



Original Investigation

Functional & Stereotactic Neurosurgery

Received: 25.11.2025

Accepted: 28.06.2026

Published Online: 29.09.2026

Evaluation of Group 1 Metabotropic Glutamate Receptors Expression After Lacosamide Treatment in a Kainic Acid-Induced Temporal Lobe Epilepsy Model

Firat NARIN¹, Tural REHIMLI², Sahin HANALIOGLU³, Zarifa YUSIFLI⁴, Hidir OZER⁵, Ibrahim BASAR⁶, Alaeddin ACAR³, Mustafa BERKER³, Burcak BILGINER³

¹Ministry of Health, Ankara City Hospital, Department of Neurosurgery, Ankara, Türkiye

²Baku Medical Plaza Hospitals, Department of Neurosurgery, Baku, Azerbaijan

³Hacettepe University School of Medicine, Department of Neurosurgery, Ankara, Türkiye

⁴Central Custom Hospitals, Department of Pathology, Baku, Azerbaijan

⁵Ordu University School of Medicine, Department of Neurosurgery, Ordu, Türkiye

⁶Dicle University School of Medicine, Department of Neurosurgery, Diyarbakır, Türkiye

Corresponding author: Firat NARIN ✉ drfiratnarin@gmail.com

ABSTRACT

AIM: To investigate the effects of lacosamide (LCM), an antiepileptic agent, on group 1 metabotropic glutamate receptors (mGluR1 and mGluR5) expression in a temporal lobe epilepsy (TLE) model.

MATERIAL and METHODS: Thirty-two rats were divided into 4 groups: (1) only burr hole without intrahippocampal injection, (2) burr hole and stereotaxic intrahippocampal saline injection, (3) burr hole, stereotaxic intrahippocampal kainic acid injection and 2 weeks of intraperitoneal saline treatment, and (4) burr hole, stereotaxic intrahippocampal KA injection and 50mg/kg intraperitoneal LCM treatment for 2 weeks.

RESULTS: No hippocampal sclerosis was detected in group 2 but sclerosis was observed in Groups 3 and 4. Despite increase in mGluR1 and mGluR5 expressions on the side of kainic acid injection, no significant effect of LCM was found on mGluR1 or mGluR5 expression. In the experimental rat model of TLE with intrahippocampal kainic acid injection in the present study, LCM was found to have no effect on mGluR expression in Group 1.

CONCLUSION: The antiepileptic effect of LCM does not seem to be mediated through group I metabotropic glutamate receptors. Since the efficacy of mGluR1 and mGluR5 antagonists as monotherapy in drug-resistant TLE remains uncertain, these findings suggest that lacosamide may act through alternative mechanisms, which require further investigation.

KEYWORDS: Kainic acid, lacosamide, temporal lobe epilepsy, hippocampal sclerosis, glutamate receptor

ABBREVIATIONS: LCM: Lacosamide, mGluR1, mGluR5: Group 1 metabotropic glutamate receptors, TLE: Temporal lobe epilepsy, m TLE: Mesial temporal lobe epilepsy, TBS: Tris-buffered saline, SDS: Sodium dodecyl sulfate, H&E: Hematoxylin-eosin, KA: Kainic acid, iGluR: Ionotropic glutamate receptor

Firat NARIN : 0000-0002-5985-4460

Tural REHIMLI : 0009-0002-6898-6817

Sahin HANALIOGLU : 0000-0003-4988-4938

Zarifa YUSIFLI : 0000-0002-3424-8085

Hidir OZER : 0000-0002-1017-2389

Ibrahim BASAR : 0000-0003-3674-4864

Alaeddin ACAR : 0009-0006-0417-6785

Mustafa BERKER : 0000-0002-3628-7977

Burcak BILGINER : 0000-0001-9667-3709



This work is licensed by "Creative Commons Attribution-NonCommercial-4.0 International (CC)".

■ INTRODUCTION

Temporal lobe epilepsy (TLE) constitutes approximately two-thirds of all types of epilepsy cases observed in adulthood (15). Mesial temporal lobe epilepsy (mTLE), in which seizures originate from the hippocampus or amygdala or lateral involving the temporal neocortex, is characterized by sclerosis that indicates neuronal loss in CA1, CA3, CA4, and dentate granule cells of the hippocampus. This is the most common drug-resistant epilepsy (9). Even though the molecular mechanisms of mTLE are still not clear, several clinical studies showed the effect of lacosamide (LCM) on long-term adjustments, intractable epilepsy, and also treatment of epilepsy in pregnant women (5,23).

The existing literature emphasizes that modulation of glutamate receptors may play a role in epileptogenesis, as well as both induction and prevention of seizures (6,13). In the present study, it is predicted that group 1 receptors (mGluR1 and mGluR5), which are commonly present in the hippocampal CA1 and CA2 regions, are biochemical parameters in terms of revealing the pathophysiology of mTLE. To date, only a few studies investigated metabotropic glutamate receptor expression and function in mTLE (12,22). This experimental study aims to investigate the effects of LCM, a new-generation antiepileptic agent, on the expression of mGluR1 and mGluR5 in the kainic acid-triggered status epilepticus model of TLE.

■ MATERIAL and METHODS

Animals

Thirty-two Sprague-Dawley albino male rats weighing between 200 and 250g were investigated. All experimental procedures involving rats were approved by the Hacettepe University Ethics Committee (approval number: 2013/57-01), and those procedures were conducted by following the institutional and international guidelines for the care and use of laboratory animals.

The rats were divided into four groups (n=32)

- **Group I, Sham group (n=8):** Scalp incision and burr hole on the right side without any injection.
- **Group II, Control group (n=8):** Intrahippocampal saline injection after scalp incision and burr hole on the right side.
- **Group III, Kainic acid group (n=8):** Intrahippocampal kainic acid injection after scalp incision and burr hole on the right side and simultaneous intraperitoneal saline injection for 2 weeks instead of LCM.
- **Group IV, Kainic acid and LCM group (n=8):** Intrahippocampal kainic acid injection after scalp incision and burr hole on the right side and simultaneous intraperitoneal 50mg/kg/day LCM injection for 2 weeks.

Experimental Design

Intraperitoneal 1mg/kg ketamine (Ketalar, Pfizer) and 1mg/kg xylazine (Alfazyne, Alfasan) were administered for anesthesia. Then, the heads of rats were fixed in a stereotaxic frame above both external auditory canals and chin. After the midline scalp incision, a burr hole was opened 5.5mm posterior and 4mm

lateral to the bregma under the stereotaxic guidance of the Rat Brain Atlas to reach the dorsal aspect of the hippocampus (30). Hamilton syringe 90° vertically penetrated burr hole to a 5mm depth, and 0.2 µl (0.4µgr) of kainic acid (Sigma-Aldrich, St, Louis) was injected slowly within 2 minutes (0.1 µL per minute) into the CA3 region of the dorsal hippocampus (31). Then, status epilepticus was observed in all individuals. Diazepam (0.2 mg/kg) was intraperitoneally injected to control the seizures for individuals, seizures of which did not end spontaneously within maximum 10 minutes.

After the diazepam injection, Group IV was immediately intraperitoneally given 50mg/kg LCM (repeated two times a day for 14 days), whereas Group III was given intraperitoneal saline in the same volume for the same duration. The dose of LCM (50 mg/kg/day, intraperitoneal) was selected after reviewing previous experimental epilepsy and neuroprotection studies demonstrating anticonvulsant efficacy and adequate brain tissue penetration in rodents without excessive sedation or toxicity (20,26,32).

Tissue Processing and Allocation

At the end of the 14-day experimental period, animals were allocated to different analyses. In each group (n=8), four rats were used for histopathological and immunohistochemical examinations and underwent intracardiac perfusion fixation. Brains were removed and fixed in formaldehyde for 24-48 h. The remaining four rats in each group were used for Western blot analysis. These animals were deeply anesthetized and decapitated without perfusion, and brain tissues were rapidly dissected and stored at -80°C until protein extraction.

Histopathology

Histopathological analyses were conducted in perfusion-fixed animals to evaluate late (2-week) histological changes following intrahippocampal kainic acid injection and the effect of LCM. The brains fixed in the paraformaldehyde were separated by cutting 3 mm thick coronal slices containing the hippocampus sections and embedded in paraffin. Coronal sections of 6µm thickness were taken from paraffin blocks using a microtome device.

Immunohistochemistry

Specific mGluR1 (Abcam, Ab82211) and anti- mGluR5 (Abcam, ab76316) antibodies were used to demonstrate the expression of mGluR1 and mGluR5 receptor subtypes in extracted tissues. After fixation with paraformaldehyde, the tissues were embedded in paraffin and cut using a microtome at a thickness of 5 µm. Before staining, the sections were deparaffinized and rehydrated using 100% xylene and 95% ethanol. The glasses were stored in tap water until the administration of antigen. Tris/EDTA pH 9.0 solution was applied to the formalin-fixed tissues for antigen retrieval before staining. Then, the sections were washed twice using TBS and 0.025% tritonX-100 for 5 minutes. After that, blocking was performed using 10% saline and 1% bovine serum albumin and they were kept at 25°C for two hours in TBS. After removing the supernatant on the sections, primary antibodies (metabotropic glutamate receptors 1 and 5) diluted with 1% bovine serum albumin and TBS were applied. The antibody-treated sections

were incubated overnight at 4°C. Then, they were rinsed twice with TBS and 0.025% Triton for 5 minutes each, and 3 times with TBS alone for 5 minutes. After the administration of a secondary antibody conjugated with horseradish, peroxidase chromogen (peroxide/DAB) was administered at room temperature for 10 minutes. Counterstain was performed and the signal was amplified with the avidin-biotin complex in order to improve the signal quality.

Western Blot

Western blot analysis was conducted using fresh brain tissue obtained from non-perfused animals (n=4 per group) to quantitatively evaluate protein expression. Hippocampal and cortical tissues were rapidly dissected on ice and immediately frozen at -80°C to preserve protein integrity. For this purpose, the brain tissues were first removed after sacrifice without being fixed. Then, homogenization lysis buffer (RIPA buffer: 150 mM sodium chloride, 0.1% triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM tris-HCl pH8.0, and protease inhibitors) and sonication were used to destroy cell membranes. To prevent proteolysis, dephosphorylation, and denaturation that occur following the cellular lysis, this procedure was performed on ice, and protease inhibitors were added to the lysis buffer. Consisting of fragmented cells, lysate was centrifuged at 12000 rpm for 20 minutes at +4°C and the ultrafiltrate was separated from the cellular component. To denature the proteins, the samples were boiled for 5 minutes at 95-100°C with anionic buffer (Laemmli 2Xbuffer) and SDS, a denaturing detergent. Then, the macromolecules were separated using polyacrylamide gel electrophoresis. Proteins, divided into bands based on their molecular weight, were transferred to a second matrix (nitrocellulose membrane). The transfer (blotting) process was performed using the sandwich model consisting of direct contact of protein-containing polyacrylamide gel with nitrocellulose membrane or another protein-binding matrix. Milk powder was used to block non-specific binding sites on the membrane. Primary antibodies (mGluR1 and 5 antibodies) and secondary antibodies conjugated with horseradish peroxidase were applied for immunoblotting. Finally, the membranes were treated with a chemiluminescent substance (ECL WB Detection Reagent, Amersham) and images were taken.

Statistical Analysis

The results of the western blot analysis were presented as the mean $\pm$ standard deviation and analyzed using SPSS 26.0 (IBM, Armonk, NY, USA). Comparative data between the two groups were analyzed using the student's t-test. Experimental data were compared among groups using the One-Way Analysis of Variance, followed by the least significant difference test. Statistical significance was set at $p < 0.05$. Western blot analyses were conducted using four animals per group.

■ RESULTS

Histopathological and Immunohistochemical Results

Group I, Sham group

- Hematoxylin and eosin (H&E) stained tissues did not reveal any pathology in the entorhinal cortex. No neuronal loss or

gliosis was observed in the CA region pyramidal neurons and the dentate gyrus granular neurons.

- mGluR1 somatodendritic staining patterns were observed in cortical neurons, hippocampal pyramidal neurons, and neurons in the dentate gyrus.
- mGluR5 dendritic staining patterns were observed in cortical, hippocampal pyramidal, and dentate gyrus neurons.

Group II, Control group

- H&E-stained tissues taken from the hippocampus on both sides did not reveal any pathology in the entorhinal cortex. No neuronal loss and gliosis were observed. There were no changes in the dentate gyrus granular neurons and CA pyramidal neurons (Figure 1A,E, and 2A).
- mGluR1 somatodendritic staining patterns were observed in cortical (Figure 2B), hippocampal pyramidal, and dentate gyrus neurons.
- mGluR5 dendritic staining patterns were observed in cortical (Figure 2C), hippocampal pyramidal, and dentate gyrus neurons.

Group III, Kainic acid group

- H&E-stained bilateral hippocampus yielded no changes in the dentate gyrus granular neurons. Sclerosis was observed in the CA1 and CA3 regions on the injection side (right) (Figure 1B,F), and severe ischemic changes occurred in the entorhinal cortex (Figure 2D). CA2 was partially preserved.
- mGluR1 somatodendritic expression loss was observed in CA3 pyramidal neurons and somatodendritic expression loss in CA1, whereas there was no expression loss in dentate gyrus neurons (Figure 1C,G). mGluR1 expression loss was observed in the entorhinal cortex on the injection side (right) (Figure 2E).
- mGluR-5 dendritic expression decreased in CA1 and CA3 pyramidal cells due to neuronal loss. There was no decrease in expression in CA2 pyramidal neurons and dentate gyrus neurons (Figure 1D,H). mGluR1 expression loss was observed in the entorhinal cortex on the injection side (right) (Figure 2F).

Group IV, Kainic acid, and LCM group

- H&E-stained bilateral hippocampus showed no changes in the dentate gyrus granular cells. Sclerosis was observed in the CA1 and CA3 regions on the injection side (right). CA2 was partially preserved. The injected side of the entorhinal cortex was preserved in two individuals, and ischemic changes occurred in the remaining subjects (Figure 2G, Figure 3).
- mGluR1 somatodendritic expression loss was observed in CA1 and CA3 pyramidal neurons, whereas there was no somatodendritic expression loss in dentate gyrus neurons.
- mGluR-5 dendritic expression in CA1 and CA3 pyramidal cells decreased due to neuronal loss. There was no decrease in expression in CA2 pyramidal neurons and dentate gyrus neurons.

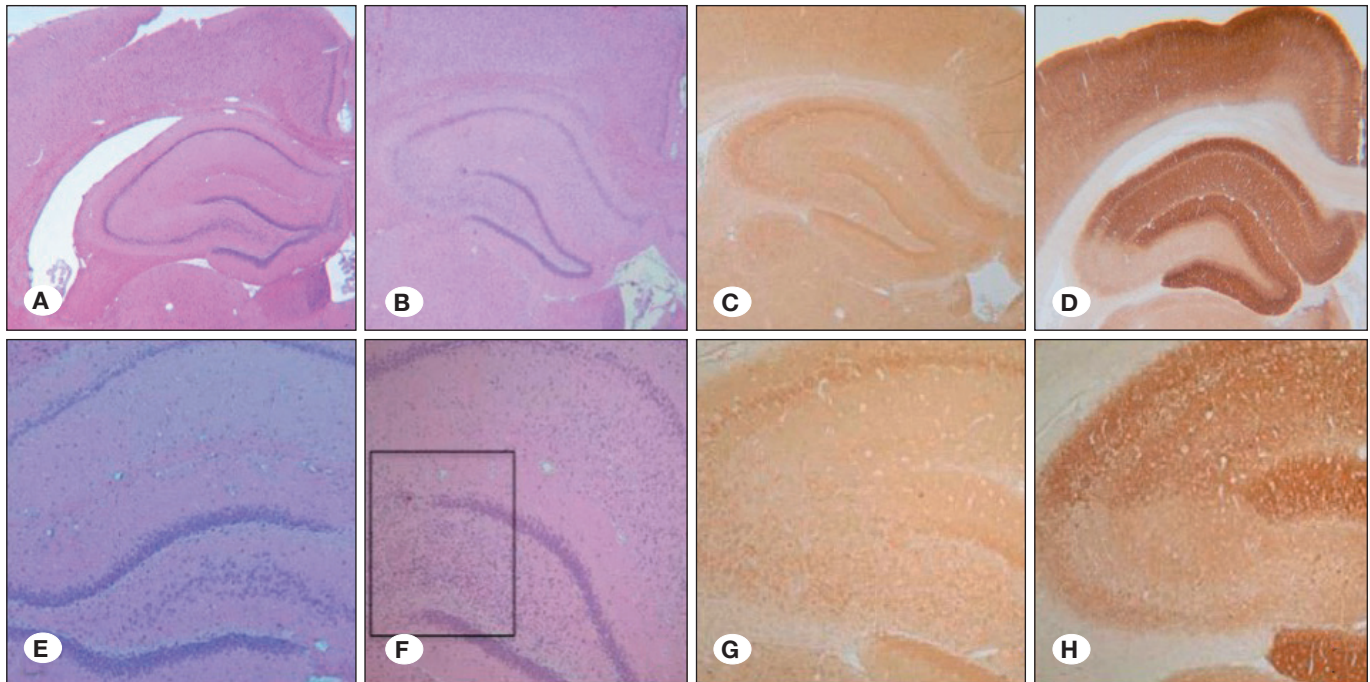


Figure 1: Hematoxylin-eosin-stained sections of normal hippocampus (A, E). Hematoxylin and eosin (B, F), mGluR-1 (C, G), and mGluR-5 (D, H) stained sections of hippocampal sclerosis after kainic acid injection on lesion side (right). (A, B, C, D: 4X and E, F, G, H: 10X magnification).

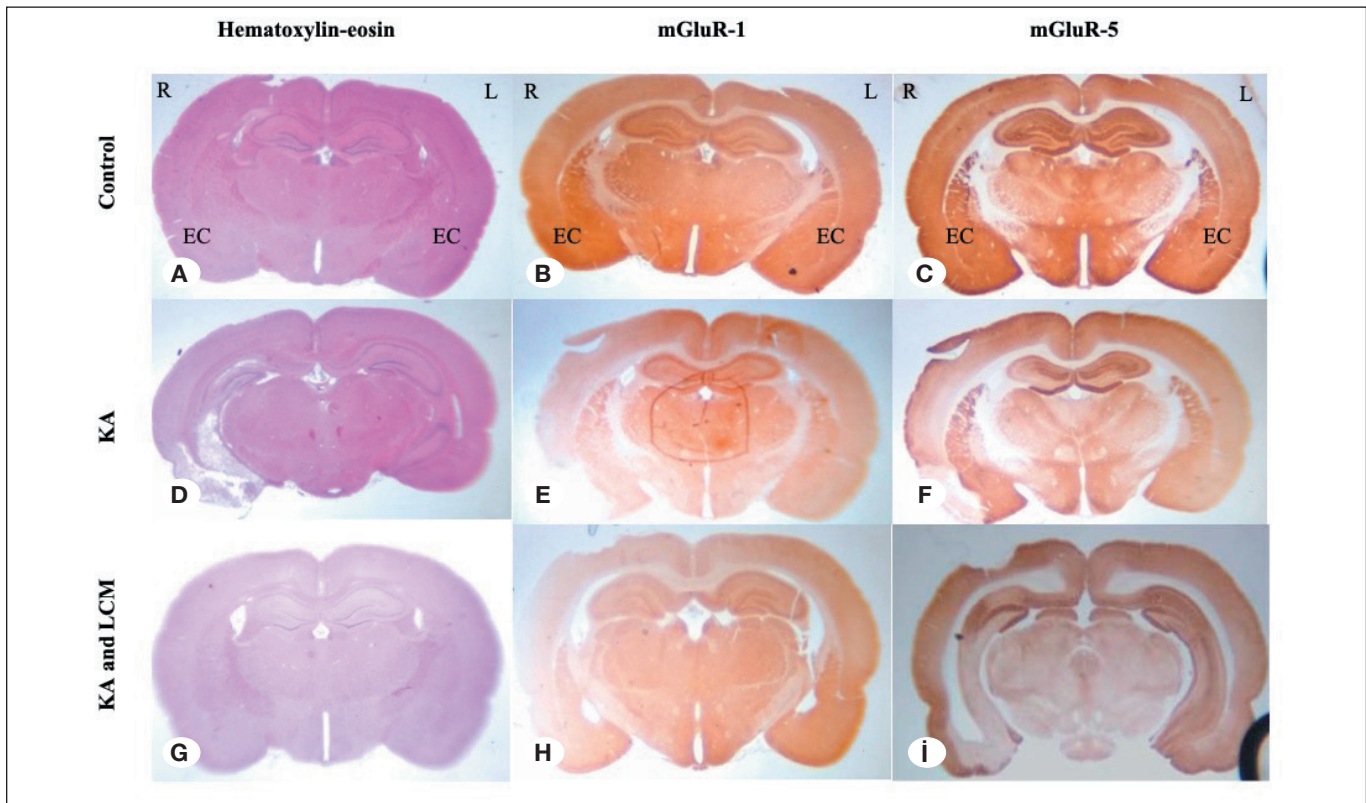


Figure 2: Differences in hematoxylin-eosin (A, D, G), mGluR-1 (B, E, H), and mGluR-5 (D, F, I) staining in the entorhinal cortex (EC) and hippocampus of the experimental groups are shown bilaterally. In the KA group, there was a decrease in hematoxylin and eosin (D), mGluR-1 (E), and mGluR-5 (F) staining of EC on lesion side (right) compared to left. After LCM administration, there is an increase in hematoxylin and eosin (G), mGluR-1 (H), and mGluR-5 (I) staining compared to the KA group. **KA:** Kainic acid, **LCM:** Lacosamide.

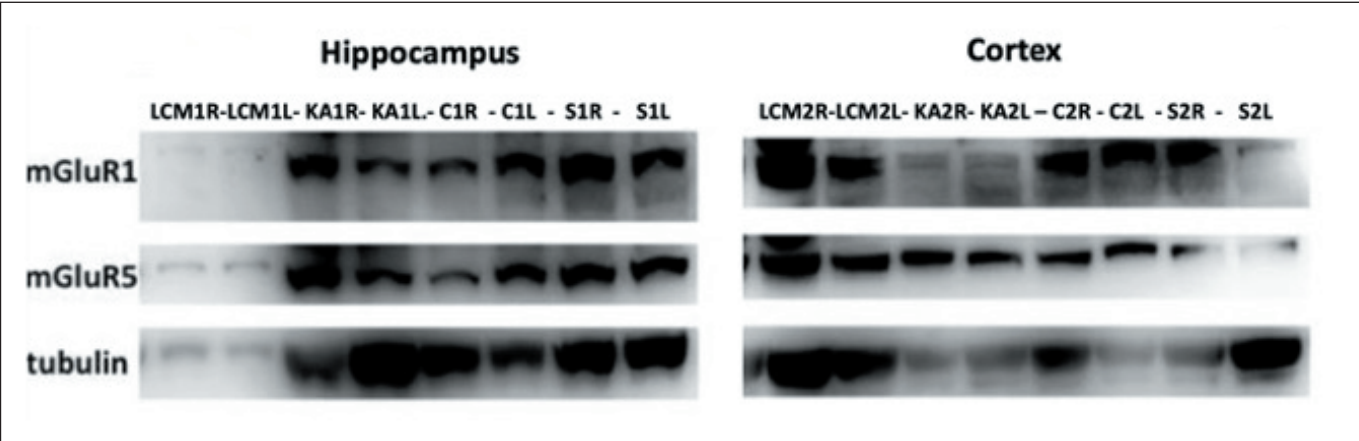


Figure 3: Image of Western Blot membranes in hippocampal and cortex samples. Bands around 130kDa were observed with mGluR-1 and mGluR-5 antibodies on the membranes. Bands around 50kDa were observed with tubulin and used as control. **LCM:** Lacosamide, **KA:** Kainic acid, **C:** Control, **S:** Sham, **R:** Right, **L:** Left.

Table I: Distribution of the Sclerosis Degree by Affected Hippocampus Regions and Entorhinal Cortex

	Dentate gyrus		CA1		CA2		CA3		Entorhinal Cortex	
	n	Sclerosis	n	Sclerosis	n	Sclerosis	n	Sclerosis	n	Sclerosis
Sham	8	-	8	-	8	-	4	-	8	-
Control	8	-	8	-	8	-	4	-	8	-
KA	8	-	8	++	8	-	4	++	8	++
KA+LCM	8	-	8	+	8	-	4	++	4	++

(-) No neuronal loss, (+) Moderate neuronal loss and gliosis, (++) Severe neuronal loss and gliosis
LCM: Lacosamide, **KA:** Kainic acid, **mGluR:** Metabotropic glutamate receptor

From the immunohistochemical aspect, the mGluR-1 staining pattern is in the form of a somatodendritic pattern in neurons of the dentate gyrus, hippocampal pyramidal, and cortex. The mGluR-5 staining pattern, on the other hand, appears as a dendritic pattern in the same regions. A decrease in mGluR-1 and mGluR-5 staining in sclerotic areas was created by kainic acid injection. The loss of expression may possibly have occurred due to neuronal loss. However, in the entorhinal cortex, there was an increase in expression of mGluR-1 and mGluR-5 staining in the kainic acid and LCM group (Figure 2H,I) in comparison to the kainic acid group (Figure 2E,F). No change was observed in samples taken from different brain regions of the same subject. These data covering different parts of the hippocampus are summarized in Table I.

Western Blotting

The hippocampus and cortical tissues (Figure 4) obtained from the right and left hemispheres were examined separately. There was no significant difference in mGluR1 and mGluR5 protein contents between the right side (injection side) and the left side. B-tubulin antibodies were applied as a control to standardize the amount of protein in each sample. Bands around 130kDa were observed with mGluR1 and mGluR5 antibodies on the membranes. Before measuring band intensities with the chemiluminescence method, they

were compared to the tubulin control. Comparing all data of groups, it was determined that there was no significant difference between hippocampus-cortex and right-left samples ($p>0.05$). However, in the kainic acid group, normalized mGluR1 and mGluR5 values in the right (injected) hippocampus (mGluR1:0.39, mGluR5: 0.32) were relatively higher than the left (mGluR1:0.19, mGluR5: 0.20) ($p>0.05$). Even though not consistently significant ($p>0.05$), in the LCM groups, this difference between right (mGluR1:0.38, mGluR5: 0.31) and left (mGluR1:0.46, mGluR5: 0.30) side seems to have changed in favor of the injected side. There was no such relationship between cortices.

DISCUSSION

Hippocampal sclerosis, which accompanies most mTLE, is thought to be both the cause and the result of seizures. Decreased cellular density in the hippocampus of patients with mTLE indicates a relationship between epilepsy and hippocampal sclerosis (2,29). The most important parameters in determining the relationship between hippocampal sclerosis and seizures were mostly obtained from experimental models (37). Although the main events responsible for its pathophysiology have not been fully elucidated, interaction with both ionotropic and metabotropic receptors as a result of synaptic stimulation

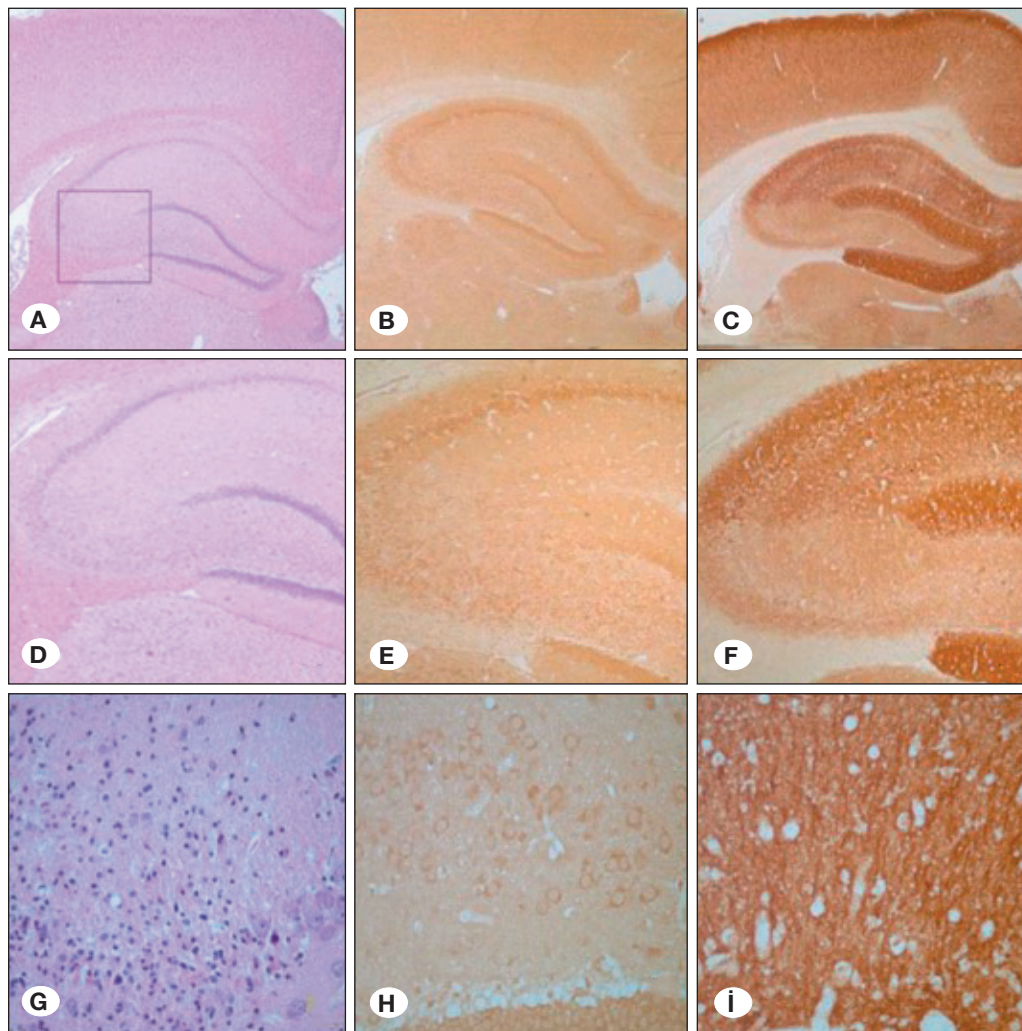


Figure 4: Image of Western Blot membranes in hippocampal and cortex samples. Bands around 130kDa were observed with mGluR-1 and mGluR-5 antibodies on the membranes. Bands around 50kDa were observed with tubulin and used as control. **LCM:** Lacosamide; **KA:** Kainic acid; **C:** Control; **S:** Sham; **R:** Right; **L:** Left.

caused by glutamate plays a significant role in mTLE. It was found that, in the inter-ictal period, the concentration of glutamate was 5 times higher in the epileptogenic hippocampus in comparison to the non-epileptogenic hippocampus (19). Since group I metabotropic glutamate receptors in the hippocampus (mGluR1 and mGluR5) are active in the pathophysiology of seizures, these receptors are seen as potential targets in the treatment of seizures (10,28).

LCM was considered an add-on therapy for children with refractory epilepsy, and it was found that there was a reduction of >50% in the frequency of seizures in 73.1% of the children after 3 months of treatment (33). Examining the efficacy and tolerability of LCM in drug-resistant epilepsy, it was concluded that LCM is an effective anti-seizure medication with acceptable side effects and adherence in drug-resistant epilepsy (17,40). In animal experiments, LCM stabilizes neuronal hyperexcitability primarily by enhancing slow inactivation of voltage-gated sodium channels (20). Beyond seizure control, potential protective effects were reported across different neurological injury models, including neurodegenerative and traumatic brain injury settings (8,14,24,26). These observations were associated with reduced oxidative stress and in-

flammatory responses (16,21). However, the molecular pathways responsible for these observations remain incompletely understood. Since group I metabotropic glutamate receptors (mGluR1 and mGluR5) play a central role in excitotoxicity, synaptic plasticity, and epileptogenesis, this study hypothesized that they might represent a potential mechanism mediating LCM effects in temporal lobe epilepsy. In the present kainic acid-induced epilepsy model, LCM did not significantly alter mGluR1 or mGluR5 expression by either immunohistochemistry or Western blot analysis. The limited histological preservation observed in some regions may reflect indirect or secondary mechanisms rather than a receptor-specific neuroprotective effect, and should therefore be interpreted cautiously. Future studies should incorporate region-specific protein quantification, EEG-based seizure burden assessment, and quantitative neuronal injury markers to determine whether LCM confers measurable neuroprotection independent of mGluR signaling.

In literature, epilepsy models with systemic injections are widely criticized nowadays. This study aims to obtain a model that mimics human TLE by injecting kainic acid directly into the dorsal hippocampus. In contrast to the systemic models,

the changes are unilateral and focal partial seizures originating from the hippocampus. Although animal models give valuable insights into the relationship between mGluR and epilepsy, further validation through human studies, including clinical trials, is essential to overwhelm the species-specific glutamate receptor differences and dose dependent effects of LCM (7,34).

Group I mGluR expression was previously shown to be increased in the hippocampus in patients with epilepsy and a rat epilepsy model (18,38). They play an important role in initiating epileptic seizures and accelerating the onset of epileptic discharges. By revealing the structural properties of these receptors, new synthetic compounds interacting with relevant receptors can provide a new avenue for the control of epileptic discharges (39). Group I mGluR are located particularly in the CA3 region of the hippocampus. Activation of these receptors causes phosphorylation and inactivation of some types of potassium channels, which ultimately leads to the depolarization of neurons. An increase was reported in the release of these receptors in hippocampal sclerosis (10,36). The potential role of mGluR1 and mGluR5 antagonists in the management of drug-resistant TLE has garnered increasing interest due to their modulatory effects on excitatory neurotransmission. Given the critical role of mGluR in synaptic plasticity and excitotoxicity, their pharmacological blockade may offer a novel therapeutic avenue for refractory epilepsy cases.

In a study carried out on Wistar Albino rats, a genetic model of absence epilepsy, the expression/ activity of mGluR1 and mGluR5 was found to be reduced in the cortico-thalamic network (27). Therefore, the literature suggests that group I mGluR leads to the induction of epilepsy, and focuses on the role of group I mGluR in seizure prolongation (25). In a study carried out on autopsy samples, it was observed that mGluR5 expression increased in the hippocampus of TLE patients in comparison to non-epileptic patients. Moreover, it was shown that mGluR5 immunoreactivity increased in the outer molecular layer of the dentate gyrus in the group with and without hippocampal sclerosis. In the inner molecular layer, a decrease was found in mGluR5 immunoreactivity in hippocampal sclerosis (4,11). Microglial glutamate also plays an important role in epilepsy, neurodegenerative diseases, and neuroprotective processes (1,41).

An active mGluR5, which is highly expressed in rat hippocampus, decreases proinflammatory cytokine with oxidative stress level. The systemic kainite model of epilepsy demonstrated that the mGluR5 expression in astrocytes could determine epilepsy development and modulate the interaction between astrocytes and neurons. However, in both systemic rat lithium-pilocarpine and kainic acid models of epilepsy, MTEP (selective mGluR5 antagonist) had a dose-dependent neuroprotective effect but did not prevent spontaneous recurrent seizures (18). In the neurodegenerative process, the upregulation of mGluR1 in rat hippocampal neurons may trigger paracrine activation of microglia and contribute to neuroinflammation and neurodegeneration. In the present study, it was hypothesized that mGluR1 and 5 blockades could suppress the seizures with a neuroprotective effect. In contrast,

although the LCM-treated group showed some reduction in sclerosis in the CA1 region of the hippocampus, no changes were identified in hippocampal mGluR1 and mGluR5 immunoreactivity. Moreover, the entorhinal cortex was protected in a significant portion of subjects given LCM through the prevention of ischemic changes. This regional preservation suggests a possible protective effect; however, it is descriptive and should be considered hypothesis-generating in the absence of quantitative neuroprotection measures. The data obtained in this study support a negative finding for mGluR1/5 modulation and do not establish a receptor-mediated neuroprotective effect. On the other hand, focal protein alterations may have been diluted in homogenized samples since Western blot analyses were performed on whole hippocampal and cortical tissue. Accordingly, the absence of a significant Western blot difference should be interpreted as a lack of a global receptor effect rather than the absence of regional biological changes. Histologically, partial preservation of the entorhinal cortex and reduced ischemic alterations were observed in LCM-treated animals. These findings may reflect indirect effects related to reduced neuronal stress or seizure burden rather than direct modulation of group I mGluR expression. The number of human studies examining group I in these patients is quite limited. In the present study, a decrease was found in group I mGluR immunoreactivity on hippocampal sclerosis. There was no difference in expression between the injected side and the intact side of dentate gyri. No significant changes were observed in the expression of mGluR1 and mGluR5 in the intact regions. In a gerbil model of ischemic stroke, the neuroprotective effects of LCM on cerebral ischemia-induced neuronal cell damage were associated with decreased activation of glial cells in the ischemic CA1 region of the hippocampus (3). In the present study, sclerosis developed in the hippocampus regions of individuals who received LCM treatment after kainic acid injection. Even though significant cell losses were observed in CA3, CA1 (regionally) and CA2 (completely) were preserved. There were no significant changes in the dentate gyrus.

No inhibitory effect of LCM on epileptogenesis was demonstrated in previous post-status epilepticus models. Cellular electrophysiological studies reported that LCM does not interact with excitatory or inhibitory ligand-gated ion channels (32). However, no study reported its interaction with mGluR in the literature. The present study yielded no significant effect of LCM on mGluR1 or mGluR5 expression by immunohistochemistry or Western blotting. In contrast, histopathological examination suggested less prominent ischemic changes in the entorhinal cortex in a subset of LCM-treated animals in comparison to the kainic acid group. Taken together, these findings do not support the initial hypothesis that the anticonvulsive effects of LCM are mediated through group I metabotropic glutamate receptors. The regional histological preservation observed in the entorhinal cortex should therefore be interpreted cautiously and considered hypothesis-generating, given the absence of quantitative neuroprotection or oxidative stress measurements in the current study. If confirmed in future studies, such an effect could plausibly explain mechanisms independent of mGluR1/5, including pathways involv-

ing oxidative stress modulation, as suggested in prior experimental studies (35). Following seizures that induce neuronal ischemia, LCM may counteract early ischemic changes by enhancing antioxidant enzyme activity, thereby having a neuroprotective effect.

Elucidating the underlying therapeutic mechanisms of LCM and gaining a deeper understanding of mGluR's role in epilepsy pathogenesis will facilitate the development and clinical implementation of more effective antiepileptic therapies in the future. The efficacy of mGluR1 and mGluR5 antagonists as monotherapy in drug-resistant TLE remains inconclusive, highlighting the need for further investigation into their potential use in combination with established antiepileptic drugs (32). Rational polytherapy approaches may include LCM, which not only targets distinct seizure propagation mechanisms but also offers neuroprotective benefits while minimizing adverse effects. Clinical trials and larger cohorts are required to assess the long-term safety and effectiveness of these combinations in drug-resistant TLE, particularly in patients with pharmacoresistant mesial temporal sclerosis or altered glutamatergic signaling.

The present study has several limitations. First, seizure activity was not quantitatively assessed using electroencephalography or standardized behavioral seizure scoring systems; therefore, antiepileptic effects could not be directly measured. A focal intrahippocampal kainic acid model with multiple control groups was used to reproduce temporal lobe epilepsy-like pathology under controlled conditions. Second, Western blot analyses were performed using whole hippocampal and cortical tissue, which may have obscured region-specific protein alterations suggested by immunohistochemistry. Regional changes were additionally examined through detailed histopathological and immunohistochemical evaluation. Finally, the relatively small sample size may have limited statistical power and increased the risk of type II error. Accordingly, the absence of statistically significant differences in Western blot analyses should be interpreted with caution and may reflect limited statistical power. However, findings were interpreted using complementary qualitative and semi-quantitative observations across different tissue regions. On the other hand, both Western blot and immunohistochemical analyses yielded concordant findings indicating that LCM had no measurable effect on mGluR1 and mGluR5 expression.

CONCLUSION

LCM did not significantly modify mGluR1 or mGluR5 expression in this intrahippocampal kainic acid model of temporal lobe epilepsy. These findings suggest that the antiepileptic action of LCM is unlikely to be mediated through group I metabotropic glutamate receptors. Histological preservation observed in the entorhinal cortex may indicate indirect or secondary effects; however, this observation should be considered hypothesis-generating rather than definitive evidence of neuroprotection. Further experimental and clinical studies are needed to clarify the alternative mechanisms of action of LCM in temporal lobe epilepsy.

ACKNOWLEDGEMENTS

We are deeply indebted to the many experimental animals whose bodies were sacrificed for the sake of science. This study was not supported by any institute.

Declarations

Funding: The authors declare no competing financial interests.

Availability of data and materials: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Disclosure: The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

AUTHORSHIP CONTRIBUTION

Study conception and design: FN, BB

Data collection: FN, HO, SH, AA, IB

Analysis and interpretation of results: SH, TR, AA, MB, ZY

Draft manuscript preparation: FN, TR

Critical revision of the article: FN, BB

Other (study supervision, fundings, materials, etc...): SH, TR, AA, IB

All authors (FN, TR, SH, ZY, HO, IB, AA, MB, BB) reviewed the results and approved the final version of the manuscript.

REFERENCES

1. Abd-Elrahman KS, Sarasija S, Ferguson SSG: The role of neuroglial metabotropic glutamate receptors in Alzheimer's disease. *Curr Neuropharmacol* 21: 273-283, 2023. <https://doi.org/10.2174/1570159X19666210916102638>
2. Abosch A, Bernasconi N, Boling W, Jones-Gotman M, Poulin N, Dubeau F, Andermann F, Olivier A: Factors predictive of suboptimal seizure control following selective amygdalohypocampectomy. *J Neurosurg* 97: 1142-1151, 2002. <https://doi.org/10.3171/jns.2002.97.5.1142>
3. Ahn JY, Yan BC, Park JH, Ahn JH, Lee DH, Kim IH, Cho JH, Chen BH, Lee JC, Cho YS, Shin MC, Cho JH, Hong S, Won MH, Kim SK: Novel antiepileptic drug lacosamide exerts neuroprotective effects by decreasing glial activation in the hippocampus of a gerbil model of ischemic stroke. *Exp Ther Med* 10: 2007-2014, 2015. <https://doi.org/10.3892/etm.2015.2794>
4. Akbar MT, Rattray M, Powell JF, Meldrum BS: Altered expression of group I metabotropic glutamate receptors in the hippocampus of amygdala-kindled rats. *Mol Brain Res* 43: 105-116, 1996. [https://doi.org/10.1016/S0169-328X\(96\)00162-3](https://doi.org/10.1016/S0169-328X(96)00162-3)
5. Aulická S, Česká K, Šána J, Siegl F, Brichtová E, Ošlejšková H, Hermanová M, Hendrych M, Michu EP, Brázdil M, Slabý O, Nestršil I: Cytokine-chemokine profiles in the hippocampus of patients with mesial temporal lobe epilepsy and hippocampal sclerosis. *Epilepsy Res* 180: 106858, 2022. <https://doi.org/10.1016/j.epilepsyres.2022.106858>
6. Barker-Haliski M, White HS: Glutamatergic mechanisms associated with seizures and epilepsy. *Cold Spring Harb Perspect Med* 8: a022863, 2015. <https://doi.org/10.1101/cshperspect.a022863>

7. Beed P, Ray S, Velasquez LM, Stumpf A, Parthier D, Swaminathan A, Nitzan N, Breustedt J, Las L, Brecht M, Schmitz D: Species-specific differences in synaptic transmission and plasticity. *Sci Rep* 10: 16557, 2020. <https://doi.org/10.1038/s41598-020-73547-6>
8. Bilal B, Kirazlar M, Erdogan MA, Yigitturk G, Erbas O: Lacosamide exhibits neuroprotective effects in a rat model of Parkinson's disease. *J Chem Neuroanat* 132: 102311, 2023. <https://doi.org/10.1016/j.jchemneu.2023.102311>
9. Blümcke I, Pauli E, Clusmann H, Schramm J, Becker A, Elger C, Merschhemke M, Meencke HJ, Lehmann T, von Deimling A, Scheiwe C, Zentner J, Volk B, Romstöck J, Stefan H, Hildebrandt M: A new clinicopathological classification system for mesial temporal sclerosis. *Acta Neuropathol* 113: 235-244, 2007. <https://doi.org/10.1007/s00401-006-0187-0>
10. Blümcke I, Becker AJ, Klein C, Scheiwe C, Lie AA, Beck H, Waha A, Friedl MG, Kuhn R, Emson P, Elger C, Wiestler OD: Temporal lobe epilepsy-associated up-regulation of metabotropic glutamate receptors: Correlated changes in mGluR1 mRNA and protein expression in experimental animals and human patients. *J Neuropathol Exp Neurol* 59: 1-10, 2000. <https://doi.org/10.1093/jnen/59.1.1>
11. Bordi F, Ugolini A: Group I metabotropic glutamate receptors: Implications for brain disease. *Prog Neurobiol* 59: 55-79, 1999. [https://doi.org/10.1016/s0301-0082\(98\)00095-1](https://doi.org/10.1016/s0301-0082(98)00095-1)
12. Celli R, Santolini I, Van Luijckelaar G, Ngomba RT, Bruno V, Nicoletti F: Targeting metabotropic glutamate receptors in the treatment of epilepsy: Rationale and current status. *Expert Opin Ther Targets* 23: 341-351, 2019. <https://doi.org/10.1080/14728222.2019.1586885>
13. Chen TS, Huang TH, Lai MC, Huang CW: The role of glutamate receptors in epilepsy. *Biomedicines* 11: 783, 2023. <https://doi.org/10.3390/biomedicines11030783>
14. Chen YS, Lai MC, Chen TS, Tseng YH, Li YJ, Huang CW: Effectiveness and safety of lacosamide, a third-generation anti-seizure medication, for poststroke seizure and epilepsy: A literature review. *Curr Neuropsychopharmacol* 21: 2126-2133, 2023. <https://doi.org/10.2174/1570159X21666230616114255>
15. Commission on Classification and Terminology of the International League Against Epilepsy: Proposal for revised clinical and electroencephalographic classification of epileptic seizures. *Epilepsia* 22: 489-501, 1981.
16. Demiröz S, Ur K, Ulucan A, Bengü AS, Ur FD, Öz Gergin O, Erdem S: Neuroprotective effects of lacosamide in experimental traumatic spinal cord injury in rats. *Türk Neurosurg* 29: 695-702, 2019. <https://doi.org/10.5137/1019-5149.JTN.25891-19.2>
17. Driessen JT, Wammes-van der Heijden EA, Verschuure P, Fasen KCFM, Teunissen MWA, Majoie HJM: Effectiveness and tolerability of lacosamide in children with drug-resistant epilepsy. *Epilepsy Behav Rep* 21: 100574, 2023. <https://doi.org/10.1016/j.ebr.2022.100574>
18. Dyomina AV, Kovalenko AA, Zakharova MV, Postnikova TY, Griflyuk AV, Smolensky IV, Antonova IV, Zaitsev AV: MTEP, a selective mGluR5 antagonist, had a neuroprotective effect but did not prevent the development of spontaneous recurrent seizures and behavioral comorbidities in the rat lithium-pilocarpine model of epilepsy. *Int J Mol Sci* 23: 497, 2022. <https://doi.org/10.3390/ijms23010497>
19. Eid T, Williamson A, Lee TS, Petroff OAC, De Lanerolle NC: Glutamate and astrocytes—Key players in human mesial temporal lobe epilepsy? *Epilepsia* 49: 42-52, 2008. <https://doi.org/10.1111/j.1528-1167.2008.01492.x>
20. Fukuyama K, Ueda Y, Okada M: Effects of carbamazepine, lacosamide and zonisamide on gliotransmitter release associated with activated astroglial hemichannels. *Pharmaceuticals (Basel)* 13: 117, 2020. <https://doi.org/10.3390/ph13060117>
21. He Z, Li J: The therapeutic effects of lacosamide on epilepsy-associated comorbidities. *Front Neurol* 14: 1063703, 2023. <https://doi.org/10.3389/fneur.2023.1063703>
22. Huang L, Xiao W, Wang Y, Li J, Gong J, Tu E, Long L, Xiao B, Yan X, Wan L: Metabotropic glutamate receptors (mGluRs) in epileptogenesis: An update on abnormal mGluRs signaling and its therapeutic implications. *Neural Regen Res* 19: 360-368, 2024. <https://doi.org/10.4103/1673-5374.379018>
23. Inoue Y, Liao W, Wang X, Du X, Tennigkeit F, Sasamoto H, Osakabe T, Hoshii N, Yuen N, Hong Z: Safety and efficacy of adjunctive lacosamide in Chinese and Japanese adults with epilepsy and focal seizures: A long-term, open-label extension of a randomized, controlled trial. *Epilepsy Res* 176: 106705, 2021. <https://doi.org/10.1016/j.eplepsyres.2021.106705>
24. Keservani RK, Jain PK, Premalatha SJ: Epilepsy and neurodegeneration. In: *A Review on Diverse Neurological Disorders: Pathophysiology, Molecular Mechanisms, and Therapeutics*. 1st ed. Elsevier, 2024: 281-290.
25. Merlin LR: Differential roles for mGluR1 and mGluR5 in the persistent prolongation of epileptiform bursts. *J Neurophysiol* 87: 621-625, 2002. <https://doi.org/10.1152/jn.00579.2001>
26. Mete M, Alpaya S, Aydemir I, Unlu Unsal U, Collu F, Özel HF, Duransoy YK, Kutlu N, Tuglu MI: Therapeutic effects of lacosamide in a rat model of traumatic brain injury: A histological, biochemical and electroencephalography monitoring study. *Injury* 52: 713-723, 2021. <https://doi.org/10.1016/j.injury.2021.02.055>
27. Nicoletti F, Bruno V, Ngomba RT, Gradini R, Battaglia G: Metabotropic glutamate receptors as drug targets: What's new? *Curr Opin Pharmacol* 20: 89-94, 2015. <https://doi.org/10.1016/j.coph.2014.12.002>
28. Notenboom RGE, Hampson DR, Jansen GHJ, van Rijen PC, van Veelen CWM, van Nieuwenhuizen O, de Graan PNE: Up-regulation of hippocampal metabotropic glutamate receptor 5 in temporal lobe epilepsy patients. *Brain* 129: 96-107, 2006. <https://doi.org/10.1093/brain/awh673>
29. Olivier A: Temporal resections in the surgical treatment of epilepsy. *Epilepsy Res Suppl* 5: 175-188, 1992. PMID: 1418447.
30. Paxinos G, Watson C: *The Rat Brain in Stereotaxic Coordinates Compact*. 3rd ed. San Diego: Academic Press, 1997.
31. Raedt R, Van Dycke A, Van Melkebeke D, De Smedt T, Claeys P, Wyckhuys T, Vonck K, Wadman W, Boon P: Seizures in the intrahippocampal kainic acid epilepsy model: Characterization using long-term video-EEG monitoring in the rat. *Acta Neurol Scand* 119: 293-303, 2009. <https://doi.org/10.1111/j.1600-0404.2008.01108.x>
32. Rogawski MA, Tofighty A, White HS, Matagne A, Wolff C: Current understanding of the mechanisms of action of the antiepileptic drug lacosamide. *Epilepsy Res* 111: 189-205, 2015. <https://doi.org/10.1016/j.eplepsyres.2014.11.021>

33. Romão TT, Angelim AIM, Prado HJP, Goes FV, Pires MEP, Fontana RS, Pires LC, D'Andrea-Meira I: Use of lacosamide in children: Experience of a tertiary medical care center in Brazil. *Arq Neuropsiquiatr* 80: 1090-1096, 2022. <https://doi.org/10.1055/s-0042-1758366>
34. Rusina E, Bernard C, Williamson A: The kainic acid models of temporal lobe epilepsy. *eNeuro* 8: ENEURO.0337-20.2021, 2021. <https://doi.org/10.1523/ENEURO.0337-20.2021>
35. Sadeghzadeh J, Hosseini L, Mobed A, Soltani Zangbar H, Jafarzadeh J, Pasban J, Shahabi P: The impact of cerebral ischemia on antioxidant enzymes activity and neuronal damage in the hippocampus. *Cell Mol Neurobiol* 43: 3915-3928, 2023. <https://doi.org/10.1007/s10571-023-01413-w>
36. Sayin U, Rutecki PA: Group I metabotropic glutamate receptor activation produces prolonged epileptiform neuronal synchronization and alters evoked population responses in the hippocampus. *Epilepsy Res* 53: 186-195, 2003. [https://doi.org/10.1016/S0920-1211\(03\)00020-2](https://doi.org/10.1016/S0920-1211(03)00020-2)
37. Sloviter RS: The neurobiology of temporal lobe epilepsy: Too much information, not enough knowledge. *C R Biol* 328: 721-722, 2005. <https://doi.org/10.1016/j.crv.2004.10.010>
38. Tang FR, Lee WL, Yeo TT: Expression of the group I metabotropic glutamate receptor in the hippocampus of patients with mesial temporal lobe epilepsy. *J Neurocytol* 30(5): 403-411, 2001. <https://doi.org/10.1023/A:1015065626262>
39. Ure J, Baudry M, Perassolo M: Metabotropic glutamate receptors and epilepsy. *J Neurol Sci* 247: 1-9, 2006. <https://doi.org/10.1016/j.jns.2006.03.018>
40. Yang C, Liu Z, Peng Y, Zhang L, Yu D: Effectiveness and safety of lacosamide therapy for children with focal epilepsy: A real-world study. *Front Pharmacol* 14: 1186768, 2023. <https://doi.org/10.3389/fphar.2023.1186768>
41. Zhang X, Wang D, Zhang B, Zhu J, Zhou Z, Cui L: Regulation of microglia by glutamate and its signal pathway in neurodegenerative diseases. *Drug Discov Today* 25: 1074-1085, 2020. <https://doi.org/10.1016/j.drudis.2020.04.001>