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Short-term alterations in hippocampal glutamate transport system caused by one-single neonatal seizure episode: Implications on behavioral performance in adulthood

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ABSTRACT

Impairment in the activity and expression of glutamate transporters has been found in experimental models of epilepsy in adult animals. However, there are few studies investigating alterations on glutamate transporters caused by epilepsy in newborn animals, especially in the early periods after seizures. In this study, alterations in the hippocampal glutamate transporters activity and immunocontent were investigated in neonatal rats (7 days old) submitted to kainate-induced seizures model. Glutamate uptake, glutamate transporters (GLT-1, GLAST, EAAC1) and glutamine synthetase (GS) were assessed in hippocampal slices obtained 12 h, 24 h, 48 h, 72 h and 60 days after seizures. Immunoreactivity for hippocampal GFAP, NeuN and DAPI were assessed 24 h after seizure. Behavioral analysis (elevated-plus maze and inhibitory avoidance task) was also investigated in the adult animals (60 days old). The decrease on glutamate uptake was observed in hippocampal slices obtained 24 h after seizures. The immunocontent of GLT-1 increased at 12 h and decreased at 24 h (+62% and −20%, respectively), while GLAST increased up to 48 h after seizures. No alterations were observed for EAAC1 and GS. It should be mentioned that there were no long-term changes in tested glutamate transporters at 60 days after kainate treatment. GFAP immunoreactivity increased in all hippocampal subfields (CA1, CA3 and dentate gyrus) with no alterations in NeuN and DAPI staining. In the adulthood, kainate-treated rats showed anxiety-related behavior and lower performance in the inhibitory avoidance task. Our findings indicate that acute modifications on hippocampal glutamate transporters triggered by a single convulsive event in early life may play a role in the behavioral alterations observed in adulthood.

1. Introduction

Epileptic seizures in children are a common and frightening neurological condition. The incidence of seizures is significantly higher in children than in adults, with the highest incidence in the first year of life (Holmes and Ben-Ari, 2001). This higher susceptibility to seizure of immature brain compared to adult seems to be related to the fact that γ-aminobutiric acid (GABA), an inhibitory neurotransmitter in mammalian brain, exerts paradoxical excitatory effects in early ages (Khazipov et al., 2004; Ben-Ari, 2002). Epidemiological data suggest that prolonged seizures or *status epilepticus* (SE) in childhood may lead to increased risk of epilepsy in adulthood, through mechanisms still unknown (Haut et al., 2004).

Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system (CNS), involved in essential physiological brain functions, as synaptic plasticity, learning and memory, brain development and ageing (Tzingounis and Wadiche, 2007; Danbolt, 2001; Segovia et al., 2001; Ozawa et al., 1998). Glutamate acts through activation of *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and kainate ionotropic receptors, and metabotropic receptors (for reviews see Kew and Kemp (2005), Rothstein et al. (1996)). However, overstimulation of the glutamatergic system (by exogenous or endogenous stimuli), which occurs when glutamate levels in the synaptic cleft increase over the physiological range, is involved in various acute and chronic brain diseases (excitotoxicity), including neurodegenerative diseases, traumatic brain injury, cerebral ischemia, and seizures (Tzingounis and Wadiche, 2007; Danbolt, 2001; Maragakis and Rothstein, 2004; Beart and O'Shea, 2007; Sheldon and Robinson,

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2007). Thus, to keep glutamate at the physiologically relevant concentrations is extremely important.

There are strong evidences pointing that glutamatergic excitotoxicity may be prevented by astrocytic glutamate uptake, a process responsible for maintaining the extracellular glutamate levels below toxic levels (Rothstein et al., 1996; Chen and Swanson, 2003; Belanger and Magistretti, 2009). To date, five distinct high-affinity, sodium-dependent glutamate transporters have been cloned from animal and human tissue [GLAST (EAAT1), GLT-1 (EAAT2), EAAC1 (EAAT3), EAAT4 and EAAT5], differing in molecular structure, pharmacological properties, and tissue distribution (Danbolt, 2001; Beart and O'Shea, 2007; Bunch et al., 2009; Dunlop, 2006). Immunohistochemical studies have revealed that GLAST and GLT-1 are localized primarily in astrocytes, whereas EAAC1 is widely distributed in neurons (Danbolt, 2001; Dunlop, 2006). The impairment of these glutamate transporters has been also implicated in the pathophysiology of some brain disorders, as epilepsy and seizures (Bjornsen et al., 2007; Binder and Steinhauser, 2006; Zhang et al., 2004; Ueda et al., 2001; Tanaka et al., 1997).

Glutamate, after being taken up into astrocytes, may be converted to glutamine by glutamine synthetase (GS), which then is released to extracellular space and taken up by neurons where it is converted again to glutamate and stored in pre synaptic vesicles (Danbolt, 2001; Suarez et al., 2002). Thus, the GS activity is an essential step in the glutamate–glutamine cycle, and its impairment has been implicated in pathogenesis of temporal lobe epilepsy (TLE), since GS expression and activity is reduced in the hippocampus of TLE patients (Eid et al., 2004). In adult animals, GS was increased in the latent phase and decreased in the chronic phase of kainate-induced seizures (Hammer et al., 2008).

The consequences of *status epilepticus* (SE) in the developing brain appear to be different from those of mature brain. Comparisons of the findings obtained in the adult and newborn brain reveal a paradox, in that the immature brain has generally been considered 'resistant' to the damaging effects of hypoxia and hypoxia–ischemia, while at the same time exhibiting periods of heightened sensitivity to injury, dependent on the specific developmental stage of the brain (Holmes, 2005; Hurn et al., 2005).

Despite that, the immature brain is not immune to injury in prolonged seizure as SE. Changes in AMPA receptors and EAAC1 transporter expression were reported in SE rats at 10 days postnatal (P10) and these modifications were related to higher susceptibility to another seizure episode (Zhang et al., 2004). Despite the apparent low susceptibility of immature brain to seizure-induced cell death, seizures in the developing brain can result in irreversible alterations in neuronal connectivity (Holmes and Ben-Ari, 2001). Neonatal rats, which suffered from SE displayed synaptic alterations and memory impairment in the adulthood (Cognato et al., 2010; Cornejo et al., 2007, 2008), showing that disturbances in a critical period of brain maturation could persistently compromise its function. Furthermore, neural injuries such as hypoxic or hypoxic–ischemic insult to the developing brain will impact on subsequent maturation, with long-lasting consequences for the adult brain (Hurn et al., 2005).

Although some information is available regarding the involvement of glutamate transporters in events triggered by seizure activity in adult animals (Rothstein et al., 1996; Ueda et al., 2001; Simantov et al., 1999; Miller et al., 1997), little is known about the neonatal brain responses to seizure involving glutamate transporters, especially in the early period post-seizure. The aim of the present study was to investigate whether activity and immunocontent of glial and neuronal glutamate transporters in the hippocampus could be affected by kainate-induced seizure activity in rat pups. Behavioral tasks (anxiety-related behavior and inhibitory avoidance task) were also evaluated in adulthood (60 days after the seizures period).

2. Materials and methods

2.1. Kainate-induced seizure model

Wistar rats were maintained under controlled environment (21–22 °C, 12 h dark-light cycle, food and water *at libitum*). All experiments were in agreement with the Committee on Care and Use of Experimental Animal Resources of Federal University of Rio Grande do Sul, Brazil. Seizures were induced as previously described (Cornejo et al., 2007). Seven-day-old male Wistar rats were separated from their dams and received a single injection of kainate (KA) (1 mg/kg, s.c.) diluted in saline (NaCl 0.9 g%). Control animals received saline solution. The volume injected in each animal corresponded to 1% of body weight (ml/g). All animals presented seizures up to 30 min after KA injection. Seizures were characterized by intermittent myoclonic jerks, generalized tonic–clonic jerks, scratching, "swimming", and "wet-dog shakes". After spontaneous ending of seizures (around 3 h after KA administration), animals returned to their dams.

2.2. [^{3}H] Glutamate uptake

Hippocampal slices for glutamate uptake were obtained 12, 24, 48, 72 h and 60 days after the end of seizures episode. Animals were euthanized, the hippocampi were dissected out and immediately immersed in ice-cold Hank's balanced salt solution (HBSS) containing (in mM): 137 NaCl; 0.63 Na_2HPO_4; 4.17 $NaHCO_3$; 5.36 KCl; 0.44 KH_2PO_4; 1.26 $CaCl_2$; 0.41 $MgSO_4$; 0.49 $MgCl_2$ and 1.11 glucose, pH 7.3. Slices from each hippocampus (0.4 mm) were obtained using a McIlwain tissue chopper. They were pre-incubated at 35 °C for 15 min and the medium was replaced by HBSS. Glutamate uptake was started by adding 100 μM [^{3}H] glutamate. Incubation was stopped after 5 min by aspiration of the medium and slices were rinsed twice with ice-cold Na^+-free HBSS. Slices were then lysed in 0.5 N NaOH and kept overnight. The uptake was also carried out in Na^+-free HBSS (replaced by *N*-methyl-D-glucamine) at 4 °C. Sodium dependent uptake was considered as the difference between the uptake with and without sodium. Incorporated radioactivity was measured using a Wallac liquid scintillation counter.

2.3. Western blotting

Hippocampi were dissected out 12, 24, 48, 72 h and 60 days after the end of seizures episode and immediately homogenized in a 25 mM HEPES solution (pH 7.4) with 0.1% SDS and protease inhibitor cocktail (Sigma, USA). Samples (20 μg protein/well) were separated in an 8% SDS–PAGE mini-gel and transferred to a nitrocellulose membrane using a Trans-Blot system (Bio-Rad, São Paulo/SP, Brazil). Membranes were processed as follow: (1) blocking with 5% bovine serum albumin (Sigma, USA) for 2 h; (2) incubation with primary antibody overnight: 1:1000 rabbit anti-GLAST, rabbit anti-GLT-1 or rabbit anti-EAAC1 glutamate transporters (AlphaDiagnostic International); 1:10,000 rabbit anti-Glutamine synthetase (Sigma); 1:2000 mouse anti-β-Actin (Sigma); (3) incubation with horseradish peroxidase-conjugated secondary antibody for rabbit 1:3000 and mouse 1:3000 (Amersham Pharmacia Biotech) for 2 h; (4) chemioluminescence (ECL, Amersham Pharmacia Biotech, São Paulo/SP, Brazil) was detected using X-ray films (Kodak X-Omat, Rochester, NY, USA). The films were scanned and bands intensities were analyzed using Image J software (developed at the US National Institutes of Health and available on the web site (http://rsb.info.nih.gov/nih-image/). In order to determine the adequate amount of protein to be assayed, different protein concentrations were carried out in the same gel for each antibody tested.

2.4. Immunohistochemistry

Perfusion and fixation of the brain from 4 animals/group were performed 24 h after the end of seizures period through transcardiac perfusion with 4% paraformaldehyde and 0.25% glutaraldehyde, followed by cryoprotection in 30% sucrose solution overnight. Brain was sectioned (50 μm coronal sections) using a Leica VT1000S microtome (Leica Microsystems, São Paulo, Brazil). Coronal sections were separated in 4 series throughout the dorsal hippocampus with 300 μm interval between each section and collected in PBS. Free-floating sections of rat brain were processed for immunohistochemistry against the neuronal specific protein neuronal nuclei (NeuN) and glial fibrillary acidic protein (GFAP), using a primary mouse anti-NeuN (1: 500, Chemicon International, São Paulo/SP, Brazil) as well as rabbit anti-GFAP antibodies (1:500, Dako, Denmark A/S). Antibodies were diluted in Tris buffer saline (TBS, 0.5 M NaCl and 30 mM Tris, pH 7.4) containing 0.2% Triton X-100 and 10% normal goat serum and incubated for 48 h at 4 °C. After incubation, sections were rinsed 4 times for 10 min in TBS and subsequently incubated with secondary fluorescent antibodies overnight: Alexa fluor anti-rabbit 488 and anti-mouse 594 (1:500, Invitrogen, Porto Alegre/RS), in 0.1 M TBS containing 0.2% Triton X-100 and 10% normal goat serum for 24 h at 4 °C. After rinsing 4 times for 10 min in TBS, the sections were mounted on slides coated with 2% gelatin with chromium and potassium sulfate. The slices were mounted in a Vectashield mounting medium containing the nuclear marker DAPI (4′-6-diamidino-2-phenylindole dilactate) (Vector Laboratories, São Paulo/SP, Brazil). The CA1, CA3 and dentate gyrus (DG) subfields of each hippocampus were examined in the Olympus FluorView 1000 system and the fluorescence was quantified using ImageJ software. The images were captured and a square region of interest (ROI) was created considering the pyramidal layer size. The ROI square of 8019 μm^2 was overlaid on the analyzed subfields with blood vessels and other artifacts being avoided, using a magnification of 20x. Six ROI were analyzed per subfield.

2.5. Behavioral analysis

2.5.1. Elevated plus-maze task

Rats (60-day-old) were exposed to the elevated plus-maze apparatus that consisted of a central platform (10 cm × 10 cm) with 2 open and 2 closed arms (45 cm × 10 cm), arranged in such a way that the 2 arms of each type were opposite to each other. The apparatus was kept 88 cm above the floor and sessions were carried out in a room lighted only with a dim red light. Animals were individually placed in the central platform facing an open arm and observed for 5 min. Two observers blinded to treatments recorded the number of entries and the time spent in the open arms as measurements of anxiety-related behavior (Walf and Frye, 2007).

2.5.2. Inhibitory avoidance task

Rats (60-day old) were placed on a 5.0 cm-high, 8.0 cm-wide platform located in the left side of a 50 cm × 25 cm × 25 cm inhibitory avoidance task apparatus, with floor composed by a series of parallel bronze bars 1.0 cm apart. In the training session, the latency to step down from the platform to the grid with all four paws was measured; immediately after stepping down onto the grid animals received a 0.4 mA, 1.0-s scrambled foot shock. The test session was performed 1.5 h (short-term memory) and 24 h (long-term memory) after training and procedures were the same, except that the foot shock was omitted. Differences between training and test latencies to step down were taken as an index of memory.

2.6. Statistical analysis

For glutamate uptake, western blot data and immunohistochemistry, the results were expressed as mean ± standard deviation, and statistical analysis was performed by one-way ANOVA followed by Tukey's test as post-hoc. For elevated plus maze task, the results were expressed as mean ± standard deviation and the Student's *t* test was applied. For inhibitory avoidance task, the results were expressed as median ± interquartile range and Wilcoxon test was used for analysis within groups. For statistical significance, the value of $P < 0.05$ was adopted. The statistical analysis was performed using SPSS 15.0 software.

3. Results

3.1. Effect of kainate-induced seizures on hippocampal glutamate uptake activity and on glutamate transporters immunocontent

Fig. 1 shows that the glutamate uptake by hippocampal slices obtained 12 h after kainate-induced seizures showed a trend to be higher ($P = 0.082$), and those obtained 24 h after seizures decreased 20%, when compared to control group. Glutamate uptake by hippocampal slices was not affected by seizures after 48 h. The immunocontent of astrocytic glutamate transporters (GLAST and GLT-1) and of neuronal glutamate transporter (EAAC1) was determined in the whole hippocampus obtained 12, 24, 48, 72 h and 60 days after seizures (Fig. 2). GLT-1 increased (37%) in hippocampi obtained 12 h after the seizures period, followed by a decrease (20%) at 24 h (Fig. 2A). GLT-1 showed no alterations after 48 h. The immunocontent of GLAST increased around 2 fold in hippocampi obtained from KA group only up to 48 h after seizures (Fig. 2B). The immunocontent of the neuronal EAAC1 glutamate transporter was not affected by KA-induced (Fig. 2C). We next investigated the long-term modifications of the density of glutamate transporters in the hippocampus; in 60-day-old rats the GLT-1 and GLAST immunocontent increased, and the EAAC1 immunocontent decreased, compared with younger animals. However, there was no difference between the immunocontent of GLT-1, GLAST or EEAC-1 in kainate-treated compared to control rats at 60 days of age. Additionally, the immunocontent of hippocampal glutamine synthetase (GS) was not affected by KA-induced seizures at any time point investigated (Fig. 3).

3.2. Effect of kainate-induced seizures on hippocampal GFAP immunoreactivity

As the hippocampal glutamate uptake and the immunocontent of astrocytic (GLT1 and GLAST) glutamate transporters were

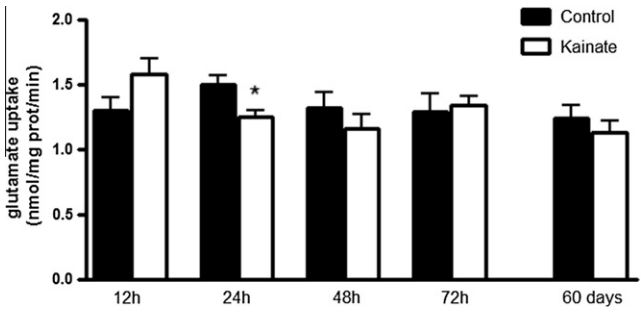

Fig. 1. Effects of a single episode of neonatal seizures on the hippocampal [^{3}H] glutamate uptake. Hippocampal slices were obtained at 12, 24, 48 and 72 h and 60 days post-seizures. Data are expressed as mean ± SD from 6 animals/group. $^*P < 0.05$, significant difference from control group within the time analyzed (One-way ANOVA followed by Tukey's post-hoc test).

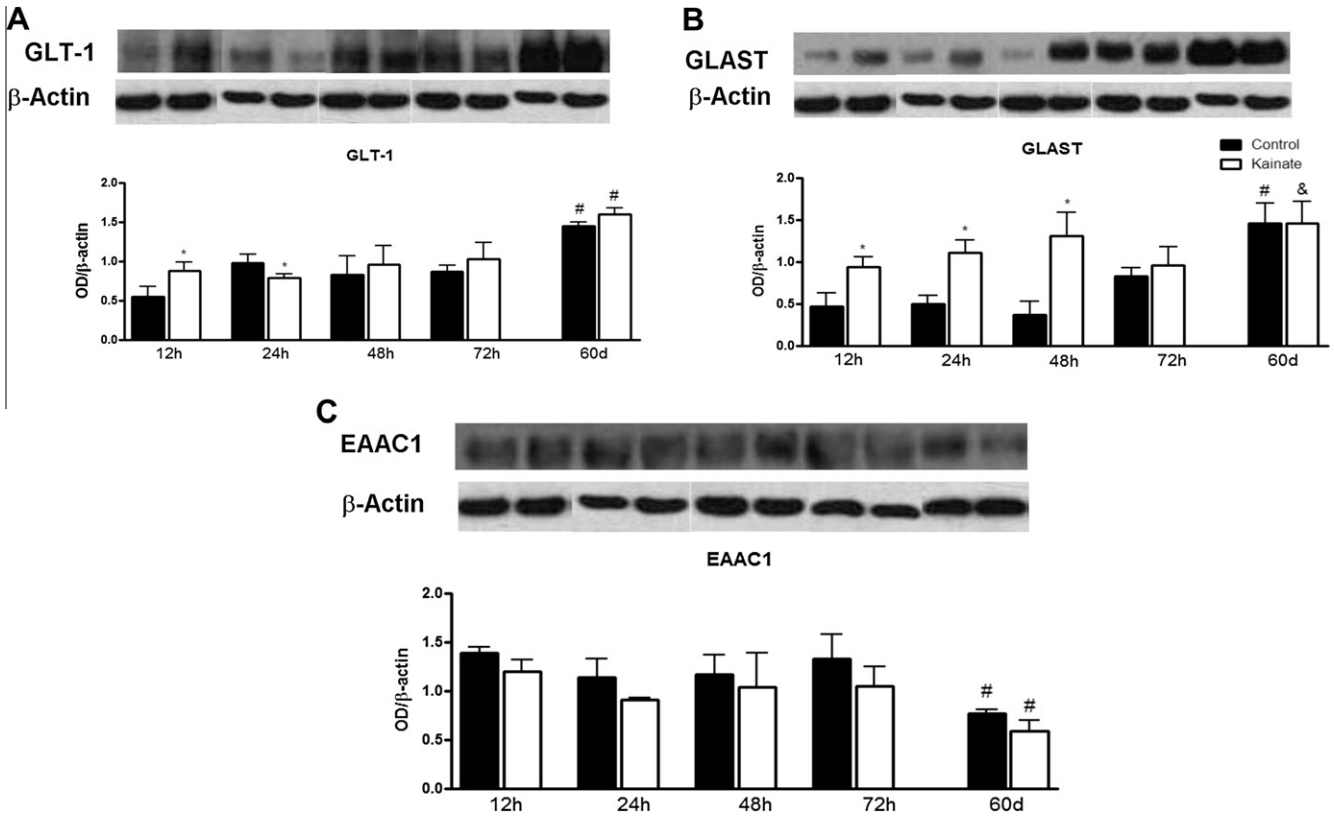

Fig. 2. Effects of a single episode of neonatal seizures on the immunocontent of the glutamate transporters (GLT-1, GLAST and EAAC1), analyzed at 12, 24, 48 and 72 h and 60 days post-seizure. Control (black bars) and kainate (KA, white bars). Data are expressed as mean ± SD from 6 animals/group. At the top of the figure are representative images of the immunocontent of transporters. β-Actin was used as a protein loading control. $^{*}P < 0.05$, significant difference from control group within the specific time. $^{\#}P < 0.05$, significant differences within the group from other times analyzed; $^{\&}P < 0.05$, significant difference from K72h (One-way ANOVA followed by Tukey's post-hoc test).

modified in the hippocampus 24 h after the end of seizures episode, immunohistochemical analysis for GFAP, NeuN and DAPI was performed in this time in all subfields of the hippocampus [CA1, CA3 and dentate gyrus (DG)]. There was an increase in the GFAP immunoreactivity in KA group as compared to control group in all subfields (Fig. 4). In the regions surrounding pyramidal layer (SPL) and over pyramidal layer (PL) of CA3 there was an increase of 147% and 100% for GFAP immunoreactivity compared to control group, respectively (Fig. 4; first panel). Likewise, surrounding pyramidal layer (SPL) and over pyramidal layer (PL) of CA1 there was an increase of 100% and 40% for GFAP immunoreactivity compared to control group, respectively (Fig. 4; second panel). GFAP immunoreactivity increased 100% compared to saline-treated rats in the dentate gyrus (DG) (Fig. 4; third panel). NeuN immunoreactivity and DAPI staining were similar between both groups, indicating absence of neuronal loss 24 h after seizure (data not shown).

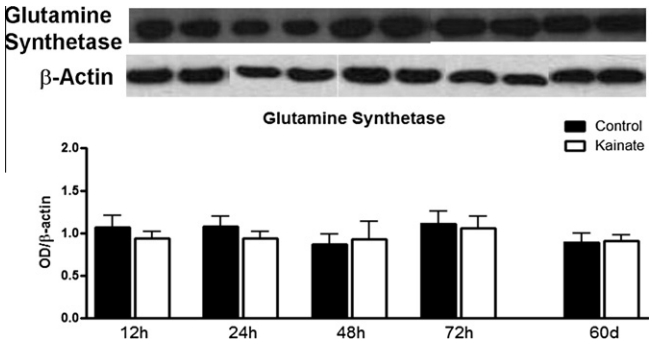

Fig. 3. Effects of a single episode of neonatal seizures on the immunocontent of glutamine synthetase (GS). GS immunocontent was analyzed at 12, 24, 48 and 72 h and 60 days post-seizure. Control (black bars) and kainate (KA, white bars). Data are expressed as mean ± SD from 6 animals/group. At the top of the figure are representative images of the immunocontent of GS. β-Actin was used as a protein loading control.

3.3. Kainate-induced seizures caused anxiety-like behavior and aversive memory impairment in adulthood

Sixty days after the seizures episode, male rats were submitted to behavioral tasks. In elevated plus-maze task, aiming to assess anxiety-related behavior (Fig. 5), kainate-treated rats presented a decrease on the time spent and the number of entries in open arms compared to saline-treated rats (Fig. 5). Kainate-treatment abolished the short- (1.5 h after training) and long- (24 h after training) term memory, evaluated in an inhibitory avoidance task (Fig. 6).

4. Discussion

The present study shows that rats presenting KA-induced seizures in early periods of development presented brain acute molecular and biochemical alterations related to the glutamatergic system, and long-term behavioral impairment in adulthood.

The short-term effects investigated were on hippocampal glutamate uptake and on astrocytic glutamate transporters immunocontent. At 12 h after seizures, there was an increase in the glutamate uptake (that did not reach statistical significance) and in both GLT-1 and GLAST immunocontent. At 24 h after seizures, the GLAST levels remained up regulated, while the glutamate uptake activity and the GLT-1 levels became diminished. The EAAC1 and glutamine synthetase levels did not vary.

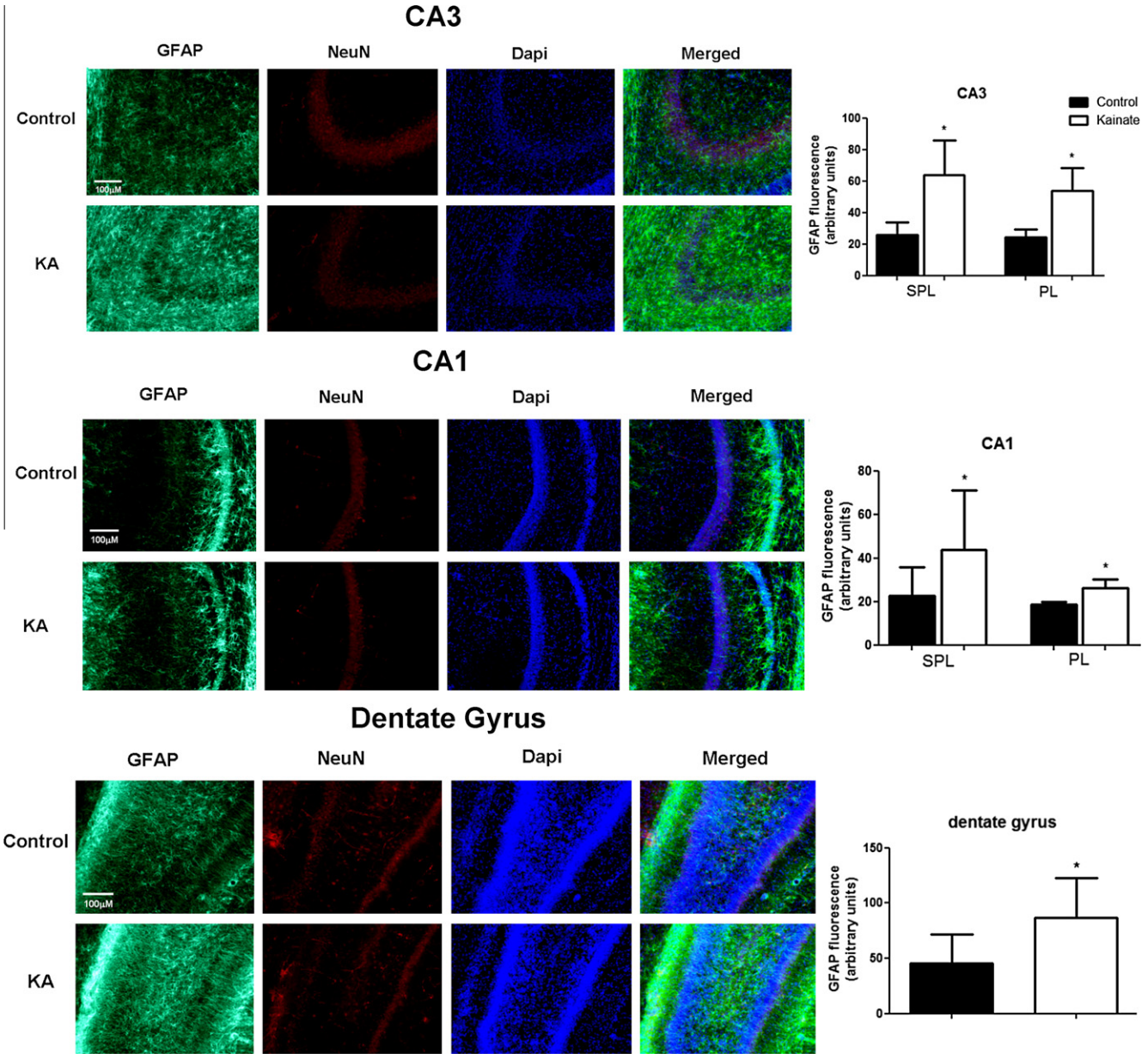

Fig. 4. Immunohistochemistry analysis for GFAP, NeuN and DAPI in the rat hippocampus after a single episode of neonatal seizures. GFAP, NeuN and DAPI reactivities were evaluated 24 h after kainate-induced seizures. Fluorescence quantification was achieved at the regions surrounding pyramidal layer (SPL) and over pyramidal layer (PL), except for dentate gyrus (DG), using pictures acquired in 20x of magnification. Control (black bars) and kainate (KA, white bars). Data are expressed as mean ± SD from 4 animals per group. $^{*}P < 0.005$; significant difference from control group (Student's *t*-test).

Based upon the common pattern of temporal adaptation, GLT-1 seems to be responsible for the transient increase and further decrease on glutamate uptake observed in the hippocampus obtained 12 and 24 h after the end of seizures, respectively. Considering that in the forebrain regions, it was demonstrated that GLT-1 accounts for more than 95% of the total high-affinity glutamate uptake capacity by cerebrocortical synaptosomal preparation and it is the most abundant in CNS (Tanaka et al., 1997; Chao et al., 2010), this correlation may embody a relevant pathophysiological response to seizures (Ueda et al., 2002). Previous study had already been conducted on the expression of glutamate transporters following kainate treatment during brain development and no differences were found for hippocampal GLT-1 mRNA levels 4, 8 and 16 h after kainate-induced seizures in rats at 7 days old (Simantov et al., 1999). These differences between the studies could be due to the required time course for changes in the mRNA expression (measured in the Ref. Simantov et al., 1999) and in the detection on the translated protein (measured in our study). Interestingly, GLAST was the only glutamate transporter in newborn rats treated with kainate that remains up regulated and the same profile for GLAST mRNA levels was also observed in adult animals (Nonaka et al., 1998). Additionally, it is noteworthy that the glutamate uptake apparently follows the ontogeny of GLT-1 during brain development (Ullensvang et al., 1997). Although it remains to be determined if glutamate uptake in acutely isolated slices from rat pups could be related to nerve terminals, glial cells or both cellular compartments, a recent study reported that the uptake activity into acutely dissociated slices from adult animals was related to nerve terminals rather than glial uptake (Furness et al., 2008). More investigations need to be performed helping to elucidate this topic. Our findings ruled out the participation of EAAC1 transporter in the kainate-induced seizures in newborns. Interestingly, the

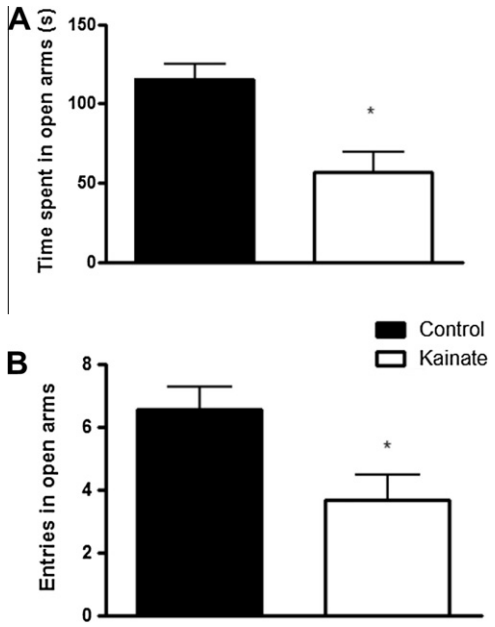

Fig. 5. Behavioral performance in adult rats submitted to a single episode of neonatal seizure. Animals were exposed to 5 min of exploration in the elevated plus-maze. (A) Time spent in seconds (s) in the open arms; (B) number of entries in the open arms. Data are expressed as means ± SD from 10 animals per group. *$P < 0.05$; significant difference from control group (Student's *t*-test).

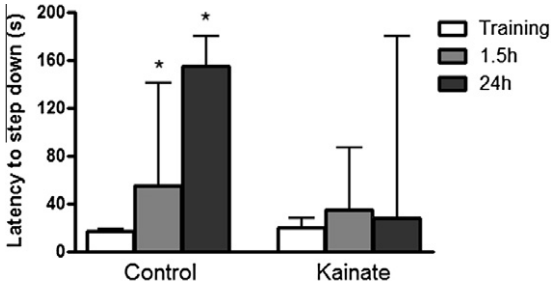

Fig. 6. Performance in the inhibitory avoidance task in adult rats submitted to a single episode of neonatal seizure. Data are expressed as median ± interquartile range ($n = 10$ per group) of the latencies to step down in seconds (s) in the training and test session performed 1.5 h (short-term memory) and 24 h (long-term memory) later. *$P < 0.05$; significant difference from training and test session within group (Wilcoxon test).

same could not be observed in adult animals submitted to kainate-induced seizures, since hippocampal EAAC1 mRNA expression remains increased up to 5 days after seizures (Nonaka et al., 1998).

As the kainate toxicity depends on the release of endogenous excitatory amino acids (Ben-Ari, 1985; Coyle, 1983; Sperk et al., 1983) and *in vitro* studies indicated that glutamate stimulates glutamate transport in primary astrocyte cultures (Gegelashvili et al., 1996), it can be hypothesized that the transient up regulation of both transporters could reflect an attempt to remove the excess of extracellular glutamate that accumulate during seizure periods (Ueda et al., 2002). As the GLAST immunocontent was more specifically involved in short (Duan et al., 1999) and prolonged (Gegelashvili et al., 1996) stimulatory effect triggered by glutamate on its own uptake by cultured astrocytes, the longer lasting increase in the GLAST immunocontent after KA-induced seizures here observed (up to 48 h) could be interpreted as a neuroprotective response to the increase of hippocampal glutamate extracellular levels.

It is interesting to note that the increase in the immunoreactivity for GFAP-positive astrocytes, which was measured 24 h after the end of seizures, accomplished the increase in the GLAST immunocontent. Epilepsy is characterized by hippocampal sclerosis, which one striking hallmark is astrocytic "reactive gliosis", accompanied by neuronal cell loss, microvascular proliferation and synaptic reorganization (Binder and Steinhauser, 2006; Seifert et al., 2010). The occurrence of seizures affects astrocytes functions generating abnormal glutamatergic and GABAergic neurotransmission activities, which precedes neuronal death (Kang et al., 2006). Accordingly, it has been shown that kainate treatment caused detectable cell damage 72 h after seizures, in 10 days old rats (Dunleavy et al., 2010). The hippocampal damage can also be observed in other seizure models in 15 days old animals (de Oliveira et al., 2008; Sankar et al., 1998; Sperber et al., 1999). In our study, astrogliosis was present in the hippocampus 24 h after seizures, with no evident signs of neuronal damage; however, it cannot be discarded the occurrence of neuronal damage after this time.

The ontogenetic profile of glutamate transporters levels observed in our findings is in agreement with previous data (Ullensvang et al., 1997; Bar-Peled et al., 1997; Furuta et al., 1997), since GLT-1 and GLAST levels increased, whereas EAAC1 decreased in adult animals. Interestingly, seizures at 7-day old did not modify the immunocontent of glutamate transporters in the adulthood.

It has been reported that patients with medical intractable mesial temporal lobe epilepsy (MTLE) present deficiency in the hippocampal glutamine synthetase (GS) Eid et al., 2004. Likewise, animals treated with methionine sulfoximine, which leads to deficiency in the GS activity, presented recurrent seizures, hippocampal atrophy and neuronal loss (Eid et al., 2008). These findings suggest that GS may play a role in the pathogenesis of MTLE that could contribute to glutamate accumulation observed in this condition. In our study, GS hippocampal levels were not affected by kainate-induced seizures.

Even though the short-term alterations in the hippocampal glutamatergic parameters were not persistent over time, in adulthood the rats presented anxiety-related behavior and memory decline in an inhibitory avoidance task. Behavioral alterations caused by kainate-induced seizure were investigated in other studies. The performance in behavioral tasks was analyzed using different paradigms, and they indicated that poor memory performance is observed in adulthood after seizure (Cognato et al., 2010; Cornejo et al., 2007, 2008; Sun et al., 2009). These behavioral findings were related to synaptic alterations, such as reduction of synaptic proteins SNAP-25, syntaxin, PSD-95 and NMDA receptor (Cognato et al., 2010; Sun et al., 2009). In our study, besides memory impairment, we also observed anxiety-like behavior in adulthood after seizure episode, although we recognize that this is not a common finding compared to other studies (Cognato et al., 2010; Cornejo et al., 2008). These long-lasting behavioral alterations might be related to early changes in hippocampal glutamatergic neurotransmission. A previous study of our group also suggested that alterations in early periods after birth could be involved in behavioral deficits in adulthood (Moreira et al., 2010). The exact mechanism involved in the long-term effects of KA-induced seizures on behavioral performance in adulthood is still unknown, but appears to involve impairment of the long-term potentiation, enhanced long-term depression and reduction on synaptic proteins levels (Cognato et al., 2010; Cornejo et al., 2007; Sun et al., 2009). Apparently, astrogliosis is not persistent up to adulthood in this model (Cognato et al., 2010).

5. Conclusion

The early periods of brain development are of great relevance and determine adequate brain function late in lifespan. Our study indicates that a single convulsive event in early life could induce

short-term alterations in relevant parameters involved in the homeostasis of glutamatergic neurotransmission in the hippocampus, which could be involved in the behavioral alterations in adulthood animals. Our findings can contribute to better understand the role of glutamate transporters in seizures during childhood. From clinical point of view, our data suggest that interventions on the glutamatergic system during seizures in children may be relevant for prevention of brain impairment in adulthood.

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