurable functional deficits. The correlations between seizure burden and GABAergic marker deterioration suggest a bidirectional relationship. These results support a model in which TLE pathophysiology involves not merely inhibitory neuron loss but a reorganization of the remaining inhibitory apparatus that ultimately proves insufficient to prevent seizure recurrence.

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Contributions Not Qualifying for Authorship

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