Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

High-intensity interval training attenuates neuroinflammation and seizure activity via miR-146a/NF-κB signaling in chronic mesial temporal lobe epilepsy

Leila Saremi [1], Mohammad Reza Kordi [1]*, Amir Hossein Saffar Kohneh Quchan [1]*, Younes Sarkabood [1], Siroos Choobineh [1], Zahra Javaheri [1]

[1] Department of Exercise Physiology, Faculty of Sport Sciences and Health, University of Tehran, Tehran, Iran

ARTICLE INFO

Article type:
Original

Article history:
Received: Feb 12, 2026
Accepted: Jun 17, 2026

Keywords:
Convulsion
Epilepsy
Exercise training
Hippocampus
Inflammation

ABSTRACT

Objective(s): Regular exercise training has emerged as a non-pharmacological approach that can beneficially impact disease progression in epileptic patients, but the underlying molecular pathways remain unclear. In this study, we investigated the effects of eight weeks of high-intensity interval training (HIIT), recognized as a time-efficient form of aerobic exercise, on hippocampal neuroinflammation and seizure activity in a kainic acid rat model of chronic Mesial temporal lobe epilepsy (MTLE).

Materials and Methods: After induction of epilepsy via intrahippocampal injection of kainic acid, rats were randomly divided into 4 groups: Control, Sham, Epilepsy, and Epilepsy+HIIT. After an acclimatization period, rats in the exercise group performed 8 weeks of HIIT. The HIIT protocol consisted of running at 90% of Vmax, with active rest periods at 30% of Vmax. At the end of week 8, rats were sacrificed, and hippocampal tissue was extracted for Western blotting and real-time qPCR.

Results: After 8 weeks of HIIT, our data showed that IL-1β (P<0.0001), IRAK1 (P<0.0001), NF-κB (P<0.0001), and p-NMDA (P<0.0001) levels were significantly lower than in the epileptic group. Additionally, miR-146a levels were significantly lower than in the epileptic group (P<0.001). Seizure intensity also decreased in parallel with the reduction in inflammation.

Conclusion: Our results indicate that HIIT could serve as a potent anti-inflammatory and neuroprotective tool in chronic MTLE through regulating the miR-146a/NF-κB/pNMDA pathway, highlighting that regular chronic exercise training could be a complementary therapeutic option for drug-resistant epilepsy.

▶ Please cite this article as:
Saremi L, Kordi MR, Saffar Kohneh Quchan AH, Sarkabood Y, Choobineh C, Javaheri Z. High-intensity interval training attenuates neuroinflammation and seizure activity via miR-146a/NF-κB signaling in chronic mesial temporal lobe epilepsy. Iran J Basic Med Sci 2026; 29:

Introduction

Epilepsy is one of the common neurological diseases worldwide, with an estimated prevalence of 0.67 %. Further psychiatric co-morbidities are common in people with epilepsy, approximately 37.5 %, encompassing disorders such as depression and anxiety (1). Epilepsy includes various types and classifications. Mesial temporal lobe epilepsy (MTLE) is recognized as one of the most common forms, which often becomes drug-resistant (2).

Drug resistance is defined as the failure to respond to at least two appropriately selected treatments. This clinical subtype is strongly associated with neuroinflammation, a crucial phenomenon in the pathophysiology of disease. Up-regulated levels of inflammatory factors, such as IL-1β (IL-1β) and interleukin-6 (IL-6), have been observed in some brain regions, especially the hippocampus (3). The inflammatory environment results in neural hyperexcitability and injury, disruption of neural function, and ultimately cell death. These processes facilitate the onset and progression of epileptic activity. Furthermore, recent evidence demonstrates that inflammation contributes to the formation of epileptogenic networks, which increases seizure frequency, severity, and resistance to antiepileptic drugs (AEDs) (3, 4).

The Toll-like receptor (TLR) and interleukin-1 receptor type I (IL-1R1) initiate an important signaling pathway in the central nervous system (CNS) immune system. IL-1β, by binding to its receptor IL-1R1, activates an inflammatory signaling pathway that augments seizures and the progression of epilepsy through transcriptional and non-transcriptional mechanisms. In non-transcriptional pathways, IL-1β increases calcium influx by enhancing N-methyl-D-aspartate (NMDA) receptor activity, thereby inducing neuronal hyperexcitability and excitotoxicity (5, 6). Transcriptionally, IL-1β acts through the Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway by binding to IL-1R1 and initiating an inflammatory signaling cascade involving the adaptor protein MyD88 and interleukin-1 receptor-associated kinase 1 (IRAK1). This leads to the activation and translocation of

***Corresponding authors:** Mohammad Reza Kordi. Department of Exercise Physiology, University of Tehran, Tehran, Iran. Email: mrkordi@ut.ac.ir; Amir Hossein Saffar Kohneh Quchan. Department of Exercise Physiology, University of Tehran, Tehran, Iran. Email: amirsaffar@ut.ac.ir

NF-κB to the nucleus. NF-κB then promotes the expression of pro-inflammatory mediators, perpetuating a pro-epileptogenic environment (3, 7). Evidence indicates that neuroinflammation functions as both a secondary response to seizures and an active contributor to epileptogenesis and disease progression. Targeting inflammatory signaling pathways may provide a strategy for developing more effective treatments, especially for pharmacoresistant MTLE.

Recent studies have identified short noncoding RNAs, known as microRNAs, as important diagnostic and therapeutic targets in various diseases. Notably, microRNA-146a (miR-146a) has been recognized as an inflammation-associated miRNA that plays an important role in the development of epilepsy (8). Research consistently reports up-regulation of miR-146a in both epileptic patients and animal models, suggesting its involvement in modulating inflammatory responses associated with seizures. Abnormal levels of miR-146a contribute to the overexpression of inflammatory and neurotoxic factors, including IL-1β, TNF-α, nitric oxide (NO), and hydrogen peroxide (H_2O_2), ultimately resulting in neuronal injury and neurodegeneration (9, 10). However, in TLE, miR-146a can also enhance IL-1β expression by suppressing complement factor H (CFH), thereby creating a miR-146a–CFH–IL-1β feed-forward loop that results in chronic inflammation (9).

However, miR-146a plays a significant role in the inflammatory pathway by targeting IRAK1 and suppressing the NF-κB pathway (11). During the chronic phase of MTLE, recurrent seizure activity and sustained inflammatory signaling result in prolonged NF-κB activation and elevated miR-146a expression. Despite this elevation, mir146a expression is insufficient to suppress the inflammatory cascade (3, 12, 13). This dysregulation of mir146a results in consistent up-regulation of inflammatory cytokines, including IL-1β, and increased phosphorylation of the NMDA receptor. These molecular changes enhance neuronal hyperexcitability and finally lead to recurrent seizures (5, 14).

In chronic MTLE, elevated miR-146a expression likely reflects a maladaptive response, indicating unresolved neuroinflammation and ineffective negative feedback regulation of immune pathways in the epileptic hippocampus. Chronic overexpression of miR-146a suggests a failed compensatory mechanism in the chronic epileptic state. Considering the evidence from research, it could be conclusive that miR-146a is a central regulator of inflammation-related disorders in epilepsy and supports its ability to be a promising therapeutic target in drug-resistant epilepsy.

Exercise and physical activity have gained attention for their beneficial role as an adjunctive therapy for various chronic diseases, and one of their outstanding features is their anti-inflammatory effects (15). Numerous clinical and experimental studies have shown that exercise reduces seizure susceptibility and enhances behavioral outcomes, including quality of life (QOL), memory, learning, mood, and self-esteem (16, 17). Despite these benefits, Physical inactivity is common among people with epilepsy, frequently driven by apprehension about seizures triggered by exercise, and family members or healthcare professionals usually advise them to avoid vigorous physical activity(17). However, several studies have shown that exercise does not increase seizure frequency or the risk of injury, even during high-intensity training (18-20).

High-intensity interval training (HIIT) has emerged as a popular and time-efficient form of aerobic exercise (21, 22). HIIT is a type of exercise that alternates periods of high-intensity activity with rest or low-intensity activity (23). Its growing popularity is supported by research demonstrating that HIIT can produce physiological and metabolic benefits equal to or greater than those of continuous aerobic training, but in a shorter duration (21). Importantly, studies have shown that HIIT may help reduce inflammatory processes in the central nervous system (CNS) (24). It may help regulate microglial and astrocyte balance and pro-inflammatory activity, both of which are critical for managing neuroinflammation and slowing disease progression (24, 25). Previous research has shown that high-intensity exercise can reduce inflammation and brain injury. However, there have been no investigations into the effects of this exercise method on epilepsy to date. We intended to determine the impact of eight weeks of HIIT on inflammation and seizure-related manifestations in the KA-induced epileptic rat model.

Materials and Methods

Experimental animals

Thirty-two male Wistar rats were purchased from the Pasteur Institute of Tehran. Following a one-week acclimatization period, the animals were randomly assigned to five groups: control (n=8), epilepsy (KA-injected, n=8), epilepsy+ high intensity interval training (n=8), sham (Saline-injected, n=8). Animals were housed in a controlled environment with a temperature range of 22-26 °C, humidity between 55% and 65%, and a 12-hr light/dark cycle. The experimental procedures are illustrated in Figure 1. Ethical approval for all procedures was granted by the Ethics Committee at the Faculty of Sport Sciences and Health, University of Tehran, Iran. All procedures were carried out in accordance with institutional guidelines and national regulations for the care and use of laboratory animals.

Epilepsy induction

Epilepsy induction was performed by intrahippocampal (IH) injection of kainic acid (KA) into anesthetized rats using a combination of ketamine (100 mg/kg, IP) and

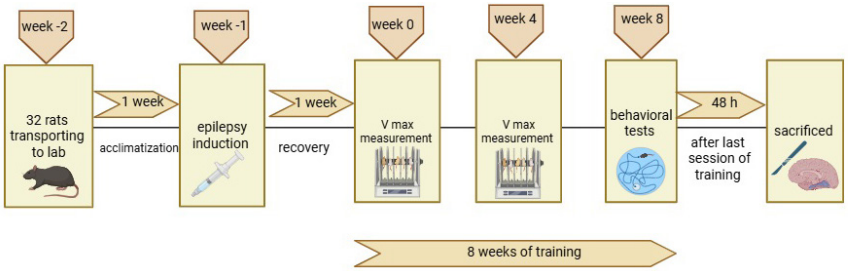

Figure 1. Epilepsy induction and experimental procedures in rats

xylazine (10 mg/kg, IP). Rats were placed in a stereotaxic apparatus, and KA (0.4 µg in 0.2 µl sterile saline) was injected unilaterally into the CA3 region using a Hamilton microsyringe, according to the Paxinos and Watson atlas. The syringe was left in place for an additional 2–3 min to prevent reflux before being slowly withdrawn. Following the injection, the first signs of seizure appeared within 5–30 min after regaining consciousness, and rats were monitored for 2 hr. Rats that showed stages 3 or above were included in the experiment (based on the Racine scale). After the first spontaneous seizures, the seizures disappeared. After a latent period of approximately 2 weeks, more than half of the rats exhibited spontaneous recurrent seizures (SRS), marking the chronic phase of epilepsy (26). Seizure activity was evaluated using Racine's classification. The stages are as follows: 0, no response; 1, stereotyped mounting, eye blinking, or mild facial clonus; 2, head bobbing or facial clonus; 3, forelimb myoclonic jerks; 4, events of stage 3 plus forelimb and rearing; 5, generalized myoclonic convulsion and falling (27).

Training protocol

To reduce stress from surgery and the new environment, the animals underwent a one-week acclimatization period. After this period, the animals were familiarized with the apparatus for three consecutive days by placing them on a treadmill at 12 m/min for 10 min/day. A HIIT protocol was performed 5 days/week for 8 weeks. Each session lasted 45 min, including a 5-minute warm-up and 40 min of the main exercise. High-intensity intervals were conducted at 90% of maximal running velocity (Vmax), while active rest periods were performed at 30% of Vmax. The protocol was carried out for 8 weeks, and Vmax was evaluated twice—first at week 0 (before training) and again at week 4—to adjust training speed. To determine Vmax, a graded treadmill exercise test was performed. The test began with a warm-up (5 min at 12 m/min). After the warm-up, treadmill speed was increased by 3 m/min every 3 min until the animal could no longer continue running (28, 29).

Seizure monitoring

Seizure monitoring was conducted during week 8 of the study, from 6 a.m. to 6 p.m. 5 specialist personnel monitored the rat cages, and all subjects were blinded to group assignments. Seizure scores were recorded based on the modified Racine's scale as previously described.

Tissue preparation

Anesthesia was induced with an intraperitoneal (IP) injection of sodium pentobarbital (150 mg/kg). Anesthesia depth was monitored by assessing the absence of the pedal withdrawal reflex and the lack of response to a mild pinch of the footpad. The hippocampi were carefully dissected, placed in sterile tubes, and immediately frozen in liquid nitrogen. The hippocampal tissue was stored at -80 °C until further analysis.

RNA isolation

For RNA isolation, we added 1 ml of TRIzol to the homogenized material and incubated it at room temperature for 5 min. We then added 250 µl of chloroform, and the mixture was centrifuged at 12000 RPM for 15 min. After centrifugation, three phases were observed, with RNA in the top layer. We transferred the top layer to DNase/RNase using a filter tip, then added cold isopropanol to a volume equal to the sample volume. Samples were washed twice with 75% ethanol.

RNA extraction and cDNA synthesis

We assessed miRNA expression in the rat hippocampus using real-time PCR. miRNA was extracted from total RNA isolated from hippocampal slices using QIAzol Lysis Reagent (Qiagen, USA). RNA quantity and purity were determined using a Nanodrop 2000 Vis spectrophotometer (Thermo Scientific, USA) based on the A260/A280 and A260/A230 absorbance ratios. cDNA synthesis was performed using a Hyper Script RT Premix with Random Hexamer (Gene All, South Korea) for miR-146a gene expression analysis. A 1 ng input of RNA was used for the reverse transcription reaction. cDNA was synthesized using the stem-loop reverse transcription method, which incorporates four to six nucleotides complementary to the 3' end of the target microRNA.

Quantitative RT-PCR

The resulting cDNA was used as a template for real-time PCR with SYBR Green master mix (Ampliqon, Denmark) on a Rotor-Gene Q cycler (Qiagen, USA) using a 40-cycle amplification program. Thermal cycling included an initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 15 sec, annealing at 60 °C for 30 sec, and extension at 72 °C for 30 secs. The PCR reaction mixture contained 2 µl of cDNA, 5 µl of SYBR Green PCR master mix, and 3 µl of primer mix, for a total reaction volume of 10 µl. The relative expression levels of mRNA and miRNA for each target gene were calculated by comparing the Ct values of the target genes to the Ct value of the housekeeping gene (U6 for miR-146a). Quantification was performed using the 2-ΔΔCt method. PCR specificity was verified by melting curve analysis. Primer sequences used for real-time PCR are provided in Table 1.

Western blotting

To evaluate inflammation, the levels of inflammatory factors, including IL-1β, IRAK-1, NF-κB, and pNMDA, were measured by Western blotting. Tissue lysates were prepared in RIPA buffer and centrifuged at 14,000 rpm for 20 min at 4 °C to remove debris. Protein concentration was measured using the Bradford Protein Quantification kit (DB0017, DNAbioTech, Iran) according to the manufacturer's instructions. Tissue lysates were mixed with an equal volume of 2X Laemmli sample buffer. Lysates (20 µg) were boiled for five min, subjected to SDS-PAGE, and transferred to a 0.2 µm Immobilon-Blot polyvinylidene difluoride (PVDF) membrane (Cat No: 162-017777; Bio-Rad Laboratories, CA, USA). Membranes were incubated with 5% BSA (Cat. No. A-7888; Sigma-Aldrich, MO, USA) in 0.1% Tween-20 for 1 hr. After this step, the membranes

Table 1. Forward and reverse primer sequence of mir146a for R-TPCR

U6
Forward CTCGCTTCGGCAGCACATATACT
Reverse ACGCTTCACGAATTTGCGTGTC
Stem loop
CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGACnnnnnnnn
mir146a
Forward GTTGAGGAGTGAATTCCA
Reverse AACTGGTGTCGTGGAGTC

were treated with anti-GAPDH loading control antibodies for 1 hr at room temperature. The membranes were then washed three times with TBST and incubated with goat anti-rabbit IgG H&L (HRP) (1/10000, Cat No: ab6721; Abcam) as the secondary antibody. The membranes were then incubated with enhanced chemiluminescence (ECL) for 1–2 min. Normalization to β-actin was performed for protein expression levels. For densitometry of protein bands, the gel analyzer Version 2010a software (NIH, USA) was used; the Area Under the Curve (AUC) for each protein band was divided by the AUC of its corresponding β-actin band, and the resulting values were compared between groups.

Statistical analysis

Data analyses were performed using IBM SPSS Statistics version 27, and the Significance level was set at $P \leq 0.05$. Data are presented in the text and figures as means±standard error of the mean (SEM). One-way ANOVA was performed to compare groups for miR-146a, IL-1β, pNMDA, NF-κB, and IRAK-1. Following the identification of significant differences by the ANOVA, Tukey's *post hoc* test was applied to determine specific pairwise differences. To compare seizure severity between the Epilepsy and Epilepsy+ HIIT groups, we used Racine's scale. The comparison was performed for each day during the last week of the experiment. Given that the data were non-parametric and involved two independent groups, the Mann-Whitney U test was used for statistical analysis.

Results

Measuring mir146a expression

Results of One-way ANOVA revealed a significant difference in hippocampal miR-146a expression among the groups (F (3,16)=30.30, *P*<0. 001, η^2=0.850, Figure 2). *Post hoc* tests revealed significant differences between the Epilepsy and control groups (miR-146a, *P*-value<0.001), Epilepsy and Sham groups (miR-146a, *P*-value<0.001), and between the Epilepsy and Epilepsy+HIIT groups (miR-146a, *P*-value<0.001) (Figure 2).

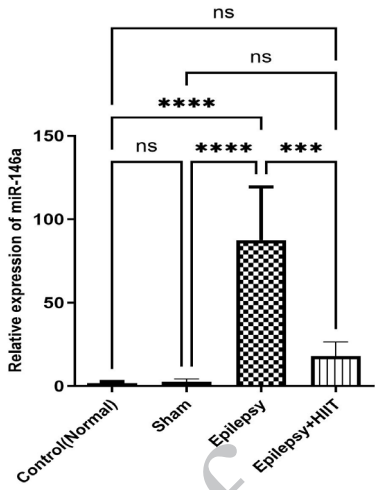

Figure 2. Results of real-time q-PCR for mir146a in rats
HIIT significantly reduced miR-146a expression in the hippocampus compared to the epilepsy group. Values are presented as mean±standard deviation (n=5 per group. * *P*<0.05, ** *P*<0.01, *P*<0.001, and **** *P*<0.0001.
HIIT: High intensity interval training, mir146a: MicroRNA 146a

Measuring hippocampal IL-1β expression

Analysis of variance showed a significant difference in hippocampal IL-1β expression among the groups (F(3,16) = 30.81, *P*<0.001, η^2=0.852; Figure 3). Pairwise comparisons indicated significant differences between the Epilepsy and control groups (IL-1β, *P*-value<0.001), Epilepsy and Sham groups (IL-1β, *P*-value<0.001), and Epilepsy and Epilepsy + HIIT groups (IL-1β, *P*-value<0.001) (Figure 3).

Evaluating hippocampal pNMDA expression

One-way ANOVA showed a significant difference in hippocampal pNMDA receptor expression among the experimental groups (F (3,16)=37.73, *P*<0.001, η^2=0.876, Figure 4). Further, the *post hoc* test showed significant differences between the Epilepsy and control groups (pNMDA, *P*-value<0.001), Epilepsy and Sham groups (pNMDA, *P*-value<0.001), and Epilepsy and Epilepsy+ HIIT groups (pNMDA, *P*-value=0.002) (Figure 4).

Evaluating hippocampal NF-κB expression

One-way ANOVA revealed a significant difference in hippocampal NF-κB expression between the groups (F

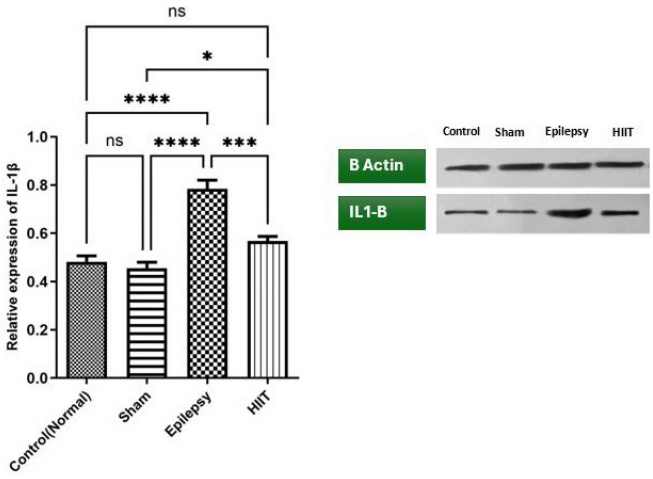

Figure 3. Results of western blotting for evaluation of rat hippocampal IL-1β expression
HIIT significantly reduced IL-1β expression in the hippocampus compared to the epilepsy group. Values are presented as mean ± standard deviation (n = 5 per group. * *P*<0.05, ** *P*<0.01, *P*<0.001, and **** *P*<0.0001.
IL-1β: Interlukin-1β; HIIT: High-intensity interval training

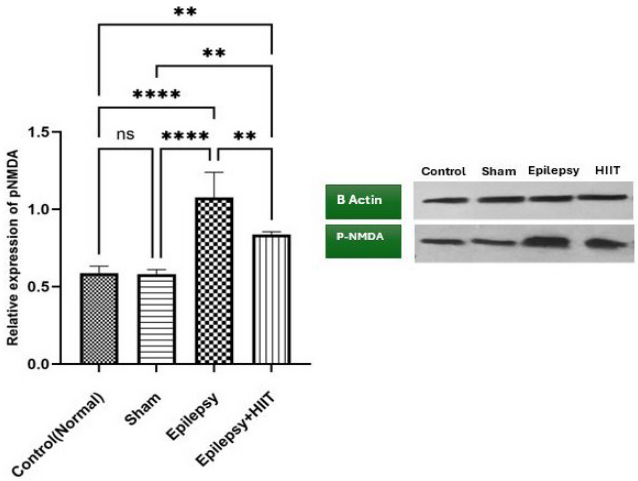

Figure 4. HIIT significantly reduced rat pNMDA receptor levels in the hippocampus compared with the epilepsy group
Values are presented as mean±standard deviation (n=5 per group. * *P*<0.05, ** *P*<0.01, *** *P*<0.001, and **** *P*<0.0001.
pNMDA: phosphorylated N-methyl-D-aspartate; HIIT: high-intensity interval training

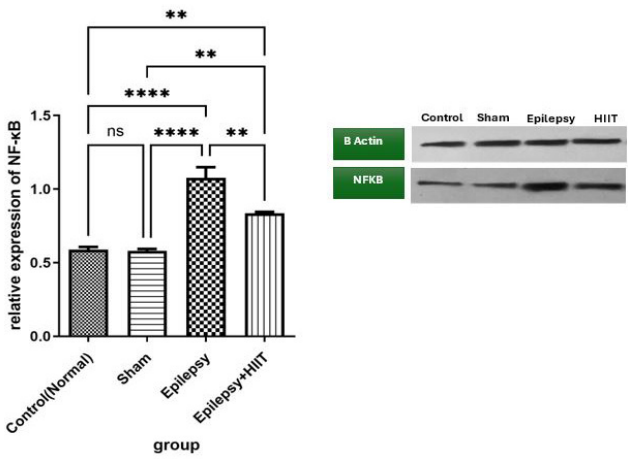

Figure 5. Results of Western blotting for measuring rat NF-κB expression in hippocampus tissue
HIIT significantly reduced NF-κB expression in the hippocampus compared to the epilepsy group. * P<0.05, ** P<0.01, ***P<0.001, and ****P<0.0001.
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; HIIT: High-intensity interval training

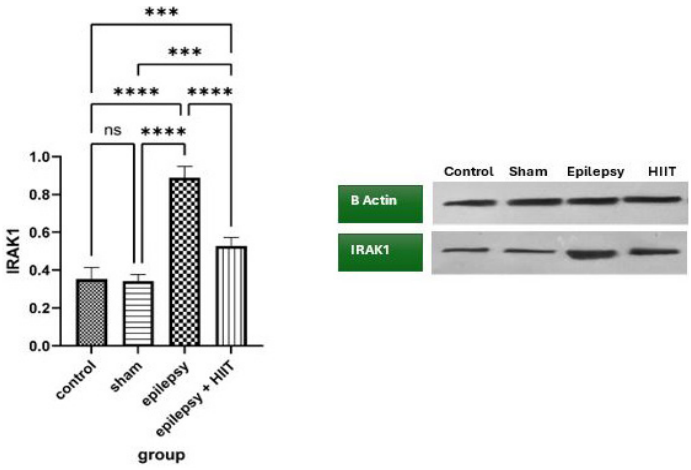

Figure 6. Results of evaluation of IRAK-1 hippocampal expression by western blot
HIIT significantly reduced IRAK-1 expression in the hippocampus compared to the epilepsy group. * P<0.05, ** P<0.01, ***P<0.001 and ****P<0.0001
IRAK-1: Interleukin-1 receptor-associated kinase 1; HIIT: High intensity interval training

(3,16)=71.68, P<0.0001, η^2=0.931, Figure 5). Pairwise comparisons indicated significant differences between the Epilepsy and control groups (NF-κB, P-value<0.001), Epilepsy and Sham groups (NF-κB, P-value<0.001), and between the Epilepsy and Epilepsy+HIIT groups (NF-κB, P-value<0.001) (Figure 5).

IRAK-1

significant difference was detected by one-way ANOVA test in hippocampal IRAK-1 expression among the groups (F (3,16)=123.1, P<0.001, η^2= 0.958, Figure 6). Tukey's *post hoc* test indicated significant differences between the Epilepsy and control groups (IRAK-1, P-value<0. 001) Epilepsy and Sham groups (IRAK-1, P-value<0. 001), and between the Epilepsy and Epilepsy+ HIIT groups (IRAK-1, P-value=<0. 001) (Figure 6).

Seizure severity

Epilepsy+ HIIT groups across the seven consecutive days of the eighth experimental week. On Day 1, the Epilepsy+ HIIT group showed significantly lower seizure severity than the Epilepsy group (U=19.5, Z=-2.400, P=0.016). This difference remained significant on Day 2 (U=15.5, Z= -2.692, P=0.007) and Day 3 (U=20.5, Z=-2.285, P=0.022). A significant reduction in seizure severity was observed in the Epilepsy+ HIIT group on Day 4 (U=12.0, Z=-2.948, P=0.003). Similarly, on Day 5 (U= 20.0, Z = -2.327, P=0.020) and Day 6 (U=18.0, Z=-2.515, P=0.012), the difference between groups remained significant. On Day 7 (U=11.0, Z=-3.040, P=0.002), the Epilepsy+ HIIT group showed significantly lower seizure severity. Overall, significant between group differences, with lower seizure severity in the Epilepsy+ HIIT group, were observed on each day of the final experimental week (Figure 7).

Discussion

MTLE is a complex neurological condition defined by spontaneous recurrent seizures and progressive neurodegeneration, especially in the hippocampus. Recent studies demonstrate that neuroinflammation significantly contributes to both the development and persistence of epileptic activity in the chronic phase of MTLE (30, 31).

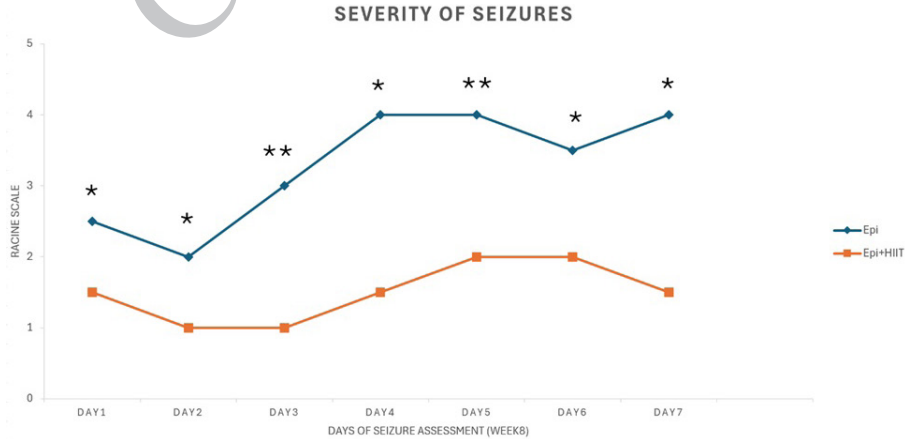

Figure 7. Effect of HIIT on the seizure severity in rats
Results are expressed as median. * a statistically significant difference from the Epilepsy group on the same day (P<0.01). * * a statistically significant difference from the Epilepsy group on the same day (P<0.05). Mann–Whitney U test.
HIIT: High-intensity interval training

Among the various regulators of neuroinflammation, miR-146a has emerged as a key molecular mediator with potential dual roles in modulating both protective and pathological responses in the epileptic brain (9, 32). Our study demonstrated significant upregulation of miR-146a, NF-κB, IL-1β, and IRAK1 in the hippocampus during the chronic phase of MTLE, accompanied by increased seizure activity.

Our findings align with previous research, which reported that chronic seizure activity activates TLR signaling and sustains neuroinflammation in MTLE (33, 34). Specifically, TLR4 activation in glial cells triggers downstream signaling via the MyD88–IRAK1–TRAF6 pathway, leading to NF-κB translocation to the nucleus. NF-κB then enhances the transcription of inflammatory factors, such as IL-1β and TNF-α, and induces the expression of miR-146a as well (3, 11, 34). Once expressed, miR-146a typically acts as an anti-inflammatory regulator by targeting IRAK1 and TRAF6 to dampen NF-κB signaling (11). Considering our data and prior evidence, it appears that in the chronic phase of epilepsy, inflammation is so persistent and robust that miR-146a becomes ineffective at suppressing it, ultimately leading to overactivation of the NF-κB pathway and the expression of inflammatory factors. This chronic inflammatory state leads to seizure augmentation, and seizures in turn further enhance inflammation. Furthermore, our study showed increased NMDA receptor phosphorylation (pNMDA) in the hippocampus, a key mechanism of glutamate-mediated excitotoxicity. IL-1β has been shown to enhance phosphorylation of the NR2A and NR2B subunits of NMDA receptors, leading to increased calcium influx, neural hyperexcitability, and neuronal injury (5). This hyperexcitable environment leads to seizure occurrence. Our study indicated enhancements in both inflammatory and excitatory pathways, highlighting the mutual interaction between neuroinflammation and excitotoxicity in MTLE.

A main finding is that HIIT could effectively reverse these molecular alterations. Our data showed that HIIT reduced the expression of NF-κB, IL-1β, IRAK1, and miR-146a, as well as NMDA receptor phosphorylation in the hippocampus, and decreased seizure activity. These results propose that HIIT exhibits anti-inflammatory and neuroprotective characteristics in chronic epileptic rats. On the other hand, the reduction in miR-146a expression may appear paradoxical. Still, NF-κB induces miR-146a, and its down-regulation in response to HIIT likely reflects reduced inflammatory signaling, thereby diminishing the need for miR-146a–mediated negative feedback. HIIT may suppress TLR4/NF-κB signaling in the hippocampus by reducing microglial activity, regulating systemic immune responses, and increasing anti-inflammatory cytokines such as IL-10. Overall, these mechanisms attenuate neuroinflammation (35, 36). Furthermore, we observed a reduction in NMDA phosphorylation in response to HIIT, which may be due to reduced IL-1β. As NMDA phosphorylation decreases, calcium influx and neuronal excitability decrease, ultimately attenuating seizure frequency and intensity (6). Some studies suggest that regular exercise can modulate neuroinflammation and seizure severity in experimental models of epilepsy. Yu *et al.* have shown that 2 weeks of treadmill running improves cognitive deficits and enhances hippocampal neuroplasticity in epileptic mouse models. These results were aligned with exercise-induced reductions in neuroinflammation and protection of parvalbumin-positive neurons in the hippocampus, a region known as the center of learning, memory, and seizure regulation (37).

In summary, these findings indicate that HIIT may reduce neural excitation and seizures by modulating neuroinflammation in chronic MTLE. Suppression of IL-1β and IRAK1 pathway and subsequently reduction of NF-κB, alongside reducing pNMDA, by HIIT, indicating attenuation of inflammation and neural hyperexcitability. Furthermore, the reduction of mir146a serves as a robust marker of declining neuroinflammation. Collectively, these results demonstrate that HIIT is not detrimental in epilepsy and also could be a beneficial non-pharmacological complementary tool for this disease. We acknowledge several limitations in this study. First, we were unable to record seizures electronically. Future studies incorporating electroencephalography (EEG) and 24-hr video monitoring are needed to confirm our findings and draw more robust conclusions. Second, another limitation is the absence of an exercise-only (sham exercise) group. Therefore, we cannot completely rule out the possibility that some of the observed effects might be partially attributable to exercise per se in the absence of the disease. However, the primary aim of this study was to investigate the modulatory effect of exercise on epilepsy-related outcomes, not to evaluate its impact on healthy subjects. Nevertheless, future studies should include an exercise-only group to further dissect the independent effects of physical activity from its disease-modifying interactions.

Acknowledgment

This research received no specific grant or financial support from any funding agency in the public, commercial, or non-profit sectors. The results presented in this paper were part of a student's thesis.

Data availability

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Authors' Contributions

L S performed formal analysis, investigation, developed the methodology, and wrote the original draft. MR K managed the project, provided administration, and supervised the work. AH SKQ supervised, contributed to the methodology, and reviewed and edited the writing. Y S conducted investigations. S Ch supervised. Z J conducted investigations.

Conflicts of Interest

The authors confirm that they have no conflicts of interest.

Declaration

We have not used any AI tools or technologies to prepare this manuscript.

References

1. Christensen J, Dreier JW, Sun Y, Linehan C, Tomson T, Marson A, *et al.* Estimates of epilepsy prevalence, psychiatric co-morbidity and cost. Seizure 2023;107:162-171.
2. Tatum WOI. Mesial temporal lobe epilepsy. J Clin Neurophysiol 2012;29:356-365.
3. Vezzani A, Aronica E, Mazarati A, Pittman QJ. Epilepsy and

brain inflammation. Exp Neurol 2013;244:11-21.
4. Marchi N, Granata T, Ghosh C, Janigro D. Blood-brain barrier dysfunction and epilepsy: Pathophysiologic role and therapeutic approaches. Epilepsia 2012;53:1877-1886.
5. Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, *et al.* Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. J Neurosci 2003;23:8692-8700.
6. Balosso S, Maroso M, Sanchez-Alavez M, Ravizza T, Frasca A, Bartfai T, Vezzani A. A novel non-transcriptional pathway mediates the proconvulsive effects of interleukin-1beta. Brain 2008;131:3256-3265.
7. Allan SM, Tyrrell PJ, Rothwell NJ. Interleukin-1 and neuronal injury. Nat Rev Immunol 2005;5:629-640.
8. Fan W, Liang C, Ou M, Zou T, Sun F, Zhou H, Cui L. MicroRNA-146a is a wide-reaching neuroinflammatory regulator and potential treatment target in neurological diseases. Front Mol Neurosci 2020;13:90.
9. Mao S, Wu J, Yan J, Zhang W, Zhu F. Dysregulation of miR-146a: A causative factor in epilepsy pathogenesis, diagnosis, and prognosis. Front Neurol 2023;14:1094709.
10. Long Y, Liu J, Wang Y, Guo H, Cui G. The complex effects of miR-146a in the pathogenesis of Alzheimer's disease. Neural Regen Res 2025;20:1309-1323.
11. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci U S A 2006;103:12481-12486.
12. Yakovleva KD, Dmitrenko DV, Panina IS, Usoltseva AA, Gazenkampf KA, Konovalenko OV, *et al.* Expression profile of mirs in mesial temporal lobe epilepsy: Systematic review. Int J Mol Sci 2022; 23:951.
13. Li G, Bauer S, Nowak M, Norwood B, Tackenberg B, Rosenow F, *et al.* Cytokines and epilepsy. Seizure 2011;20:249-256.
14. Dong Y, Zhang X, Wang Y. Interleukins in epilepsy: Friend or foe. Neurosci Bull 2024;40:635-657.
15. Scheffer DdL, Latini A. Exercise-induced immune system response: Anti-inflammatory status on peripheral and central organs. Biochim Biophys Acta Mol Basis Dis 2020;1866:165823.
16. Arida RM, Passos AA, Graciani AL, Brogin JAF, Ribeiro MdAL, Faber J, *et al.* The potential role of previous physical exercise program to reduce seizure susceptibility: A systematic review and meta-analysis of animal studies. Front Neurol 2021;12: 2021.
17. Zhang CQ, Li HY, Wan Y, Bai XY, Gan L, Sun HB. Effect of different physical activity training methods on epilepsy: A protocol for systematic review and meta-analysis. Medicine 2022;101: e29085.
18. Johnson EC, Helen Cross J, Reilly C. Physical activity in people with epilepsy: A systematic review. Epilepsia 2020;61:1062-1081.
19. McAuley JW, Long L, Heise J, Kirby T, Buckworth J, Pitt C, *et al.* A prospective evaluation of the effects of a 12-week outpatient exercise program on clinical and behavioral outcomes in patients with epilepsy. Epilepsy Behav 2001;2:592-600.
20. Sırtbaş G, Yalnızoğlu D, Livanelioğlu A. Comparison of physical fitness, activity, and quality of life of the children with epilepsy and their healthy peers. Epilepsy Res 2021;178:106795.
21. Milanović Z, Sporiš G, Weston M. Effectiveness of high-intensity interval training (HIT) and continuous endurance training for VO2max improvements: A systematic review and meta-analysis of controlled trials. Sports Med 2015;45:1469-1481.
22. Elliott AD, Rajopadhyaya K, Bentley DJ, Beltrame JF, Aromataris EC. Interval training versus continuous exercise in patients with coronary artery disease: A meta-analysis. Heart Lung Circ 2015;24:149-157.
23. Feito Y, Heinrich KM, Butcher SJ, Poston WSC. High-intensity functional training (HIFT): Definition and research implications for improved fitness. Sports 2018;6:76.
24. Zaychik Y, Fainstein N, Touloumi O, Goldberg Y, Hamdi L, Segal S, *et al.* High-intensity exercise training protects the brain against autoimmune neuroinflammation: Regulation of microglial redox and pro-inflammatory functions. Front Cell Neurosci 2021;15:640724.
25. Goldberg Y, Segal S, Hamdi L, Nabat H, Fainstein N, Mediouni E, *et al.* High-intensity interval training attenuates development of autoimmune encephalomyelitis solely by systemic immunomodulation. Sci Rep 2023;13:16513.
26. Rusina E, Bernard C, Williamson A. The kainic acid models of temporal lobe epilepsy. eNeuro 2021;8:ENEURO.0337-20.2021.
27. Racine RJ. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalogr Clin Neurophysiol1972;32:281-294.
28. Orumiyehei A, Khoramipour K, Kezaei MH, Madadizadeh E, Meymandi MS, Mohammadi F, *et al.* High-intensity interval training-induced hippocampal molecular changes associated with improvement in anxiety-like behavior but not cognitive function in rats with type 2 diabetes. Brain Sci 2022;12:1280.
29. Saberi S, Ataripour M, Khaksari M, Rajizadeh MA, Bejeshk MA, Akhavari M, *et al.* Exercise training improves diabetic renal injury by reducing fetuin-A, oxidative stress and inflammation in type 2 diabetic rats. Heliyon 2024;10:e27749.
30. Li W, Wu J, Zeng Y, Zheng W. Neuroinflammation in epileptogenesis: From pathophysiology to therapeutic strategies. Front Immunol 2023;14:1269241.
31. O'Dell CM, Das A, Wallace Gt, Ray SK, Banik NL. Understanding the basic mechanisms underlying seizures in mesial temporal lobe epilepsy and possible therapeutic targets: A review. J Neurosci Res 2012;90:913-924.
32. Gilyazova I, Asadullina D, Kagirova E, Sikka R, Mustafin A, Ivanova E, *et al.* MiRNA-146a-A key player in immunity and diseases. Int J Mol Sci 2023;24:12767.
33. Aronica E, Fluiter K, Iyer A, Zurolo E, Vreijling J, van Vliet EA, *et al.* Expression pattern of miR-146a, an inflammation-associated microRNA, in experimental and human temporal lobe epilepsy. Eur J Neurosci 2010;31:1100-1107.
34. Iyer A, Zurolo E, Prabowo A, Fluiter K, Spliet WG, van Rijen PC, *et al.* MicroRNA-146a: A key regulator of astrocyte-mediated inflammatory response. PLoS One 2012;7:e44789.
35. Arida RM, Scorza FA, Cavalheiro EA. Favorable effects of physical activity for recovery in temporal lobe epilepsy. Epilepsia 2010;51:76-79.
36. Mota Bibiana C, Kelly Áine M. Exercise alters LPS-induced glial activation in the mouse brain. Neuronal Signal 2020;4:NS20200003.
37. Yu H, Shao M, Luo X, Pang C, So KF, Yu J, Zhang L. Treadmill exercise improves hippocampal neural plasticity and relieves cognitive deficits in a mouse model of epilepsy. Neural Regen Res 2024;19:657-662.