Received: 8 June 2018 | Revised: 8 August 2018 | Accepted: 20 August 2018

DOI: 10.1002/dev.21781

RESEARCH ARTICLE

WILEY Developmental Psychobiology

Effect of early-life inflammation and magnesium sulfate on hyperthermia-induced seizures in infant rats: Susceptibility to pentylenetetrazol-induced seizures later in life

Ehsan Saboory[1,2] | Maryam Ghadimkhani[1] | Shiva Roshan-Milani[1,2] | Leila Derafshpour[1,2] | Sedra Mohammadi[3] | Sina Dindarian[3] | Hozan Mohammadi[3]

[1]Neurophysiology Research Center, Urmia University of Medical Sciences, Urmia, Iran

[2]Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

[3]Student Research Committee, Urmia University of Medical Sciences, Urmia, Iran

Correspondence
Ehsan Saboory and Maryam Ghadimkhani, Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran.
Emails: saboory@umsu.ac.ir, e.saboory@yahoo.com (ES) and ma.ghadimkhani@gmail.com (MG)

Funding information
Research Council of Urmia University of Medical Sciences

Abstract

This study investigated the effect of inflammation and $MgSO_4$ pretreatment on behaviors caused by hyperthermia (HT) and the effect of these interventions on PTZ-induced seizure a week later. In this experimental study, rat pups experienced inflammation on postnatal day 10 (P10). On P18–19, the pups received either saline or $MgSO_4$ then subjected to hyperthermia. On P25–26, PTZ-induced seizure was initiated in the rats. Neonatal inflammation increased the susceptibility to HT-induced seizure. Inflammation and HT increased the susceptibility to PTZ-induced seizure. Pretreatment with $MgSO_4$ before hyperthermia decreased the susceptibility to both HT- and PTZ-induced seizure. Furthermore, calcium and magnesium blood levels significantly decreased compared to control rats. It can be concluded that neonatal inflammation potentiates while pretreatment with $MgSO_4$ attenuates HT-induced seizures. Also, neonatal inflammation and HT potentiate PTZ-induced seizure initiated one week later.

KEYWORDS
hyperthermia, inflammation, magnesium, neonatal, rat, seizure

1 | INTRODUCTION

Febrile seizures (FS) are the most common type of convulsive events among children. Their prevalence has been estimated at 2%–5% in children from the age of 3 months up to 5 years (Hauser, 1994). This type of seizure is accompanied with pyrexia and is not related to intracranial infection or past seizure history, but is correlated with the elevation of body temperature (Gholipoor, Saboory, Roshan-Milani, & Fereidoni, 2013). Studies suggest that FS in childhood, especially if long-lasting, increases the risk of temporal lobe seizures in adulthood (Papierkowski, Mroczkowska-Juchkiewicz, Pawłowska-Kamieniak, & Pasternak, 1999; Rajab et al., 2014). Various environmental and genetic factors, including family history, mother's disease during pregnancy, vaccines, and the elevation of body temperature from causes such as viral and respiratory infections and even hot baths can cause this kind of seizures (Mashimo et al., 2010; Vestergaard et al., 2005). In spite of several studies in this field, the mechanism of FS has not been completely recognized yet.

Studies suggest that inflammatory processes in the central nervous system (CNS) play an important role in the pathophysiology of seizures. Several inflammatory mediators and cytokines facilitate seizures with IL-1β apparently having a more important role than others (Auvin, Shin, Mazarati, & Sankar, 2010). IL-1β acts by increasing the neuronal excitability (Galic, Riazi, &

Abbreviations: CH, control hyperthermia; CNS, central nervous system; CSF, cerebrospinal fluid; FS, febrile seizures; GABA, gamma-aminobutyric acid; HIS, hyperthermia-induced seizures; HT, hyperthermia; IL-1β, interleukin 1 beta; IP, intraperitoneally; LPS, lipopolysaccharides; MHP, magnesium-hyperthermia-PTZ; NH, nonhyperthermia; NMDA, N-methyl-D-aspartic acid; P10, postnatal day 10; PTZ, pentylenetetrazol; TNF-α, tumor necrosis factor alpha.

Pittman, 2012). Findings from clinical practice and from in vivo and in vitro laboratory studies suggest that IL-1β and TNF-α can increase seizure susceptibility and may be involved in epileptogenesis. Molecular mechanisms of these effects include upregulation of glutamatergic transmission and downregulation of GABAergic transmission (Galic et al., 2012; Riazi, Galic, & Pittman, 2010). Omitting the receptor of IL-1β, a higher temperature is needed to induce seizures. Furthermore, the intensity of seizures is increased by the direct injection of IL-1β into the brain. Lipopolysaccharides (LPS) induce the release of IL-1β; and injection of lower doses of LPS causes seizures comparable to kainic acid (Dubé, Brewster, & Baram, 2009; Zheng, Zhang, Luo, & Zhu, 2011). Rats with defects in the expression of IL-1β receptors are more resistant to FS (Mazarati, 2005). The injection of TNF-α antibody into brain ventricles can block the effect of LPS on seizure intensity in adulthood. Thus, the effect of LPS on seizure intensity in adulthood can be associated with TNF-α (Galic et al., 2008).

Magnesium has an inhibitory effect on postsynaptic potentials and decreases the excitability of muscular fibers (Lee, Zhang, & Kwan, 1996). Studies show that the increased entrance of calcium ions into cells increases cell death and tissue degeneration in the CNS (Kristián & Siesjö, 1998). As a result, magnesium may have a protective effect in the CNS by blocking these channels (Heath & Vink, 1997; Hoffman, Marro, McGowan, Mishra, & Delivoria-Papadopoulos, 1994). Meanwhile, IL-1β increases the rate of calcium entrance into cells and excitability by increasing the activity of NMDA channels (Viviani et al., 2003). Magnesium is known as an NMDA channel blocker which decreases the entrance of calcium ions into cells by blocking these channels (Fawcett, Haxby, & Male, 1999). Although there is evidence for the effect of inflammation on seizures (mostly excitatory) in the acute phase during inflammation or immediately after it, its effect on seizure susceptibility a week or more later is not yet clear. These time points were selected because it is likely that a human child experiencing inflammation during the neonatal period experience a seizure later in life (for many reasons, including FS) either very soon after inflammation (e.g., in 2 months) or 2–3 years later. Therefore, this study aimed to evaluate the effect of neonatal inflammation on hyperthermia-induced seizures (HIS) 8–9 days later and the effects of these interventions on PTZ-induced seizures a week later. The present study aimed to find out whether (a) pretreatment with $MgSO_4$ before hyperthermia affects the HIS, (b) the effects of inflammation on HIS are transient during the inflammation or can alter the incidence of seizures later in life, and (c) inflammation at infancy and pretreatment with $MgSO_4$ prior to hyperthermia affects PTZ-induced seizures later in life?

2 | MATERIALS AND METHODS

This study was approved by Medical Ethics Committee at Urmia University of Medical Science, Urmia, Iran. All experimental protocols and procedures were followed according to the guidelines of 1975 Declaration of Helsinki as reflected in the Guidelines of Medical Ethics Committee, Ministry of Health, Iran.

TABLE 1 Classification of groups and interventions on rats subjected to current study

		Interventions		
Groups	**Subgroups**	**P10**	**P18–19**	**P25–26**
Nonhyperthermic =NH, *n* = 8	—	Saline injection 14 ml/kg	Blood sampling	—
Control hyperthermic =CH, *n* = 16	Control–Seizure, *n* = 8	Saline injection 14 ml/kg	Saline injection–hyperthermia induction	PTZ injection–behavior observation
	Control–Blood sampling, *n* = 8	Saline injection 14 ml/kg	Saline injection–hyperthermia induction–blood sampling	
$MgSO_4$=Mg, *n* = 8		Saline injection 14 ml/kg	$MgSO_4$ injection–hyperthermia induction	PTZ injection–behavior observation
High LPS=HLPS, *n* = 16	HLPS–seizure, *n* = 8	LPS injection 400 μg/kg	Saline injection–hyperthermia induction	PTZ injection–behavior observation
	HLPS–Blood sampling, *n* = 8	LPS injection 400 μg/kg	Saline injection–hyperthermia induction–blood sampling	
Moderate LPS=MLPS, *n* = 16	MLPS–seizure, *n* = 8	LPS injection 100 μg/kg	Saline injection–hyperthermia induction	PTZ injection–behavior observation
	MLPS–Blood sampling, *n* = 8	LPS injection 100 μg/kg	Saline injection–hyperthermia induction–blood sampling	
HLPS + Mg, *n* = 8		LPS injection 400 μg/kg	$MgSO_4$ injection–hyperthermia induction	PTZ injection–behavior observation
MLPS + Mg, *n* = 8		LPS injection 100 μg/kg	$MgSO_4$ injection–hyperthermia induction	PTZ injection–behavior observation

2.1 | Animals

Twenty virgin female Wistar rats (12–14 weeks old) were purchased from the animal facility of Urmia University of Medical Sciences. Each female rat was coupled with a male rat in the morning (8 a.m.) and removed the next morning by observing the vaginal plug. Pregnant rats were placed in groups of four per cage (20 × 32 × 43 cm) and kept in standard conditions (12-hr light–dark cycle, light on 7 a.m., 22–24°C, and food and water ad libitum). On the 21th day of pregnancy, each rat was transferred to a separate cage and the same conditions were applied for all of them. The day of maternal delivery was designated as postnatal day 1 (P1). To reduce the effect of unequal litter size on the development and growth of pups, they were culled to eight both male and female pups were retained in each litter. Also, the number of male and female pups was selected equally in order to decrease the effect of sex differentiation.

2.2 | Study groups

On P10, interventions were started and two pups from each dam were selected in each group; All the pups received either saline or lipopolysaccharide (LPS) intraperitoneally (IP) at this time point (P10); on P18 and P19, the pups were randomly divided into seven groups as follows (Table 1):

(1) Nonhyperthermia (NH) (n = 8): on P18 and P19, anesthesia was induced by ether and blood samples were immediately obtained by heart puncture; (2) control hyperthermia (CH) (n = 16): the pups received 14 ml/kg of saline IP; HT was induced 30 min later. After HT, the rats were randomly divided into two subgroups (n = 8 each): rats subjected to blood sampling from the heart immediately after HT and rats subjected to PTZ-induced seizure on P25-P26; (3) magnesium-hyperthermia-PTZ (MHP) (n = 8): Pups received $MgSO_4$ (270 mg/kg, IP) with an equal volume of saline 30 min before the HT. Then, the rats were subjected to PTZ-induced seizure on P25–26; (4 and 5) LPS groups, high dose of LPS (HLPS), and moderate dose of LPS (MLPS): On P10, HLPS and MLPS (n = 16 each) received the LPS of *Escherichia coli* 400 and 100 µg/kg with an equal volume of saline, respectively. HT was induced on P18–19. Then, in half of the rats in each group, blood samples were obtained right after HT. The other half was subjected to PTZ-induced seizure on P25-P26; (6 and 7) Combination of Mg and LPS, HLPS + Mg and MLPS + Mg: LPS was injected (similar to Groups 3 and 4) on P10. On P18–19, before HT, the pups received (270 mg/kg, IP) of $MgSO_4$. All the rats of these groups were subjected to PTZ-induced seizure a week later.

2.3 | Induction of HT

HT-induced seizures were surveyed regardless of sex as a factor. On P18 and P19, HT was induced by hot air current as previously described by Baram, Gerth, and Schultz (1997) and Gholipoor et al. (2013). Hot air was blown from the roof of the chamber at a distance of approximately 40 cm above the rats. The container temperature was monitored near the chamber wall by a digital thermometer. To keep the temperature at the constant interval of 52–56°C, the temperature inside the chamber was regulated at a desired level before the animal's entrance. All the pups were kept in their dam until 30 min prior to the experiment. Each rat was weighed and received an injection of either saline (14 ml/kg) or $MgSO_4$ (270 ml/kg, IP) with equal volumes. Then, the rats were moved to the chamber.

Immediately before and after HT, the core body temperature of the rats was measured by a rectal probe. Behaviors of the rats were monitored and recorded for 25 min and then categorized according to Table 2.

The latency of each behavioral stage was determined, and the total score of seizure was calculated. For example, if a rat showed one behavior from an identical stage, the score for that stage would be considered and, for several behaviors of a stage, the score for that stage would be multiplied by the number of behaviors, and eventually all of the values were summed. In this study, because tonic–clonic seizures were not observed, they were ignored in the calculation of the total score (Gholipoor et al., 2013). For instance, if the rat showed hyperactivity (Stage 1), ataxia (Stage 2), rotational motion (Stage 3), and tremor (Stage 3), the total score would be $1 + 2 + (3 \times 2) = 9$. Total score (TS) = $\sum$(S.NB), where S stands for stage and NB denotes for the number of behaviors in that stage. Latency to Stages 2 and 3

TABLE 2 Hyperthermia behaviors in rats

Stage	Behavior	Description
0	Normal	Normal explorative behavior
1	Hyperactivity	Hyperactive behavior, jumping, and rearing
2	Immobility ataxia	Sudden complete immobility (duration: 3–10 s) Unsteady, jerky gait
3	Circling	Running in tight circles (approx 2 circles/s)
	Shaking	Whole-body shaking
	Clonic seizures	Contractions of hindlimbs and forelimbs with reduced consciousness
4	Tonic–clonic convulsions	Continuous tonic–clonic convulsions

of HT-induced seizures and the TS of seizure among the groups were compared. Unfortunately, due to lack of sufficient instruments, EEG recording was not performed in this study.

2.4 | PTZ-induced seizure

On P25–26 between 8 and 11 a.m., all the rats were weighed, injected with PTZ (45 mg/kg, IP), and transferred to a glass chamber (30 × 30×30 cm) to evaluate their behaviors. The behaviors of each rat were monitored for 90 min. The seizure score of each rat was determined and classified according to the five-stage scale proposed by Racine et al. and Hashemi, Ebrahimi, Saboory, and Roshan-Milani (2013): 0 = normal; 1 = immobilization, sniffing; 2 = head nodding, facial, and forelimb clonus (short myoclonic jerk); 3 = continuous myoclonic jerk, tail rigidity; 4 = generalized limbic seizures with kangaroo posture or violent convulsion; and 5 = continuous generalized seizures (tonic or clonic-tonic convulsions).

Latency to first convulsion behavior, first tonic–clonic seizure, duration and number of tonic–clonic seizures, and TS of seizure (sum of all behavioral stages) were observed and recorded (Saboory, Ebrahimi, Roshan-Milani, & Hashemi, 2015). In addition, in rats which had more than one episode of tonic–clonic seizure, the sum of all behavioral levels was multiplied by the number of tonic–clonic seizures to calculate TS. For instance, if a rat showed the 4th stage of seizure, its seizure score would be measured as 1 + 2+3 + 4=10 and if it showed tonic–clonic seizure (Stage 5) twice, its seizure score would be measured as (1 + 2+3 + 4+5) × 2 = 30. Also, the duration and time of each behavioral stage were recorded for every rat (Gholami, Saboory, & Roshan-Milani, 2014; Saboory, Gholami, Zare, & Roshan-Milani, 2014).

2.5 | Collecting blood samples

Rats that were subjected to blood sampling were anaesthetized with the inhalation of ether. Afterward, blood samples were obtained by direct heart puncture. The samples were centrifuged at 3,000 *g* at 4°C for 15 min. Then, the serum was kept at −80°C until serum concentrations of Ca^{2+}, Mg^{2+}, TNF-α (kit profile), and IL_1-β (kit profile) were measured using ready-to-use ELISA kits.

2.6 | Data analysis

Data distribution was controlled using the Shapiro–Wilk test. Data related to the number of tonic–clonic seizures and latency of tail tremor which were not normally distributed were analyzed using the Kruskal–Wallis test. The rest of the data had normal distributions. Therefore, one-way and two-way analyses of variance (ANOVAs) were employed for multiple comparisons. If necessary, the Tukey post hoc test was used for between-group comparisons. The results were expressed as mean ± *SEM*, and $p < 0.05$ was considered significant.

3 | RESULTS

3.1 | HT behaviors

Results of core body temperature and weight are illustrated in Table 3. Body temperature increased during HT but did not exceed 41°C. In terms of HT behaviors, rats had a normal exploratory behavior after entering the chamber (Stage 0) which quickly changed to hyperactivity. Rats frequently showed jumping and rearing (Stage 1). Then, all activities stopped and rats stayed motionless for a while (Stage 2). Some rats repeated the behaviors of Stage 2 (oral automatism) without showing first-stage behaviors. Most rats had imbalance in motions (Stage 2) or circular motion (Stage 3). Tail tremor was observed in the group that had received LPS. No rat experienced tonic–clonic seizures (Stage 4) or died in HT. Furthermore, behaviors did not necessarily occur in order of staging.

3.2 | Prophylactic effect of $MgSO_4$ on HT-induced seizure on P18–19

To evaluate the effect of $MgSO_4$ on HT-induced seizure, behaviors were compared between CH and Mg groups. Data related to latency to Stages 2 and 3 and the total score of seizure (TS) were

TABLE 3 Mean of body temperature before and after hyperthermia at P18–19 as well as mean body weight at P18–19 and P25–26 in rats

Groups	Base *T* (°C) Mean ± *SE*	Final *T* (°C) Mean ± *SE*	Body weight(g) at P18–19 Mean ± *SE*	Body weight(g) at P25–26 Mean ± *SE*
NH	—	—	27.50 ± 0.75	—
CH	36.02 ± 0.15	40.06 ± 0.18	27.05 ± 0.85	47.18 ± 1.08
$MgSO_4$	36.04 ± 0.21	39.84 ± 0.13	27.83 ± 0.98	48.93 ± 1.75
HLPS	35.97 ± 0.24	39.43 ± 0.11	27.75 ± 1.25	46.75 ± 0.31
MLPS	35.86 ± 0.18	39.63 ± 0.22	27.00 ± 1.38	45.31 ± 1.03
HLPS + Mg	35.93 ± 0.14	39.88 ± 0.16	26.98 ± 1.16	47.00 ± 1.04
MLPS + Mg	35.98 ± 0.12	39.51 ± 0.17	28.50 ± 0.96	48.16 ± 1.32

Notes. CH: control hyperthermia; HLPS: high-dose LPS; MLPS: moderate dose LPS; NH: nonhyperthermia.
There was no significant difference between groups in terms of body T and body weight at the same stage (one-way ANOVA).

analyzed by two-way ANOVA for two factors of LPS and $MgSO_4$. In terms of latency to Stage 2, the effect of LPS ($F(1, 43) = 29.92$, $p < 0.001$) and $MgSO_4$ ($F(1, 43) = 27.07$, $p < 0.001$) was significant. The LPS*$MgSO_4$ interaction was also significant ($F(1, 43) = 13.98$, $p = 0.001$). In terms of latency to Stage 3, the effect of LPS was significant ($F(1, 43) = 33.32$, $p < 0.001$) and $MgSO_4$ ($F(1, 43) = 34.69$, $p < 0.001$) was significant. The LPS*$MgSO_4$ interaction was also significant ($F(1,43) = 6.27$, $p = 0.016$). Moreover, in terms of TS, the effect of LPS ($F(1, 43) = 18.32$, $p < 0.001$) and $MgSO_4$ ($F(1, 43) = 19.91$, $p < 0.001$) was significant, but the LPS*$MgSO_4$ interaction was not. To compare these seizure behaviors within experimental groups, baseline ANOVA and Tukey's post hoc test were performed. Latency to Stages 2 and 3 of seizure was significantly higher ($p = 0.002$, one-way ANOVA and Tukey) in the Mg group than in the CH group (Figure 1). Furthermore, the TS of seizure was significantly lower in the Mg group compared with the CH group (Figure 2).

3.3 | Effect of neonatal inflammation and pretreatment with $MgSO_4$ on HT-induced seizure

Latency to Stage 3 in HLPS + Mg and MLPS + Mg groups significantly increased ($p = 0.01$) compared with HLPS and MLPS groups (Figure 1). Also, the TS of seizure was significantly higher in HLPS and MLPS groups than HLPS + Mg and MLPS + Mg groups ($p = 0.01$ and $p = 0.03$, respectively) (Figure 2).

Tail tremor was observed only in LPS groups. This behavior consisted of continuous and intense shaking of the tail which appeared after placing the rats in the HT chamber. Pretreatment with $MgSO_4$ significantly increased latency to tail tremor in relevant groups (Figure 3).

3.4 | Effect of HT on blood calcium and magnesium levels

Data related to blood Ca^{2+}, Mg^{2+}, IL1-β, and TNF-α-α levels were analyzed by two-way ANOVA for two factors of LPS and hyperthermia.

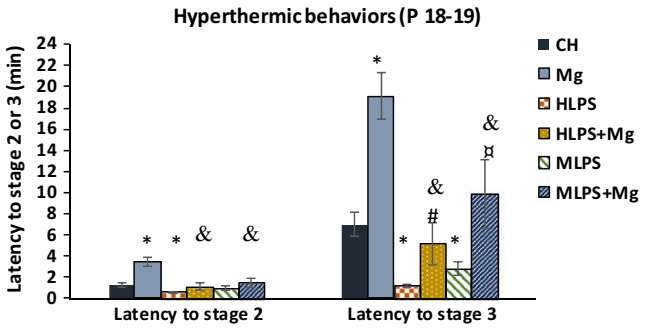

FIGURE 1 Latency to stages 2 and 3 of HT-induced seizure in control hyperthermia, $MgSO_4$, high- and moderate-dose LPS, HLPS + Mg, and MLPS + Mg groups was analyzed by one-way ANOVA and Tukey tests; *significant difference with control hyperthermia ($p < 0.05$); #significant difference with HLPS; ¤significant difference with MLPS ($p = 0.01$); and &significant difference with Mg group ($p < 0.04$)

In terms of Mg^{2+}, the effect of LPS ($F(1, 26) = 6.7$, $p = 0.016$) and that of hyperthermia ($F(1, 26) = 15.86$, $p < 0.001$) were significant. However, the LPS*hyperthermia interaction was not significant. In terms of Ca^{2+}, the effect of hyperthermia was significant ($F(1, 26) = 6.88$, $p = 0.014$) while that of LPS and the LPS*hyperthermia interaction were not. To compare blood Ca^{2+} and Mg^{2+} levels within experimental groups, baseline ANOVA and Tukey's post hoc test were performed. HT decreased blood Ca^{2+} and Mg^{2+} levels in all the groups but some decreases were insignificant (Figure 4).

3.5 | Effect of HT on blood IL1-β and TNF-α-α levels

IL1-β and TNF-α-α were compared in NH, CH, HLPS, and MLPS groups. In terms of TNF-α-α, data analysis by two-way ANOVA (LPS and hyperthermia) revealed that the effect of hyperthermia was significant ($F(1, 20) = 4.6$, $p = 0.044$) while that of LPS and the LPS*hyperthermia

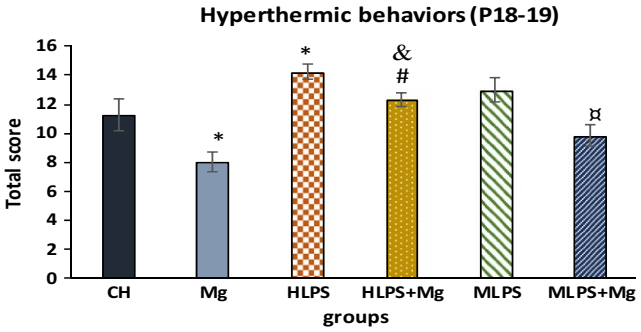

FIGURE 2 Total score of HT-induced seizure in control hyperthermia and $MgSO_4$, high- and moderate-dose LPS, HLPS + Mg, and MLPS + Mg groups was analyzed by one-way ANOVA and Tukey tests; *significant difference with CH; #significant difference with HLPS ($p = 0.01$); ¤significant difference with MLPS ($p = 0.03$); and &significant difference with Mg group ($p < 0.01$)

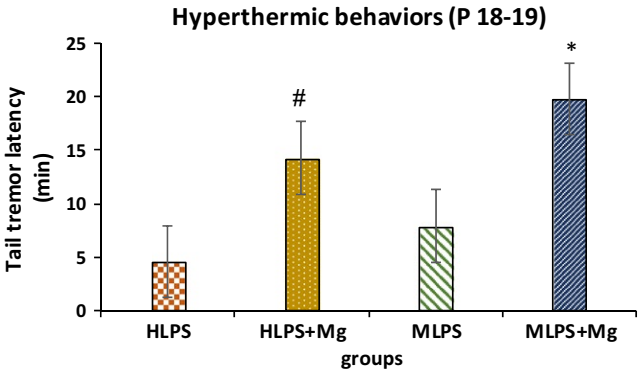

FIGURE 3 Latency to tail tremor behavior in 18- to 19-day-old rats which experienced inflammation at P10; #significant difference with HLPS ($p < 0.01$); *significant difference with MLPS ($p < 0.02$). There was no significant difference between high- and moderate-dose LPS (HLPS and MLPS) groups (Kruskal–Wallis and all pairwise comparisons)

interaction were not. In terms of IL1-β, no significant effect was detected. In order to compare blood TNF-α-α levels within experimental groups, baseline ANOVA and Tukey's post hoc test were conducted. The induction of HT decreased TNF-α-α compared with the NH group. There was a significant difference in TNF-α-α level between NH and CH groups ($p = 0.04$). The difference between NH and both LPS groups was also significant ($p = 0.008$) (Figure 5).

3.6 | PTZ-induced seizure on P25–26

After the injection of PTZ, the behaviors of rats were accurately monitored. The movements of some rats were primarily slow, and some had no movements (Stage 1). In addition, some others had unintentional head movements (Stage 2). Another commonly observed behavior in rats was the sudden opening of legs and arms. This behavior was considered as Stage 3. Also, the stiffening of the tail and face or anterior limb clonus (Stage 3) was observed in some rats. Some rats entered Stage 4 which includes severe seizure and unintentional jumps, while some entered Stage 5 which encompasses steady tonic–clonic seizure and loss of consciousness. The rats did not necessarily demonstrate all the stages in the mentioned order. Sometimes a rat entered a stage without entering the previous one or demonstrated a stage for several times. Tonic–clonic seizures were not seen in some rats and occurred more than once in some others. None of the rats died at the time of or after treatment with PTZ.

3.6.1 | Latency of first seizure behavior

Data analysis by two-way ANOVA (LPS and $MgSO_4$) revealed that the effect of LPS ($F(1, 43) = 11.6$, $p = 0.001$) and that of $MgSO_4$ ($F(1, 43) = 39.7$, $p < 0.001$) were significant. Moreover, the LPS*$MgSO_4$ interaction was significant ($F(1, 43) = 7.07$, $p = 0.011$). To compare this variable within experimental groups, baseline ANOVA and Tukey's post hoc test were conducted. Pretreatment with $MgSO_4$ before HT increased the latency to first seizure behavior (Figure 6).

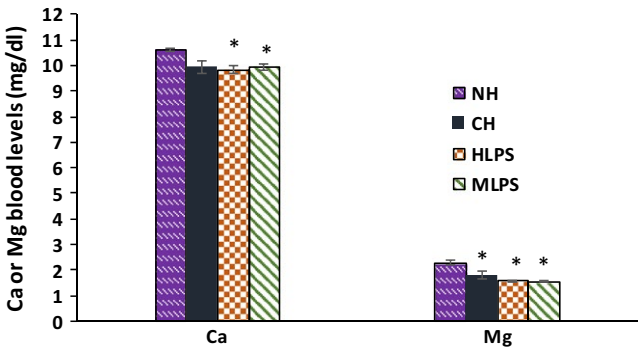

FIGURE 4 Comparison of calcium and magnesium blood levels in nonhyperthermia, control hyperthermia, high LPS, and Moderate LPS groups: *significant difference with NH group ($p < 0.05$, one-way ANOVA and Tukey)

3.6.2 | Latency to first tonic–clonic seizure

Based on the data analysis by two-way ANOVA (LPS and $MgSO_4$), the effect of $MgSO_4$ was significant ($F(1, 43) = 6.17$, $p = 0.017$). Nevertheless, the effect of LPS and the LPS*$MgSO_4$ interaction were not significant. Baseline ANOVA and Tukey's post hoc test were performed in order to compare this variable within experimental groups. Latency to first tonic–clonic seizure was lower in LPS groups than the CH group but it was not statistically significant. Pretreatment with $MgSO_4$ significantly increased latency to first tonic–clonic seizure compared with relevant control groups (Figure 7).

3.6.3 | Duration of tonic–clonic (TC) seizure

Data related to the duration of TC seizure were analyzed by two-way ANOVA for two factors of LPS and $MgSO^4$. The effect of $MgSO_4$ was significant ($F(1, 43) = 5.4$, $p = 0.025$). However, the effect of LPS and the LPS*$MgSO_4$ interaction were not significant. Baseline ANOVA and Tukey's post hoc test were performed to compare the duration of TC within experimental groups. The duration of TC seizure was significantly higher in the HLPS group than the CH group. Pretreatment with $MgSO_4$ significantly reduced the duration of TC seizure in all groups compared to relevant controls (Figure 8).

3.6.4 | Number of TC seizure

Data analysis by the Kruskal–Wallis test indicated that the Mg group had the least and the HLPS group had the most frequent TC seizures (Figure 9).

3.6.5 | Total score of PTZ-induced seizure

Based on the data analysis by two-way ANOVA (LPS and $MgSO_4$), the effect of LPS ($F(1, 43) = 8.6$, $p = 0.005$) and that of $MgSO_4$ ($F(1,$

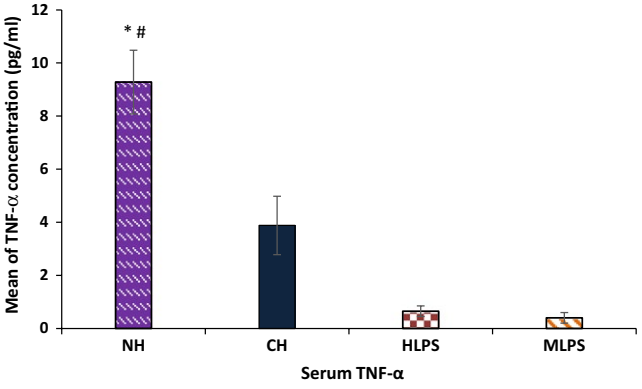

FIGURE 5 TNF-α-α serum level in 18- to 19-day-old rats that experienced an acute inflammation at P10; *significant difference with CH ($p = 0.04$); #significant difference with HLPS and MLPS groups ($p < 0.008$). Baseline analysis was performed by one-way ANOVA and Tukey

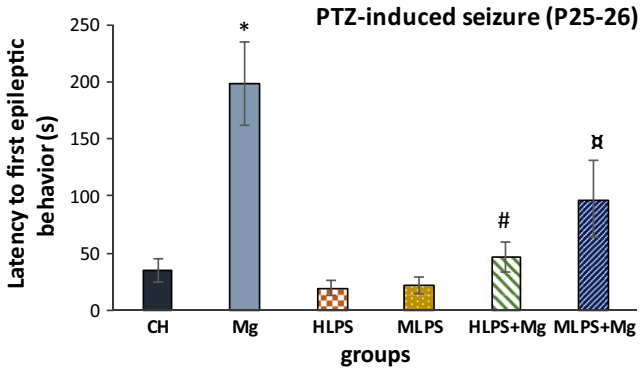

FIGURE 6 Latency to first PTZ-induced seizure in 25- to 26-day-old rats that experienced inflammation at P10 and HT at P18–19; *significant difference with CH (p = 0.001); #significant difference with HLPS (p = 0.04); and ¤significant difference with MLPS (p = 0.03). There was no significant difference between CH and LPS groups (one-way ANOVA and Tukey)

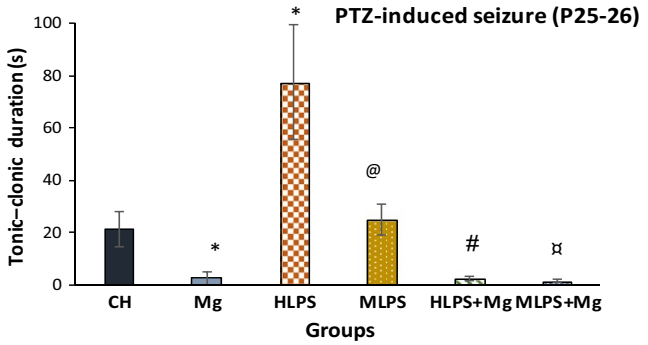

FIGURE 8 Tonic–clonic seizure duration in 25- to 26-day-old rats that experienced inflammation at P10 and HT at P18–19; *significant decrease (p = 0.02) in Mg group and a significant increase (p = 0.04) in HLPS group compared with CH group; #significant difference with HLPS; ¤significant decrease compared with MLPS (p = 0.04); @significant difference (p = 0.049) with high-dose HLPS. Data were analyzed by one-way ANOVA and Tukey tests

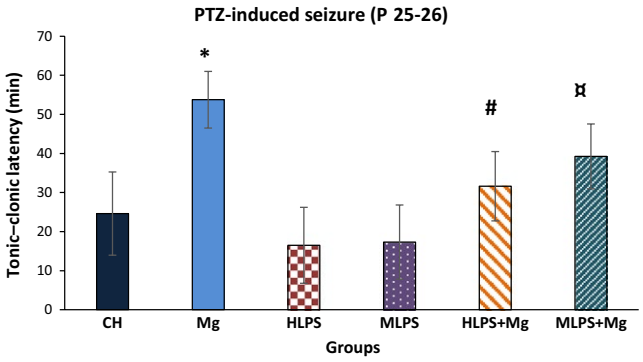

FIGURE 7 Latency to first tonic–clonic seizure in 25- to 26-day-old rats that experienced inflammation at P10 and HT at P18–19; *significant difference (p = 0.04) with CH group; #significant difference with HLPS (p < 0.05); and ¤significant difference with MLPS (p = 0.03). Data were analyzed by one-way ANOVA and Tukey test

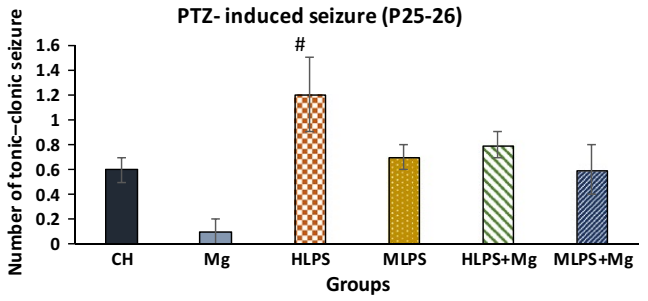

FIGURE 9 Number of PTZ-induced tonic–clonic seizure in 25- to 26-day-old rats that experienced inflammation at P10 and HT at P18–19; *significant difference with CH group (p = 0.035); and #p = 0.027 with Mg group. Pretreatment with $MgSO_4$ in inflammation groups insignificantly decreased number of seizures (Kruskal–Wallis and all pairwise comparisons)

43) = 14.1, p = 0.001) were significant, while the LPS*$MgSO_4$ interaction was not. Baseline analysis by one-way ANOVA showed that pretreatment with $MgSO_4$ significantly decreased while severe inflammation in neonatal period significantly increased the TS of seizure compared with the CH group. Furthermore, the difference between MLPS and HLPS groups and the difference between Mg group and both HLPS + Mg and MLPS + Mg groups were significant (Figure 10).

4 | DISCUSSION

In this study, rat pups were studied for early-life inflammation and the effect of $MgSO_4$ on HT- and PTZ-induced seizures. Blood samples were obtained to measure Ca^{2+}, Mg^{2+}, IL1β, and TNF-α-α concentrations. Findings of this study indicated that neonatal inflammation increased the susceptibility to HT-induced seizure. Inflammation and HT potentiated the PTZ-induced seizure, while pretreatment with $MgSO_4$ attenuated the susceptibility to both hyperthermia- and PTZ-induced seizure. In addition, both neonatal inflammation and HT reduced Ca^{2+}, Mg^{2+}, and TNF-α-α concentrations.

4.1 | Effect of $MgSO_4$ on HT-induced seizure

It is reported that the magnesium level in CSF does not change significantly up to 4 hr after receiving $MgSO_4$ (Brewer, Parra, Borel, Hopkins, & Reynolds, 2001). In one study, the effect of $MgSO_4$ on brain defects caused by ischemia was investigated in rats. Results suggested that the complications of hypoxic ischemia were significantly decreased in the group receiving $MgSO_4$ in the first hour, while the effect was not significant 2 hr later (Spandou et al., 2007). Fuchs-Buder, Tramer and Tassonyi (1997) reported that CSF Mg^{2+} level was moderately increased 30 min after receiving $MgSO_4$ in patients who had undergone anesthesia and received $MgSO_4$ (60 mg/kg, IV) in bolus form.

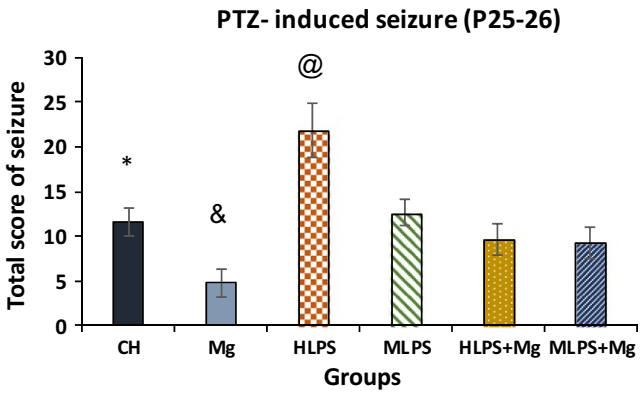

FIGURE 10 Total score of PTZ-induced seizure in 25- to 26-day-old rats that experienced inflammation at P10 and HT at P18–19; *significant difference with Mg ($p = 0.01$) and HLPS ($p = 0.02$) groups; @significant difference with MLPS ($p = 0.04$) and HLPS + Mg ($p = 0.001$); and &significant difference with HLPS + Mg ($p = 0.03$) and MLPS + Mg ($p < 0.05$). Data were analyzed by one-way ANOVA and Tukey tests

It was also gradually increased up to 240 min after $MgSO_4$ injection. Moreover, $MgSO_4$ causes a decrease in the complications of preeclampsia and an increase in seizure threshold in rats with preeclampsia by decreasing neuronal inflammation and keeping CSF permeability constant (Johnson et al., 2014). The main mechanism of the effects of $MgSO_4$ on CNS is not clearly understood. However, Mg^{2+} is known as an NMDA receptor blocker which decreases the entrance of calcium into cells and depresses synaptic transmission (Chou et al., 2016). The entrance of calcium into cells has been known as a potential reason for FS. $MgSO_4$ may decrease HT-induced seizures by decreasing calcium entrance into cell (Radzicki et al., 2013) or depressing synaptic transmission. In the majority of preeclampsia patients, pretreatment with $MgSO_4$ can control the disease, prevent the occurrence of eclampsia, or decrease its complications (Hallak, Kupsky, Hotra, & Evans, 1999; Isler, Barrilleaux, Rinehart, Magann, & Martin, 2002; Johnson et al., 2014). In a case report study, the febrile seizure (status epilepticus) was successfully controlled when lidocaine plus $MgSO_4$ was introduced in a 12-year-old girl (Chou et al., 2016).

Our study supports the findings of previous studies in this context as pretreatment with $MgSO_4$ significantly attenuated HT-induced seizures (Ghadimkhani, Saboory, Roshan-Milani, Mohammdi, & Rasmi, 2016). According to the findings of current and previous studies (Ebrahimi, Saboory, Roshan-Milani, & Hashemi, 2014; Gholami & Saboory, 2013; Rowley, Martin, & Marsden, 1995), the level of blood Mg^{2+} significantly decreases in FS and HT and the injection of $MgSO_4$ restores blood Mg^{2+} level. Thus, the correction of blood Mg^{2+} level in FS may be an appropriate method for controlling FS.

4.2 | Effect of neonatal inflammation on HT-induced seizure

Several studies have been carried out to detect the correlation between seizure and inflammation. It is reported that early-life inflammation increases the probability of adulthood seizure (Galic et al., 2008). A study by Eun et al. indicated that the injection of LPS prior to HT increases the severity of FS (Eun, Abraham, Mlsna, Kim, & Koh, 2015). Most previous studies have tested the simultaneous effect of inflammation on FS. They all suggested that inflammation and inflammatory factors have an excitatory effect on FS. Our study was somewhat different from previous studies as we tested the delayed effect of inflammation on HT-induced seizure. The result of the current study indicated that neonatal inflammation (on P10) dose-dependently increased the severity of HT-induced seizures on P19. Our results are consistent with those of others in this context.

4.3 | Effect of HT on blood Ca^{2+} and Mg^{2+} levels

It is reported that Mg^{2+} serum levels are significantly lower in children with FS than healthy children with the same age and weight, and that Ca^{2+} levels remain almost constant (Amouian, Mohammadian, Behnampour, & Tizrou, 2013). However, another study showed a significant decrease in both Mg^{2+} and Ca^{2+} serum levels in children suffering from FS compared with healthy children with the same age (Akbayram et al., 2012). In our study, Ca^{2+} level in the CH group had no significant difference with that of the NH group. In all groups, Mg^{2+} levels significantly decreased compared to the NH group. The mechanism of low Mg^{2+} serum level in FS is yet unknown.

Previously, it was stated that Mg^{2+} has antiepileptic and neuroprotective effects on the nervous system and prevents Ca^{2+} from entering the cell by blocking NMDA channels (Chou et al., 2016). It is likely that the decrease in Mg^{2+} in blood is one of the reasons for the elevation of CNS irritability and increase in Ca^{2+} entrance to cells in HT and PLS groups, thereby causing severe HT-induced seizures. Furthermore, in the present study, serum Ca^{2+} levels were considerably lower in LPS groups than NH rats. This might be, at least in part, the reason for the high intensity of HT-induced seizures in these groups.

4.4 | Effect of HT on serum IL1-β and TNF-α level

It is reported that the IL1-β level in children with fever is not different across patients with or without seizure (Behmanesh, Ashrafzadeh, Varasteh, Shakeri, & Shahsavand, 2012). The injection of IL1-β in brain ventricles potentiated FS (Heida & Pittman, 2005), while the lack of or defect in IL1-β receptors decreases the probability of FS (Dubé, Vezzani, Behrens, Bartfai, & Baram, 2005). In one study, LPS was injected to rats 2.5 hr prior to HT. After that, TNF-α and IL1-β levels were measured 3.5 and 24 hr after LPS injection. Results suggested that TNF-α and IL1-β levels increased in the first hour after HT induction. Findings of this study are not consistent with those of ours. In our study, the injection of LPS was performed 8–9 days prior to HT. Our study differed from others in this regard. In the above-mentioned study, the level of TNF-α and IL1-β decreased as time passed, reaching zero after 24 hr (It could not be detected with the used kits). In this aspect, our findings are similar to the noted finding. Moreover, the expression levels

of transient receptor potential vanilloid 1 (TRPV1) increases after FS in mice; also, TRPV1 activation results in a significant elevation in the expression of pro-inflammatory cytokines such as IL-1β and TNF-α-α in the hippocampus and cortex (Huang et al., 2015). The study conducted by Dube et al. to measure the level of inflammatory factors suggested that the level of TNF-α and IL1-β increased 24 hr after HT, contradicted by our findings (Dubé et al., 2010). In the present study, the level of TNF-α and IL1-β was lower in the HT group than the NH group. What makes our study different from other studies and can have been the cause of different results is that, in most studies, the effects of inflammatory factors have been investigated on FS and/or HT (not the effect of HT on inflammatory factors), while we checked the effects of HT on inflammatory factors. We recommend further studies in this field.

4.5 | The effect of pretreatment with $MgSO_4$ prior to HT on PTZ-induced seizure later in life

Previous studies showed the role of FS in increasing the probability of adulthood seizure (Bender, Dubé, & Baram, 2004; Dubé et al., 2012, 2010). The neuroprotective effects of $MgSO_4$ on CNS and its role in decreasing the intellectual disability of preterm infants have been described (Conde-Agudelo & Romero, 2009). A mechanism of FS induction is the entrance of calcium into cells (Radzicki et al., 2013). The uncontrolled entrance of calcium into cells can lead to cell death (Kristián & Siesjö, 1998). It is likely that blocking NMDA channels using $MgSO_4$ should prevent cell death in HT by decreasing the entrance of calcium into cells which, in turn, might lead to the decreased intensity of seizures later in life. Seizure-induced brain damage has been shown in several seizure models (Domachevsky et al., 2012; Holmes, 1991; Meldrum, 1997). The increased entrance of calcium into cells (or other mechanisms) in HT may increase cell excitability, leading to the increased intensity of HT-induced seizures. This increased severity can lead to cell damage and/or death in brain tissues, raising the probability of seizures later in life. It can be concluded that pretreatment with $MgSO_4$ in HT might decrease cell damage and/or death at the time of HT and also decrease HT-induced seizures, thereby reducing the probability of seizures later in life.

4.6 | The effect of inflammation and HT on PTZ-induced seizure

Previous studies have suggested an excitatory effect for inflammation on seizures at the same time (Auvin et al., 2010; Srivastava, Dixit, Banerjee, Tripathi, & Chandra, 2016) and the increase in seizure susceptibility in adulthood (Galic et al., 2008). It has also been stated that FS in childhood increases the probability of seizure in adulthood (Dubé et al., 2012; McClelland, Dubé, Yang, & Baram, 2011). In the present study, inflammation was induced on P10 and HT on P18–19 which can be conceptualized as early and late infancy, respectively. Then, PTZ-induced seizure was elicited on P25–26. In fact, PTZ-induced seizure occurred after two interventions having an excitatory effect on seizure susceptibility in adulthood. In most previous studies, the time gap between the induction of inflammation and/or FS and the time point for checking seizure susceptibility was longer than that of our study. Also, we studied the concomitant effects of infantile inflammation and HT on PTZ-induced seizure. These were the differences between our study and previous ones. The result of the present study is consistent with that of others. However, the synergistic effect of these two interventions on PTZ-induced seizures was revealed in the present study. The present study could be conducted with, at least, one more group of rats which would receive LPS on P10, without HT on P18, and would be subjected to PTZ-induced seizure on P25. However, this group was not included due to time and budget constraints.

5 | CONCLUSION

It can be concluded that inflammation during infancy increases both HT-induced seizures in later infancy and seizure susceptibility at older ages. Pretreatment with $MgSO_4$ can decrease both HT-induced seizures at the same time and seizure intensity later in life. The probable mechanism of these effects may be, at least in part, due to the HT-induced decrease in magnesium and calcium levels which can be compensated by $MgSO_4$ administration. Meanwhile, decreased blood IL1-β and TNF-α-α levels might be one reason for the increased seizure intensity caused by infantile inflammation.

ACKNOWLEDGMENT

This study was supported by the Research Council of Urmia University of Medical Sciences, Urmia, Iran.

CONFLICT OF INTEREST

The authors have no conflict of interests to declare regarding the study described in this article and the preparation of the article.

AUTHORS CONTRIBUTIONS

ES and MG designed the study, implemented the experiments, analyzed the data, and drafted the manuscript. SR-M, LD, SM, SD, and HM analyzed the data and drafted the manuscript.

ORCID

Ehsan Saboory http://orcid.org/0000-0003-4777-4751

REFERENCES

Akbayram, S., Cemek, M., Büyükben, A., Aymelek, F., Karaman, S., Yilmaz, F., … Caksen, H. (2012). Major and minor bio-element status in children with febrile seizure. *Bratislavske Lekarske Listy*, *113*, 421–423. https://doi.org/10.4149/BLL_2012_095

Amouian, S., Mohammadian, S., Behnampour, N., & Tizrou, M. (2013). Trace elements in febrile seizure compared to febrile children admitted to an academic hospital in Iran, 2011. *Journal of Clinical and Diagnostic Research*, *7*, 2231. https://doi.org/10.7860/JCDR/2013/5548.3478

Auvin, S., Shin, D., Mazarati, A., & Sankar, R. (2010). Inflammation induced by LPS enhances epileptogenesis in immature rat and may be partially reversed by IL1RA. *Epilepsia*, *51*, 34–38. https://doi.org/10.1111/j.1528-1167.2010.02606.x

Baram, T. Z., Gerth, A., & Schultz, L. (1997). Febrile seizures: An appropriate-aged model suitable for long-term studies. *Developmental Brain Research*, *98*, 265–270. https://doi.org/10.1016/S0165-3806(96)00190-3

Behmanesh, F., Ashrafzadeh, F., Varasteh, A., Shakeri, A., & Shahsavand, S. (2012). Evaluation of Interleukin 1 [Beta] in Febrile Convulsion. *Iranian Journal of Allergy, Asthma and Immunology*, *11*, 336.

Bender, R. A., Dubé, C., & Baram, T. Z. (2004). Febrile seizures and mechanisms of epileptogenesis: Insights from an animal model. In D. K. Binder, & H. E. Scharfman (Eds.), *Recent advances in epilepsy research* (pp. 213–225). Boston, MA: Springer.

Brewer, R. P., Parra, A., Borel, C. O., Hopkins, M. B., & Reynolds, J. D. (2001). Intravenous magnesium sulfate does not increase ventricular CSF ionized magnesium concentration of patients with intracranial hypertension. *Clinical Neuropharmacology*, *24*, 341–345. https://doi.org/10.1097/00002826-200111000-00005

Chou, I. C., Lai, H. C., Tsai, F. J., Chang, Y. T., Lin, S. S., Hong, S. Y., & Lee, I. C. (2016). Marked improvement in febrile infection-related epilepsy syndrome after lidocaine plus $MgSO_4$ treatment in a 12-year-old girl. *Epilepsy & Behavior Case Reports*, *6*, 6–9.

Conde-Agudelo, A., & Romero, R. (2009). Antenatal magnesium sulfate for the prevention of cerebral palsy in preterm infants less than 34 weeks' gestation: A systematic review and metaanalysis. *American Journal of Obstetrics and Gynecology*, *200*, 595–609. https://doi.org/10.1016/j.ajog.2009.04.005

Domachevsky, L., Pick, C. G., Arieli, Y., Krinsky, N., Abramovich, A., & Eynan, M. (2012). Do hyperbaric oxygen-induced seizures cause brain damage? *Epilepsy Research*, *100*, 37–41. https://doi.org/10.1016/j.eplepsyres.2012.01.004

Dubé, C. M., Brewster, A. L., & Baram, T. Z. (2009). Febrile seizures: Mechanisms and relationship to epilepsy. *Brain and Development*, *31*, 366–371. https://doi.org/10.1016/j.braindev.2008.11.010

Dubé, C. M., McClelland, S., Choy, M., Brewster, A. L., Noam, Y., & Baram, T. Z. (2012). Fever, febrile seizures and epileptogenesis. In J. L. Noebels, M. Avoli, M. A. Rogawski, R. W. Olsen, & A. V. Delgado-Escueta. *Jasper's basic mechanisms of the epilepsies* (pp. 497–512). Bethesda, MD: National Center for Biotechnology Information (US).

Dubé, C. M., Ravizza, T., Hamamura, M., Zha, Q., Keebaugh, A., Fok, K., … Vezzani, A. (2010). Epileptogenesis provoked by prolonged experimental febrile seizures: Mechanisms and biomarkers. *Journal of Neuroscience*, *30*, 7484–7494.

Dubé, C., Vezzani, A., Behrens, M., Bartfai, T., & Baram, T. Z. (2005). Interleukin-1β contributes to the generation of experimental febrile seizures. *Annals of Neurology*, *57*, 152–155. https://doi.org/10.1002/ana.20358

Ebrahimi, L., Saboory, E., Roshan-Milani, S., & Hashemi, P. (2014). Effect of prenatal forced-swim stress and morphine co-administration on pentylentetrazol-induced epileptic behaviors in infant and prepubertal rats. *Developmental Psychobiology*, *56*, 1179–1186. https://doi.org/10.1002/dev.21198

Eun, B. L., Abraham, J., Mlsna, L., Kim, M. J., & Koh, S. (2015). Lipopolysaccharide potentiates hyperthermia-induced seizures. *Brain and Behavior*, *5*, e00348. https://doi.org/10.1002/brb3.348

Fawcett, W., Haxby, E., & Male, D. (1999). Magnesium: Physiology and pharmacology. *British Journal of Anaesthesia*, *83*, 302–320. https://doi.org/10.1093/bja/83.2.302

Fuchs-Buder, T., Tramer, M., & Tassonyi, E. (1997). Cerebrospinal fluid passage of intravenous magnesium sulfate in neurosurgical patients. *Journal of Neurosurgical Anesthesiology*, *9*, 324–328. https://doi.org/10.1097/00008506-199710000-00006

Galic, M. A., Riazi, K., Heida, J. G., Mouihate, A., Fournier, N. M., Spencer, S. J., … Pittman, Q. J. (2008). Postnatal inflammation increases seizure susceptibility in adult rats. *Journal of Neuroscience*, *28*, 6904–6913. https://doi.org/10.1523/JNEUROSCI.1901-08.2008

Galic, M. A., Riazi, K., & Pittman, Q. J. (2012). Cytokines and brain excitability. *Frontiers in Neuroendocrinology*, *33*, 116–125. https://doi.org/10.1016/j.yfrne.2011.12.002

Ghadimkhani, M., Saboory, E., Roshan-Milani, S., Mohammdi, S., & Rasmi, Y. (2016). Effect of magnesium sulfate on hyperthermia and pentylen-tetrazol-induced seizure in developing rats. *Iranian Journal of Basic Medical Sciences*, *19*, 608–614.

Gholami, M., & Saboory, E. (2013). Morphine exposure induces age-dependent alterations in pentylenetetrazole-induced epileptic behaviors in prepubertal rats. *Developmental Psychobiology*, *55*, 881–887. https://doi.org/10.1002/dev.21080

Gholami, M., Saboory, E., & Roshan-Milani, S. (2014). Proconvulsant effects of tramadol and morphine on pentylenetetrazol-induced seizures in adult rats using different routes of administration. *Epilepsy & Behavior 36*, 90–96.

Gholipoor, P., Saboory, E., Roshan-Milani, S., & Fereidoni, J. (2013). Effect of hyperthermia on histamine blood level and convulsive behavior in infant rats. *Epilepsy & Behavior*, *29*, 269–274. https://doi.org/10.1016/j.yebeh.2013.07.026

Hallak, M., Kupsky, W. J., Hotra, J. W., & Evans, J. B. (1999). Fetal rat brain damage caused by maternal seizure activity: Prevention by magnesium sulfate. *American Journal of Obstetrics and Gynecology*, *181*, 828–834. https://doi.org/10.1016/S0002-9378(99)70309-1

Hashemi, P., Ebrahimi, L., Saboory, E., & Roshan-Milani, S. (2013). Effect of restraint stress during gestation on pentylenetetrazol-induced epileptic behaviors in rat offspring. *Iran J Basic Med Sci*, *16*, 979–984.

Hauser, W. A. (1994). The prevalence and incidence of convulsive disorders in children. *Epilepsia*, *35*, S1–S6. https://doi.org/10.1111/j.1528-1157.1994.tb05932.x

Heath, D. L., & Vink, R. (1997). Magnesium sulphate improves neurologic outcome following severe closed head injury in rats. *Neuroscience Letters*, *228*, 175–178. https://doi.org/10.1016/S0304-3940(97)00394-7

Heida, J. G., & Pittman, Q. J. (2005). Causal links between brain cytokines and experimental febrile convulsions in the rat. *Epilepsia*, *46*(12), 1906–1913.

Hoffman, D. J., Marro, P. J., McGowan, J. E., Mishra, O. P., & Delivoria-Papadopoulos, M. (1994). Protective effect of MgSO 4 infusion on NMDA receptor binding characteristics during cerebral cortical hypoxia in the newborn piglet. *Brain Research*, *644*, 144–149. https://doi.org/10.1016/0006-8993(94)90357-3

Holmes, G. (1991). Do seizures cause brain damage? *Epilepsia*, *32*, S14–S28.

Huang, W. X., Yu, F., Sanchez, R. M., Liu, Y. Q., Min, J. W., Hu, J. J., … Peng, B. W. (2015). TRPV1 promotes repetitive febrile seizures by pro-inflammatory cytokines in immature brain. *Brain Behavior, and Immunity*, *48*, 68–77. https://doi.org/10.1016/j.bbi.2015.01.017

Isler, C., Barrilleaux, P., Rinehart, B., Magann, E., & Martin, J. (2002). Repeat postpartum magnesium sulfate administration for seizure prophylaxis: Is there a patient profile predictive of need for additional therapy? *Journal of Maternal-Fetal & Neonatal Medicine*, *11*, 75–79. https://doi.org/10.1080/jmf.11.2.75.79

Johnson, A. C., Tremble, S. M., Chan, S.-L., Moseley, J., LaMarca, B., Nagle, K. J., & Cipolla, M. J. (2014). Magnesium sulfate treatment reverses seizure susceptibility and decreases neuroinflammation in a rat model of severe preeclampsia. *PloS One*, *9*, e113670. https://doi.org/10.1371/journal.pone.0113670

Kristián, T., & Siesjö, B. K. (1998). Calcium in ischemic cell death. *Stroke*, *29*, 705–718.

Lee, C., Zhang, X., & Kwan, W. (1996). Electromyographic and mechanomyographic characteristics of neuromuscular block by magnesium sulphate in the pig. *British Journal of Anaesthesia*, *76*, 278–283. https://doi.org/10.1093/bja/76.2.278

Mashimo, T., Ohmori, I., Ouchida, M., Ohno, Y., Tsurumi, T., Miki, T., … Takizawa, A. (2010). A missense mutation of the gene encoding voltage-dependent sodium channel (Nav1. 1) confers susceptibility to febrile seizures in rats. *Journal of Neuroscience*, *30*, 5744–5753. https://doi.org/10.1523/JNEUROSCI.3360-09.2010

Mazarati, A. M. (2005). Cytokines: A link between fever and seizures. *Epilepsy Currents*, *5*, 169. https://doi.org/10.1111/j.1535-7511.2005.00053.x

McClelland, S., Dubé, C. M., Yang, J., & Baram, T. Z. (2011). Epileptogenesis after prolonged febrile seizures: Mechanisms, biomarkers and therapeutic opportunities. *Neuroscience Letters*, *497*, 155–162. https://doi.org/10.1016/j.neulet.2011.02.032

Meldrum, B. (1997). First Alfred Meyer Memorial Lecture. Epileptic brain damage: A consequence and a cause of seizures. *Neuropathology and Applied Neurobiology*, *23*, 185–202. https://doi.org/10.1111/j.1365-2990.1997.tb01201.x

Papierkowski, A., Mroczkowska-Juchkiewicz, A., Pawłowska-Kamieniak, A., & Pasternak, K. (1999). Magnesium and zinc levels in blood serum and cerebrospinal fluid in children with febrile convulsions. *Polski Merkuriusz Lekarski: Organ Polskiego Towarzystwa Lekarskiego*, *6*, 138–140.

Radzicki, D., Yau, H.-J., Pollema-Mays, S. L., Mlsna, L., Cho, K., Koh, S., & Martina, M. (2013). Temperature-sensitive Cav1. 2 calcium channels support intrinsic firing of pyramidal neurons and provide a target for the treatment of febrile seizures. *Journal of Neuroscience*, *33*, 9920–9931. https://doi.org/10.1523/JNEUROSCI.5482-12.2013

Rajab, E., Abdeen, Z., Hassan, Z., Alsaffar, Y., Mandeel, M., Al Shawaaf, F., … Kamal, A. (2014). Cognitive performance and convulsion risk after experimentally-induced febrile-seizures in rat. *International Journal of Developmental Neuroscience*, *34*, 19–23. https://doi.org/10.1016/j.ijdevneu.2014.01.001

Riazi, K., Galic, M. A., & Pittman, Q. J. (2010). Contributions of peripheral inflammation to seizure susceptibility: Cytokines and brain excitability. *Epilepsy Research*, *89*, 34–42. https://doi.org/10.1016/j.eplepsyres.2009.09.004

Rowley, H. L., Martin, K. F., & Marsden, C. A. (1995). Decreased GABA release following tonic-clonic seizures is associated with an increase in extracellular glutamate in rat hippocampus in vivo. *Neuroscience*, *68*, 415–422. https://doi.org/10.1016/0306-4522(95)00159-G

Saboory, E., Ebrahimi, L., Roshan-Milani, S., & Hashemi, P. (2015). Interaction of prenatal stress and morphine alters prolactin and seizure in rat pups. *Physiology & Behavior*, *149*, 181–186. https://doi.org/10.1016/j.physbeh.2015.06.004

Saboory, E., Gholami, M., Zare, S., & Roshan-Milani, S. (2014). The long-term effects of neonatal morphine administration on the pentylenetetrazol seizure model in rats: The role of hippocampal cholinergic receptors in adulthood. *Developmental Psychobiology*, *56*, 498–509. https://doi.org/10.1002/dev.21117

Spandou, E., Soubasi, V., Papoutsopoulou, S., Augoustides-Savvopoulou, P., Loizidis, T., Pazaiti, A., … Guiba-Tziampiri, O. (2007). Neuroprotective effect of long-term $MgSO_4$ administration after cerebral hypoxia-ischemia in newborn rats is related to the severity of brain damage. *Reproductive Sciences*, *14*, 667–677. https://doi.org/10.1177/1933719107305864

Srivastava, A., Dixit, A. B., Banerjee, J., Tripathi, M., & Chandra, P. S. (2016). Role of inflammation and its miRNA based regulation in epilepsy: Implications for therapy. *Clinica Chimica Acta*, *452*, 1–9. https://doi.org/10.1016/j.cca.2015.10.023

Vestergaard, M., Wisborg, K., Henriksen, T. B., Secher, N. J., Østergaard, J. R., & Olsen, J. (2005). Prenatal exposure to cigarettes, alcohol, and coffee and the risk for febrile seizures. *Pediatrics*, *116*, 1089–1094.

Viviani, B., Bartesaghi, S., Gardoni, F., Vezzani, A., Behrens, M., Bartfai, T., … Galli, C. (2003). Interleukin-1β enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. *Journal of Neuroscience*, *23*, 8692–8700. https://doi.org/10.1523/JNEUROSCI.23-25-08692.2003

Zheng, X.-Y., Zhang, H.-L., Luo, Q., & Zhu, J. (2011). Kainic acid-induced neurodegenerative model: Potentials and limitations. *Journal of Biomedicine and Biotechnology*, *2011*, 1–10. https://doi.org/10.1155/2011/457079

How to cite this article: Saboory E, Ghadimkhani M, Roshan-Milani S, et al. Effect of early-life inflammation and magnesium sulfate on hyperthermia-induced seizures in infant rats: Susceptibility to pentylenetetrazol-induced seizures later in life. *Developmental Psychobiology*. 2019;61:96–106. https://doi.org/10.1002/dev.21781