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ORIGINAL PAPER

Effects of Pentylenetetrazole Kindling on Mitogen-Activated Protein Kinases Levels in Neocortex and Hippocampus of Mice

Juliana Ben · Paulo Alexandre de Oliveira · Filipe Marques Gonçalves · Tanara Vieira Peres · Filipe Carvalho Matheus · Alexandre Ademar Hoeller · Rodrigo Bainy Leal · Roger Walz · Rui Daniel Prediger

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Abstract The epileptogenesis process involves cell signaling events associated with neuroplasticity. The mitogen-activated protein kinases (MAPKs) integrate signals originating from a variety of extracellular stimuli and may regulate cell differentiation, survival, cell death and synaptic plasticity. Here we compared the total and phosphorylated MAPKs (ERK1/2, JNK1/2 and $p38^{MAPK}$) levels in the neocortex and hippocampus of adult Swiss male mice quantified by western blotting analysis 48 h after the last injection of pentylenetetrazole (PTZ), according to the kindling protocol (35 mg/kg, i.p., on alternated days, with a total of eight injections). The total levels of the investigated MAPKs and the phospho-$p38^{MAPK}$ in the neocortex and hippocampus were not affected by the PTZ injections. The MAPKs phosphorylation levels remain unaltered in PTZ-treated animals without convulsive seizures. The phospho-JNK2 phosphorylation, but not the phospho-JNK1, was increased in the hippocampus of PTZ-treated animals showing 1–3 days with convulsive seizures, whereas no significant changes were observed in those animals with more than 3 days with convulsive seizures. The phospho-ERK1/2 phosphorylation decreased in the neocortex and increased in the hippocampus of animals with 1–4 days with convulsive seizures and became unaltered in mice that showed convulsive seizures for more than 4 days. These findings indicate that resistance to PTZ kindling is associated with unaltered ERK1/2, JNK1/2 and $p38^{MAPK}$ phosphorylation levels in the neocortex and hippocampus. Moreover, when the PTZ kindling-induced epileptogenesis manifests behaviorally, the activation of the different MAPKs sub-families shows a variable and non-linear pattern in the neocortex and hippocampus.

Keywords Epilepsy · Kindling · Pentylenetetrazole · Mitogen-activated protein kinases (MAPKs) · Hippocampus · Neocortex

Abbreviations

PTZ	Pentylenetetrazole
MAPKs	Mitogen-activated protein kinases
ERK1/2	Extracellular signal-regulated kinases 1 and 2
JNK1/2/3	c-Jun-N-terminal kinases 1-3
$p38^{MAPK}$a/b/c/d	p38 Kinases a-d

Introduction

Epilepsies are characterized by recurrent spontaneous seizures due to abnormal hyper synchronic neuronal hyperactivity in the brain [1–3]. Clinical treatment with antiepileptic drugs enhance the threshold for seizures without a significant antiepileptogenic effect [4] and about

J. Ben · T. V. Peres · R. B. Leal · R. Walz · R. D. Prediger
Programa de Pós-Graduação em Neurociências, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

P. A. de Oliveira · F. C. Matheus · R. D. Prediger (✉)
Departamento de Farmacologia, Universidade Federal de Santa Catarina, CCB/Bloco D, Florianópolis, SC 88049-900, Brazil
e-mail: ruidsp@hotmail.com

F. M. Gonçalves · R. B. Leal
Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

A. A. Hoeller · R. Walz
Departamento de Clínica Médica, Centro de Ciências da Saúde, Hospital Universitário (HU), Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

30 % of epileptic patients remain with seizures even considering all the current pharmacological options [5]. A better understanding regarding the epileptogenesis using animal models may help to shed light new pharmacological targets and more effective treatments.

The pentylenetetrazole (PTZ) acts blocking the chloride ionophore of the gamma-aminobutyric acid type A ($GABA_A$) receptor by interacting with the picrotoxin site of this receptor [6]. The PTZ kindling model consists of progressive and persistent intensification of seizure susceptibility after repeated sub-threshold stimulation with PTZ, resulting in generalized seizures afterwards [7]. The PTZ kindling model is useful to investigate basic mechanisms related to resistance and sensibility to epileptogenesis in rodents [8, 9] which involves acute and chronic seizure-related effects including enzymatic changes, altered neurotransmitter release and modification of receptor or ion channels expression or functions which require a complex cellular biochemical interaction network of signal transduction molecules [9–11].

The mitogen-activated protein kinases (MAPKs) are a group of serine-threonine kinases that amplify and integrate signals originating from a variety of extracellular stimuli and may regulate cell differentiation, survival and death and synaptic plasticity [12–14]. They consist of three main kinases subfamilies, the extracellular signal-regulated kinases 1 and 2 (ERK1/2), the c-Jun-N-terminal kinases 1-3 (JNK1/2/3) and the $p38^{MAPK}$ [15, 16].

All these signaling enzymes are involved in the synaptic plasticity events and changes in their activities may be associated with physiologic and pathologic processes including propensity or resistance to epileptogenesis and modulation of seizures threshold. In fact, a time and structure-dependent involvement of MAPKs has been described in chronic models of epilepsy including in the pilocarpine [17], kainic acid models [18–20] and electrical kindling [21], as well as in acute models such as in PTZ-induced seizures [22] and electroconvulsive shock [23]. Despite widely studies, the possible associations between the MAPKs and the resistance or propensity to PTZ kindling remain to be investigated. Therefore, we investigated the profile of the total and phosphorylated ERK1/2, JNK1/2/3 and $p38^{MAPK}$ levels in the neocortex and hippocampus of mice according to their progression or resistance to kindling induced by systemic PTZ.

Materials and Methods

Animals

Experiments were conducted using male *Swiss* mice (8–12 weeks old, n = 49), weighing 30–60 g at the beginning of the experiments and supplied by the animal facility of the Universidade Federal de Santa Catarina (UFSC, Florianópolis, Brazil). The animals were kept in collective cages (15 animals per cage) and maintained in a room under controlled temperature (21 ± 2 °C) and 12 h light cycle (lights on at 7:00 a.m.), with free access to food and water. All procedures were conducted between 9:00 and 12:00 a.m. Efforts were made to minimize the number of animals used and their suffering. All experiments were undertaken in accordance with the guidelines on animal care of the local Ethics Committee on the use of animals (CEUA/UFSC, protocol PP00830), which follows the NIH publication "Principles of Laboratory Animal Care".

PTZ Kindling and Experimental Design

In the PTZ kindling model (Fig. 1a), a subconvulsive dose of PTZ (35 mg/kg, i.p., n = 32) dissolved in saline was administered on alternate days for 16 days comprising eight injections (the number of injections where most mice reached the kindling with at least 2 days with convulsive seizure, without a significant mortality rate). Animals were observed for 30 min immediately after each stimulation, in groups of eight animals each time, and seizure severity was

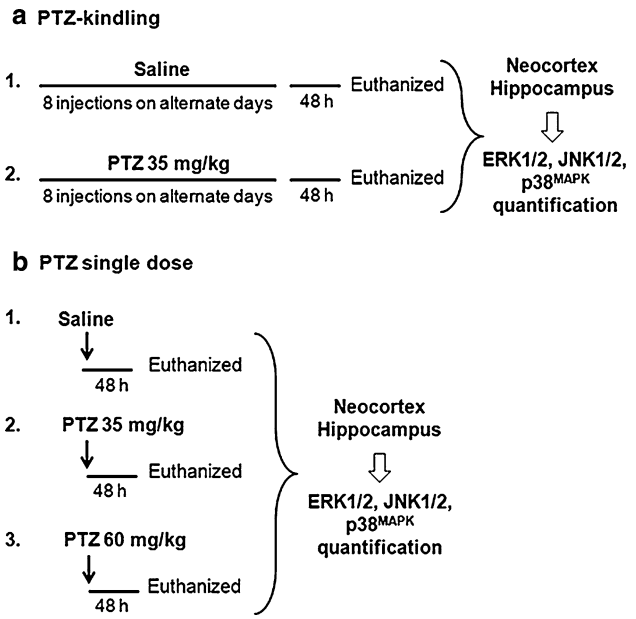

Fig. 1 Experimental design. Panel **a** shows the PTZ kindling model of epilepsy, in which animals received eight subconvulsive PTZ injections (35 mg/kg, i.p.), and were observed for 30 min every stimulation day immediately after the PTZ administration; control animals receive saline (10 ml/kg, i.p.) on alternate days (eight injections). Panel **b** shows the PTZ single dose, in which animals received one single subconvulsive (35 mg/kg, i.p.) or convulsive (60 mg/kg, i.p.) PTZ doses; control animals received one single dose of saline (10 ml/kg, i.p.). In both experiments neocortex and hippocampus were dissected for Western blotting analysis

classified according to stages of the Racine scale: 1, facial clonus; 2, head nodding; 3, myoclonic jerks; 4, rearing/falling seizures; 5, running/bouncing seizures [7, 8, 24–26]. In susceptible animals, seizure intensity increased with each administration until animals reached Racine stage 4, 5 or died. Seizure severity and mortality were recorded every day after each observation. Control animals received saline (10 mL/kg, i.p., n = 4) on alternate days (eight injections).

The behavior analyzes followed the subsequent denomination: (1) kindling progression was determined by the number of days with convulsive seizures (Racine stage 4 or 5) developed during the protocol, animals showing 8 days with convulsive seizures had the maximal and faster kindling progression; (2) resistance to PTZ kindling was considered in animals that showed no behavioral convulsive seizures during all procedure; (3) more susceptibility to kindling progression for animals that showed more than 4 days with convulsive seizure—faster kindling progression, and (4) less susceptibility to kindling progression for animals that showed convulsive seizures up to 3 days with convulsive seizure—slower kindling progression.

Between the 32 PTZ kindling-treated animals, 20 animals were randomly assigned to the following groups (n = 4 per group) for western blot analyzes: *S0*, animals without convulsive seizures; *S1,3*, animals showing 1–3 days with convulsive seizures; *S4*, animals showing 4 days with convulsive seizures; *S5*, animals showing 5 days with convulsive seizures; *S6,7*, animals showing 6 or 7 days with convulsive seizures. The animals were euthanized 48 h after the last PTZ or saline injection, and the neocortex and hippocampus dissected for protein analysis. The total and phosphorylated MAPKs (ERK1/2, JNK1/2/3, $p38^{MAPK}$) levels were analyzed by western blotting. We study the neocortex and hippocampus due the involvement of these regions in the PTZ induced-seizures initiation and spreading as well as metabolic events related to seizures [27].

To exclude the possible residual effects of PTZ or convulsive seizure *per se* on the biochemical variables, we investigated the total and phosphorylated MAPKs (ERK1/2, JNK1/2/3, $p38^{MAPK}$) levels after a single injection of subconvulsive (35 mg/kg, i.p.) or convulsive (60 mg/kg, i.p.) PTZ doses (n = 5 and 4, respectively) and compared with control animals treated with saline (n = 4) (Fig. 1b). In this protocol, the animals were sacrificed 48 h after the single PTZ administration.

Drugs and Reagents

HEPES, Triton X-100, SDS, acrylamide, bis-acrylamide, were obtained from GE Healthcare Life Science (Piscataway, NJ, USA). Glycine, Tris, TEMED, β-mercaptoethanol were obtained from Amresco Life Science (Solon, OH, USA). Bovine serum albumin (BSA) was obtained from Inlab (São Paulo, SP, Brazil). Immobilon nitrocellulose and goat anti-rabbit IgG HRP (horseradish peroxidase) were obtained from the Millipore (Temecula, CA, USA). PTZ, ammonium persulfate (APS), rabbit anti-total-ERK1/2, anti-total-JNK1/2, anti-phospho and anti-total-$p38^{MAPK}$ were purchased from the Sigma Chemical Company (St. Louis, MO, USA). Rabbit anti-phospho-ERK1/2, anti-phospho-JNK1/2/3, LumiGLO reagent (luminol chemiluminescent substrate) were obtained from Cell Signaling Technology (Beverly, MA, USA). Mouse anti-β actin as well goat anti-mouse IgG HRP conjugated antibodies were obtained from Santa Cruz Biotechnology. All other reagents were analytical grade.

Western Blot Analysis

For quantification of MAPKs activation Western blot analysis was performed as previously described by [28–30]. Animals were euthanized by decapitation, brains were collected and the neocortex and hippocampus were dissected (4 °C), placed in liquid nitrogen and then stored at −80 °C until use. Samples were prepared as previously described by Oliveira et al. [31]. Briefly, the whole neocortex and both hippocampus of each mice were mechanically homogenized in 400 μL of Tris 50 mM pH 7.0, EDTA 1 mM, NaF 100 mM, PMSF 0.1 mM, Na3VO4 2 mM, Triton X-100 1 %, glycerol 10 %, Sigma Protease Inhibitor Cocktail (P2714). Lysates were centrifuged (10,000×*g* for 10 min, at 4 °C) to eliminate cellular debris. The supernatants were diluted 1/1 (v/v) in Tris 100 mM pH 6.8, EDTA 4 mM, SDS 8 % and boiled for 5 min. Thereafter, sample dilution (40 % glicerol, 100 mM Tris, bromophenol blue, pH 6.8) in the ratio 25:100 (v/v) and β mercaptoethanol (final concentration 8 %) were added to the samples. Protein content was estimated by the method described by Peterson [32]. The same amount of protein (70 μg per lane) for each sample was electrophoresed in 10 % SDS–PAGE mini-gels and transferred to nitrocellulose membranes using a semidry blotting apparatus (1.2 mA/cm^2; 1.5 h). To verify transfer efficiency process, gels were stained with Coomassie blue and membranes with Ponceau S.

The membranes were blocked with 5 % skim milk in TBS (Tris 10 mM, NaCl 150 mM, pH 7.5). The phosphorylated and total forms of MAPKs were detected after overnight incubation with specific antibodies diluted in TBS-T containing BSA 2 % in the dilutions of 1:1,000 (anti-phospho-JNK1/2/3, anti-phospho-$p38^{MAPK}$), 1:2,000 (anti-phospho-ERK1/2), 1:5,000 (anti-total-JNK1/2) 1:10,000 (anti-total-$p38^{MAPK}$) and 1:40,000 (anti-total-ERK1/2). Moreover, the membranes were incubated for 1 h at room temperature with horseradish peroxidase

(HRP)-conjugated anti-rabbit for detection of phosphorylated and total forms of each MAPK, by the reactions developed by chemioluminescence substrate (LumiGLO). All blocking and incubation steps were followed by three times washing (5 min) with TBS-T (Tris 10 mM, NaCl 150 mM, Tween-20 0.1 %, pH 7.5). All membranes were incubated with mouse anti-β-actin (1:2,000) antibody to verify that equal amounts of proteins were loaded on the gel. The phosphorylation (activation) level of MAPKs was determined as a ratio of optical densitometry (OD) of phosphorylated band/OD of total band [30]. The bands were quantified using the Scion Image® software.

The antibody against ERK1/2 detected two bands, one at approximately 44 kDa and the second at approximately 42 kDa, corresponding respectively to the two ERK isoforms, ERK1 and ERK2. Anti-p38^{MAPK} detected a single band of approximately 38 kDa, anti-JNK 1/2/3 detected two bands, one at approximately 54 kDa and the second at approximately 46 kDa, corresponding respectively to the three JNK isoforms, JNK2/3 and JNK1. The anti-β-actin antibody detected a single band of approximately 45 kDa.

Statistical Analysis

All the continuous variables showed a normal distribution in the one sample Kolmogorov–Smirnov test. Comparisons of MAPKs levels (total and phosphorylated) in the brain structures (neocortex and hippocampus) between control and PTZ-treated groups were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. Data were expressed as the mean ± SEM, and a "p" level lower than 0.05 were considered statistically significant.

Results

The Figs. 2, 3 and 4a, b show the MAPKs activation (phosphorylation) levels in the neocortex of controls and PTZ kindling-treated animals stratified according to the number of days with convulsive seizures (Racine 4 or 5) after a total of eight injections on alternated days. The JNK1, JNK2 and p38^{MAPK} phosphorylation levels did not differ between the controls and PTZ kindling-treated animals ($p > 0.62$). There was a variable decrease in the neocortical levels of phopho-ERK1 [$F(5,18) = 6.70$; $p = 0.001$] and phospho-ERK2 [$F(5,18) = 5.18$; $p = 0.004$] in comparison to controls depending on the number of days with convulsive seizures. There were no significant differences ($p > 0.20$) in the total content of all the investigated MAPKs between the controls and PTZ-treated animals (data not shown).

The Figs. 2, 3 and 4c, d illustrate the MAPKs activation (phosphorylation) levels in the hippocampus of controls and PTZ kindling-treated animals stratified according to the number of days with convulsive seizures elicited during the 8 stimulations days. The ERK2, JNK1 and p38^{MAPK} phosphorylation levels did not differ between the controls and PTZ kindling-treated animals ($p > 0.20$). Animals treated with PTZ that displayed 1–3 days with convulsive seizures after 8 days of stimulation showed a significant increase in the hippocampal levels of phospho-ERK1 [$F(5,18) = 3,44$; $p = 0.02$] and phospho-JNK2 [$F(5,18) = 2.92$; $p = 0.04$] in comparison to controls. There was no association between the levels of phospho-ERK1 and phospho-JNK2 in the hippocampus ($r = 0.13$, $p = 0.57$, data not shown). Moreover, no significant differences ($p > 0.24$) were observed in the total content of all investigated MAPKs in hippocampus between the controls and PTZ-treated animals (data not shown).

The levels of MAPKs in the neocortex and hippocampus determined 48 h after the injection of a single subconvulsive (35 mg/kg, i.p.) or convulsive (60 mg/kg, i.p.) dose of PTZ did not differ from controls ($p > 0.20$, data not shown). Pointing out that all animals submitted to the single convulsive dose of PTZ reach Racine stage 4 or 5, and none of them died.

Discussion

The present findings show that repetitive injections of subconvulsive doses of PTZ (35 mg/kg, i.p., on alternated days, comprising a total of eight injections) differently modulates the neocortical and hippocampal MAPKs (ERK1/2, JNK1/2 and p38^{MAPK}) phosphorylation profile without affecting the total content of these enzymes. The relationship between the sensitivity to kindling induction and these signal transduction molecules are variable according to the investigated MAPKs subfamilies, as well as the brain region analyzed. The current results are not due to confounding bias resulted from residual effects of the last PTZ injection or convulsive seizure per se, since the MAPKs profiles were not affected by single subconvulsive or convulsive PTZ injections.

Most of studies performed so far using the kindling PTZ model have analyze only animals that shown convulsive seizures without stratify animals according to the number of days with convulsive seizures, and exclude animals that are resistant to kindling (0–1 convulsive seizure). To our knowledge, this is the first time that animals submitted to PTZ kindling were stratified accordingly to the numbers of days with convulsive seizure, focusing in the differences of MAPKs in animals that present different susceptibility to PTZ kindling. Of high importance, the present findings provide new evidence of a decrease in ERK1/2 activation in the neocortex of animals with faster PTZ kindling

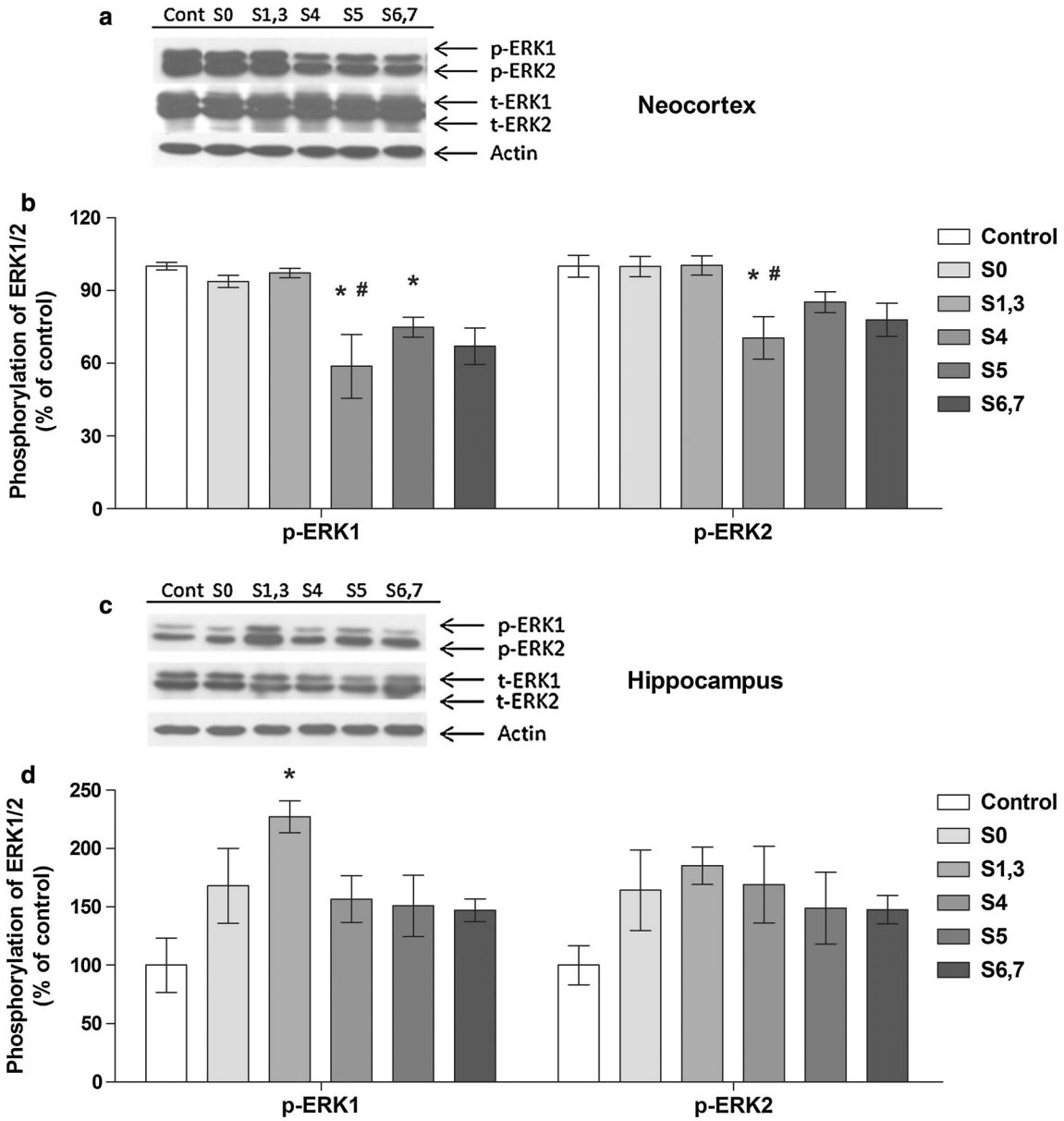

Fig. 2 Western blot analysis of ERK1/2 phosphorylation in the neocortex (**a**, **b**) and hippocampus (**c**, **d**) of mice submitted to the PTZ kindling model of epilepsy. The panel **a** and **c** show a representative blot of neocortex immunoreactivity of the phospho-ERK1/2, total-ERK1/2 and anti-β actin (used as load control) in neocortex and hippocampus, respectively. The quantification of phospho-ERK1 and phospho-ERK2 are shown in the neocortex (**b**) and hippocampus (**d**). The phosphorylation level of each protein was determined by computer-assisted densitometry as a ratio of the O.D. of the phosphorylated band over the O.D. of the total band and the data are expressed as percentage of the control. The values are presented as mean ± SEM. *Cont*, control group; *S0*, no days with convulsive seizures; *S1,3*, 1–3 days with convulsive seizures; *S4*, 4 days with convulsive seizures; *S5*, 5 days with convulsive seizures; *S6,7*, 6 and 7 days with convulsive seizures. $^{*}p < 0.05$ compared to control group and $^{\#}p < 0.05$ compared to *S0* and *S1*, *3*

progression (4, 6 and 7 days with convulsive seizures—more susceptible to PTZ kindling) and an increase in ERK1 activation in the hippocampus of animals with slower PTZ kindling progression (1–3 days with convulsive seizures—less susceptible to PTZ kindling) 48 h after the last convulsive seizure.

The ERK activation is involved in several forms of synaptic plasticity, including long-term potentiation (LTP), hippocampal-dependent learning and memory as well as regulation of neuronal excitability [8, 9, 12, 33]. More importantly, the activity-dependent plasticity related to ERK activation may be involved in the epileptogenic process in humans. For instance, it was recently demonstrated a persistent ERK1/2 activation associated with increased synaptic density in the ictal onset zone in comparison to the nearby resected regions that do not produce

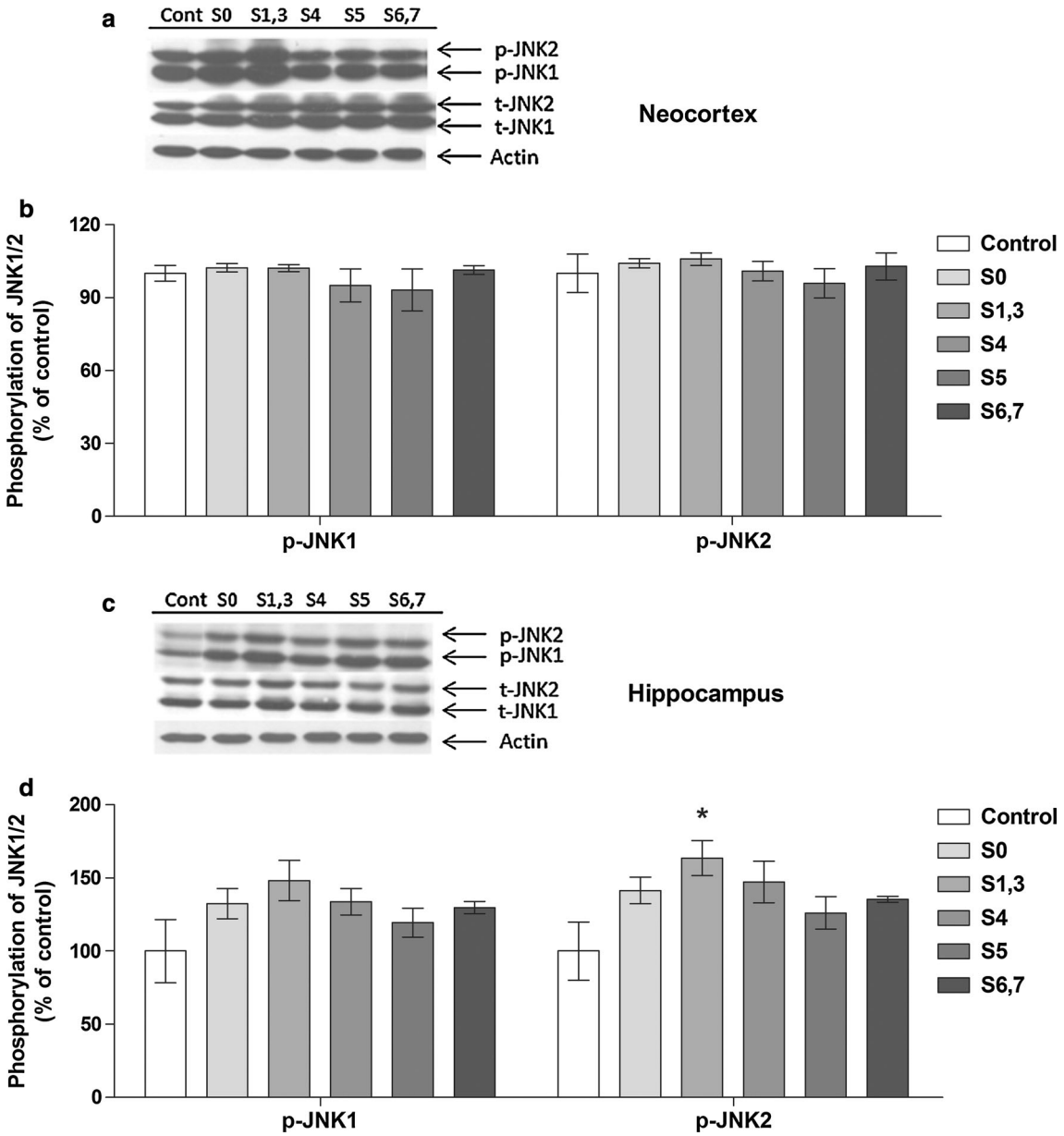

Fig. 3 Western blot analysis of JNK1/2 phosphorylation in the neocortex (**a**, **b**) and hippocampus (**c**, **d**) of mice submitted to the PTZ kindling model of epilepsy. The panel **a** and **c** show a representative blot of neocortex immunoreactivity of the phospho-JNK1/2, total-JNK1/2 and anti-β actin (used as load control) in neocortex and hippocampus, respectively. The quantification of phospho-JNK1 and phospho-JNK2 are shown in the neocortex (**b**) and hippocampus (**d**). The phosphorylation level of each protein was determined by computer-assisted densitometry as a ratio of the O.D. of the phosphorylated band over the O.D. of the total band and the data are expressed as percentage of the control. The values are presented as mean ± SEM. *Cont*, control group; *S0*, no days with convulsive seizures; *S1,3*, 1–3 days with convulsive seizures; *S4*, 4 days with convulsive seizures; *S5*, 5 days with convulsive seizures; *S6,7*, 6 and 7 days with convulsive seizures. $*p < 0.05$ compared to control group

seizures [34]. Although this previous study does not indicate a cause-effect relationship between ERK1/2 and its downstream pathway (CREB-dependent transcription genes) with epileptogenesis process, it was demonstrated a clear spatial relationship between ERK1/2 activation and probable epileptogenic zone in human neocortex documented by chronic electrocorticography [34].

Recently, our research group demonstrated the ERK1/2 activation in the rat hippocampus 1–12 h, but not chronically, after the pilocarpine-induced status epilepticus [17]. The ERK1/2 activation in the hippocampus during the acute and sub-acute periods of pilocarpine-induced status epilepticus were confirmed by other authors using immunohistochemistry essay [35, 36]. The ERK1/2 activation

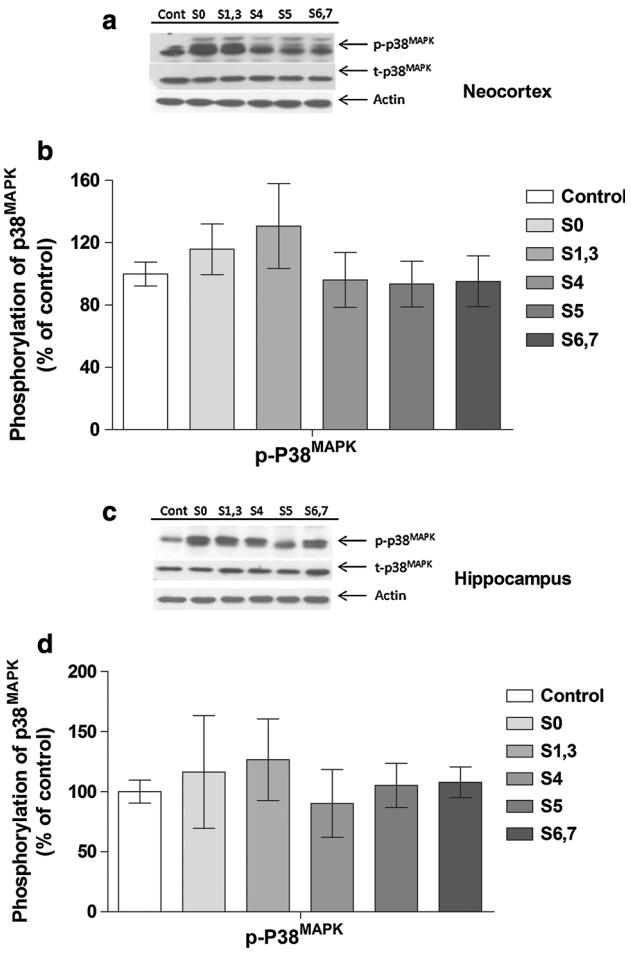

Fig. 4 Western blot analysis of $p38^{MAPK}$ phosphorylation in the neocortex (**a**, **b**) and hippocampus (**c**, **d**) of mice submitted to the PTZ kindling model of epilepsy. The panel **a** and **c** show a representative blot of neocortex immunoreactivity of the phospho-$p38^{MAPK}$, total-$p38^{MAPK}$ and anti-β actin (used as load control) in neocortex and hippocampus, respectively. The quantification of phospho-$p38^{MAPK}$ and phospho-$p38^{MAPK}$ are shown in the neocortex (**b**) and hippocampus (**d**). The phosphorylation level of each protein was determined by computer-assisted densitometry as a ratio of the O.D. of the phosphorylated band over the O.D. of the total band and the data are expressed as percentage of the control. The values are presented as mean ± SEM. *Cont*, control group; *S0*, no days with convulsive seizures; *S1,3*, 1–3 days with convulsive seizures; *S4*, 4 days with convulsive seizures; *S5*, 5 days with convulsive seizures; *S6,7*, 6 and 7 days with convulsive seizures

was also demonstrated in the hippocampus at 0.5, 3 and 5 h after the kainic acid administration [18, 37, 38] indicating that it is not specific to the status epilepticus induced by pilocarpine. In the pilocarpine model, a spontaneous kindling-like evolution is observed during the silent period (7–22 days after the status epilepticus) and once the Racine stage 5 was reached the subsequent seizures intensity became higher than stage 3, being mostly generalized [39]. These findings suggest a coincident ERK activation in the hippocampus earlier in spontaneous kindling process with a subsequent return to baseline when the kindling is fulfilled installed. When the neocortex was evaluated, once again the ERK levels were increased in the acute phase of the pilocarpine model [17] returning to normal levels in the latent period (1–5 days after the status epilepticus) and chronic periods. Altogether, the current and previous findings indicate that the time and structure-dependent ERK1/2 activation in the PTZ kindling differ from those observed in the spontaneous kindling process in the pilocarpine model. The ERK1/2 phosphorylation levels did not change in the neocortex and hippocampus of animals resistant to PTZ kindling, whereas they increased in the hippocampus and decreased in the neocortex of animals with slower PTZ kindling progression (1–4 days with convulsive seizures). Interestingly, ERK1/2 activation was not altered in the neocortex and hippocampus of those animals with faster PTZ kindling progression (more than 4 days with convulsive seizures). Therefore, these findings indicate that ERK1/2 may present opposite effects in the neocortex and hippocampus during the PTZ kindling progression.

The JNK1/2 activation in the neocortex was not significantly affected by the PTZ injections. On the other hand, the phopho-JNK2 (p54 kDa), but not the phospho-JNK1, showed a similar inverted U-shaped pattern of activation observed in ERK1, increasing phosphorylation in animals with 1–3 days with convulsive seizures, and similar to controls phosphorylation levels in kindling resistant mice and in mice with faster PTZ kindling progression (i.e., more susceptible to kindling progression). However, no significant association was observed between the ERK1 and JNK2 activation in the hippocampus, suggesting that these two kinases are functionally independent during PTZ kindling resistance or progression. JNK and ERK are involved in many biological processes, such as cellular differentiation, apoptosis, stress reaction, neurodegeneration, but also involved in neuroplasticity and regeneration [40–43]. Recently, our research group showed a decrease of JNK2/3 activation in the neocortex and hippocampus 3 h after the status epilepticus induction, as well as during the chronic period, in the pilocarpine model. The JNK isoforms may exhibit different responses, since JNK1 is more active in the basal state and JNK2 and/or JNK3 seem to participate in the mechanism of cell death [18, 40, 44]. Some authors also showed an increase of JNK1/2 activation in the CA1 sub region of the hippocampus after electrical kindling, and correlate it with neuronal death [21, 45] and reactive gliosis [21].

The $p38^{MAPK}$ activation was not altered in the current PTZ kindling protocol neither in the neocortex nor in the hippocampus. Hsieh et al. [20] also showed no alterations in $p38^{MAPK}$ activation in the neocortex and hippocampus after kainic acid treatment. In contrast, a decrease in

$p38^{MAPK}$ activation was observed 10 days after the kainic acid treatment in another study [19] as well as in the pilocarpine model, where it occurred in association with a small increase in total $p38^{MAPK}$ expression [46]. In our previous study, $p38^{MAPK}$ was activated at 1 and 12 h after the induction of the pilocarpine status epilepticus in both neocortex and hippocampus, but returned to baseline levels in both structures in the chronic period (50 days). These findings may indicate that $p38^{MAPK}$ activation is not associated to PTZ kindling maintenance as well as to the spontaneous seizure observed in the chronic period of pilocarpine model.

The PTZ administration (repeated subconvulsive doses or single convulsive dose) may cause neuronal death and astroglial modifications which are associated to seizures, and epileptogenesis [47, 48]. Although these structural changes cannot be the cause of epileptogenesis itself, they may explain the neurochemical modifications in animals that underwent to PTZ protocols. Our study design does not permit to identify the independent association among the structural (neuronal death or gliosis) and functional changes (threshold for seizures and seizures progression) and the neurochemical biomarkers analyzed. Further studies using neurochemical and histochemical analysis are required to elucidate this question.

Conclusion

Our findings demonstrate that resistance to PTZ kindling is associated to unaltered ERK1/2, JNK1/2 and $p38^{MAPK}$ phosphorylation levels in the neocortex and hippocampus of mice. When the epileptogenesis induced by PTZ kindling manifests behaviorally, the MAPKs phosphorylation profile shows a variable and a non-linear pattern in the neocortex and hippocampus. These results point out the intricate cross-links between the MAPKs in this experimental model of epileptogenesis. Further studies addressing specific signal transduction molecules and their pathways in the PTZ kindling model may be helpful to identify potential biomarkers for the treatment of epilepsies.

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Conflict of interest The authors declare that they have no conflict of interest.

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