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Effects of Lamotrigine and Topiramate on Brain Maturation and Cognitive Functions in Offspring of Pregnant Rats – Preliminary Study

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Antiepileptic drugs (AED) which are used to treat seizures in pregnant women, infants, and young children may cause cognitive impairment or other uncertain injury. However, the precise mechanisms responsible for the negative effects of new AEDs like lamotrigine (LTG) and topiramate (TPM) in the developing brain are still unclear.
Objectives. To investigate the GFAP, NCAM and S100B levels in the whole brain of newborn rats on postnatal 1 day and in the hippocampus of adult rats to find out the effect of TPM and LTG on cognitive impairment and brain maturation.
Material and Methods. Twenty eight pregnant rats were randomly divided into 7 groups with 4 animals in each group. The first group, receiving no drugs, was assigned as the control group. The study groups received intraperitoneal TPM or LTG injections in each trimester. Western blot analysis of the GFAP, NCAM and S100B was performed in the offspring. Behavioral tests were performed at postnatal day 75.
Results. The rats in the TPM-I and TPM-III groups had a significant impairment in escape latency on the 5th day as compared to the control rats in a Morris water maze test. In addition, in the expression of astrocyte derived markers, GFAP was upregulated, whereas S100β and NCAM were downregulated in the whole brain on postnatal day 1, in offspring exposed to LTG and TPM *in utero*.
Conclusions. The detrimental effects of TPM and LTG appear to be confined particularly to the early stages of brain development. And TPM seems to have a partial role in the cognitive impairment (**Adv Clin Exp Med 2014, 23, 5, 691–698**).

Key words: topiramate, lamotrigine, brain development, cognitive functions.

The new generation AEDs such as lamotrigine (LTG) and topiramate (TPM) represent a potential improvement for patients whose seizures are incompletely controlled or who experience significant adverse effects with older anticonvulsants [1]. These two drugs are teratogenic and prenatal exposure has been associated with an increased risk of congenital abnormalities affecting a variety of organs, including the central nervous system [2]. High doses of TPM have been reported to be associated with cognitive impairments [3]. Impairments in cognitive functions such as learning and memory have usually been associated with congenitally occurring structural and molecular alterations in the

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limbic system, particularly the hippocampus of offspring [4]. These alterations are not clearly seen at birth but form the basis for various functional defects noticible gradually during further maturation.

Although a limited number of animal model studies show less teratogenic effects with the new AEDs compared to the known effects in animal models exposed to the older agents, it is still not known whether these new antiepileptic drugs have deleterious effects on brain maturation and cognitive functions of the fetusus of pregnant woman. A recent study has shown that prenatal exposure to lamotrigine during the neuronal migration stage causes neocortical and hippocampal alterations [5]. It is likely that these antiepileptic drugs might affect fetal brain maturation and cognitive functions.

There are a number of markers indicating brain injury affecting cognitive functions, such as neural cell adhesion molecules (NCAM), glial fibrillary acidic protein (GFAP) and S100β. NCAM is known to participate in synaptogenesis in neuronal plasticity and particularly involved in synaptic changes underlying memory formation in adult individuals [6]. Glial fibrillary acidic protein (GFAP) is one of the intermediate filament proteins of glial cells and is involved in neuronal differentiation and maturation, which is recognized as an astrocyte maturation marker and reactive astrocytes [7]. In response to neuronal damage such as brain injury [8], stroke [9], or induced seizures [10], reactive hypertrophy and proliferation of astroglial cells can be promoted, a process known as astrogliosis. S100β is a Ca^{2+} binding protein expressed in Schwann cells and astroglia in the central nervous system, which is overexpressed in response to neuronal damage [11].

Therefore, we aimed to study the expression levels of NCAM, GFAP and S100β protein and performed Morris water maze tasks to search for possible mechanisms of brain injury and cognitive impairment in the offspring of pregnant rats exposed to intraperitoneal LTG or TPM injections.

Material and Methods

Animals and Experimental Design

The experimental protocols were approved by the local Animal Use Committees. Twenty eight healthy adult female Wistar albino rats, aged 4–5 month and weighing in the range of 210–250 g, were used, and were housed in polycarbonate cages in a room with a 12 h day–night cycle, temperature of 24 ± 3°C and humidity of 45 to 65%.

Pro-estrous animals were kept overnight with male rats and the next day was taken as gestational day 0 when spermatozoa were seen in a vaginal smear. The pregnant rats were randomly divided into 7 groups with 4 animals in each group in the following way: the first group of rats, receiving no drugs, was assigned as the control group (n = 4). The animals receiving intraperitoneal topiramate injection at 25 mg/kg in the first trimester were assigned as (TPM I), the second trimester as (TPM II) and the third trimester as (TPM III), respectively. Similarly, the animals receiving intraperitoneal lamotrigine injection at 25 mg/kg in the first trimester as (LTG I), the second trimester as (LTG II) and the third trimester as (LTG III). The pregnant rats in group LTG I had 34 offspring, LTG II had 32, LTG III had 36, TPM I had 28, TPM II had 32, TPM III had 36 and the control animals had 42. After delivery on postnatal day (PND) 1, the litter size, body weight and gender of the offspring of each litter were recorded. Half of the offspring per group were decapitated (total n = 120) and their brains were stored at –70 °C for the Western blot analysis of NCAM, GFAP and S100β on PND1. The remaining offspring from each experimental litter were weaned from their dams when they were 21 days old and they were separated into male and female cages. The Morris water maze test [12] was employed to study the cognitive status of the animals, regarding spatial learning and memory, at postnatal day 75. After 24 h, young-adult offspring (n = 120) from all groups were decapitated. Brain tissues were removed and the hippocampi were dissected and stored at –70°C.

The detailed descriptions of the methods on imminoblotting for the measurements of NCAM, GFAP and S100β, and the Morris water maze test for studying the cognitive status of the animals regarding spatial learning have been described in our earlier publication [13].

Statistical Analysis

The results were expressed as mean ± SD one--way analysis of variance (ANOVA) and *post hoc* Tukey-HSD tests were used to determine differences in all parameters between groups. Differences were considered statistically significant if the p-value was < 0.05.

Results

Learning and memory performances of the offspring of pregnant rats exposed to intraperitoneal LTG and TPM.

There were no still births in any of the groups except group topiramate I, in which there were 27% still birth. The duration of pregnancy and litter size were not statistically different between the groups. After birth, all the living animals survived the procedure, with no evidence of neurological deficits.

The effects of prenatal TPM and LTG exposure on learning and memory performance were examined using the Morris water maze test of a hidden--platform, a hippocampus-dependent task, which requires an animal to learn and remember the relationships between the platform location and multiple distal cues to escape the water [14], the mean escape latencies, which is the time for rats to reach the platform, in control, LTG-I, LTG-II, LTG-III, TPM-I, TPM-II and TPM-III groups were comparable in the first trial, suggesting that their motor performances (ability to swim) were unaffected by prenatal LTG and TPM exposures. All animals were able to swim in a normal way during all trials and control rats and rats whose mothers were exposed to LTG and TPM during pregnancy learned the task, as evidenced by a decrease in the escape latency to find the platform from the first to the last day of training (day 5) ($p < 0.001$). However, the rats in the TPM-I and TPM-III groups had a significant impairment in escape latency on the 5th day as compared to the control rats, since TPM-I and TPM-III groups tended to take longer to find the platform than the controls in the last trials ($p < 0.05$ vs $p < 0.001$) (Fig. 1 A). Whereas, there were no significant differences between the performances of LTG-I, LTG-II, LTG-III and control groups (Fig. 1B).

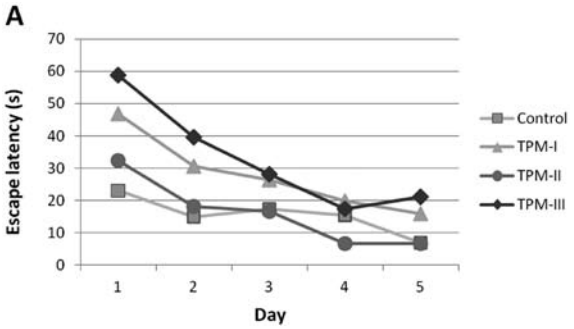

Fig. 1A. Morris water maze test results of rats in the TPM and control groups

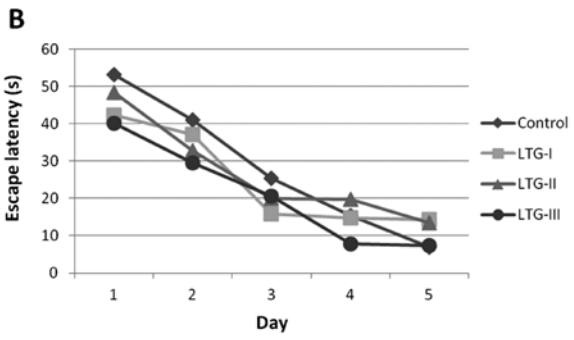

Fig. 1B. Morris water maze test results of rats in the LTG group and controls

Levels of GFAP, S100β and NCAM in the Offspring of Pregnant Rats Exposed to LTG and TPM

In order to demonstrate if LTG and TPM exposure during the first, second or third trimester causes reactive gliosis in the offspring, expression levels of GFAP, NCAM and S100β were studied using semiquantitative Western blotting.

GFAP protein had main band of relative molecular weights of 49 kDa and several bands for degradation products of smaller molecular weight at roughly 42–47 kDa (Fig. 2, shown by arrows). The main bands and degradation products of GFAP protein in the whole offspring brain was significantly elevated in all groups except the TPM-I group, compared to the control, indicating an induced glial hyperactivity (Fig. 2A2). There were also significant increases in GFAP content in the hippocampal tissues from adult rats in only LTG-I and TPM-III groups (Fig. 2B2).

The major band representing the main S100β polypeptide migrates at a relative mobility of about 50 kDa (Fig. 3, shown by arrows). The amount of S100β protein in the whole offspring brain of rats from all TPM and LTG groups were significantly lower than in the control group, suggesting a delayed maturation of astrocytes, which is linked to brain maturation (Fig. 3A2). Whereas, the amounts of S100β protein was higher in the hippocampus of adult rats from TPM and LTG groups except TPM-I compared to control (Fig. 3B2).

As shown in the figure (indicated by an arrow), two bands in each line could be detected. The 140-kDa isoform of NCAM in LTG-II and TPM-III groups were found to be significantly lower in comparison to the control group in the whole offspring brain (Fig. 4A2). Similarly, the 180-kDa isoforms of NCAM were found to be significantly lower in the whole offspring brain of the TPM-III group in comparison to the control group (Fig. 4A3). No significant difference was discerned in the 140- and 180-kDa isoforms of NCAM in the hippocampus of adult LTG&TPM rats compared to control group (Fig. 4B2 and B3).

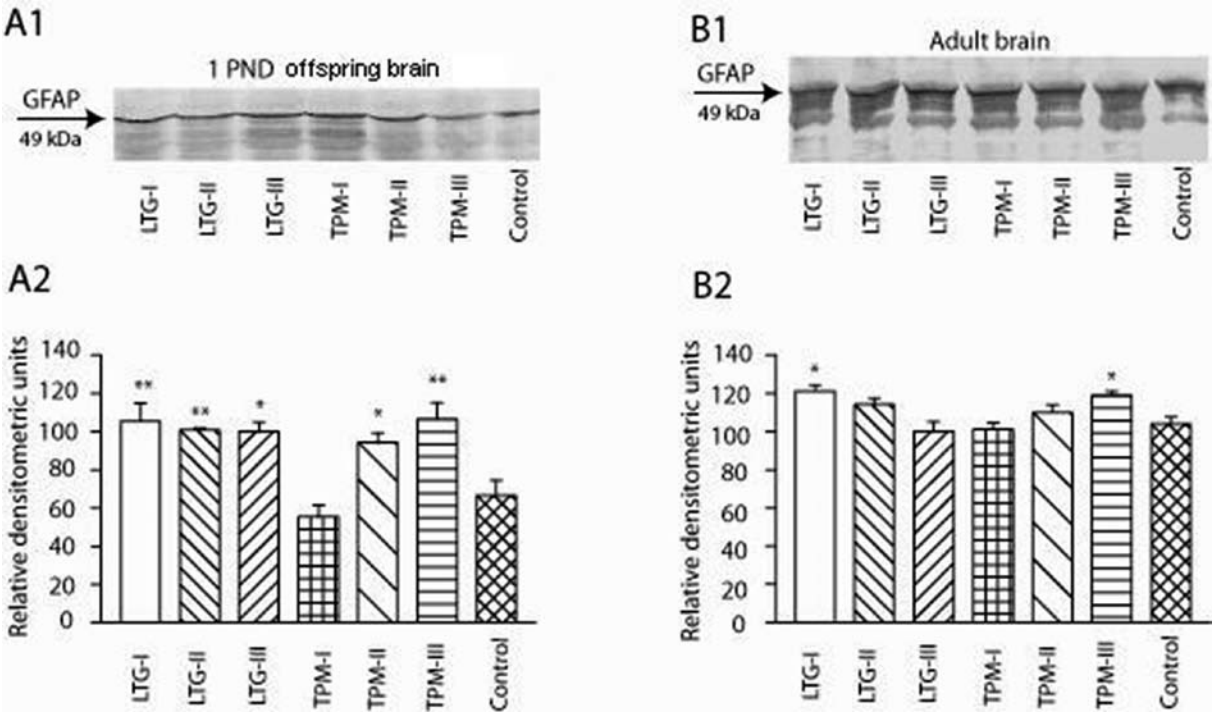

Fig. 2. Western blot analysis of GFAP. Representative bands in whole offspring brain (A1) and in the hippocampal tissues from adult rats (B1) and their respective densitometric analysis in whole offspring brain (A2) and in the hippocampal homogenates from adult rats (B2). *P, 0:05, **P, 0:01 and ***P, 0:001 vs. control values

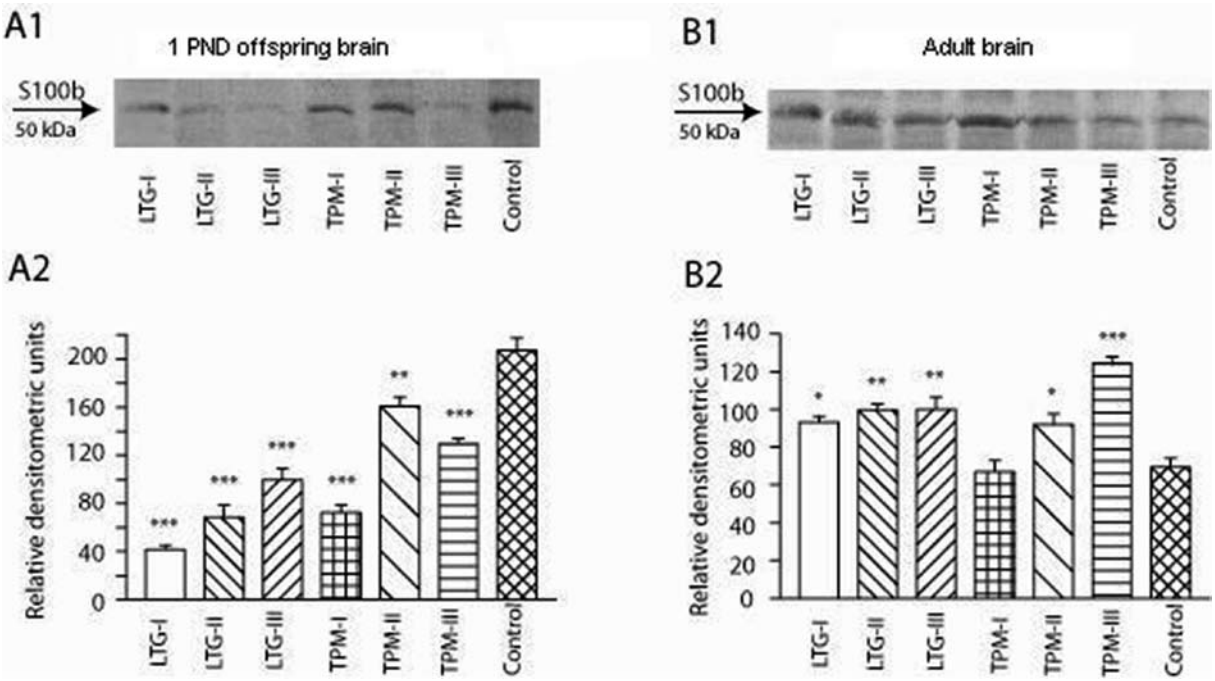

Fig. 3. Western blot analysis of S100β. Representative bands in whole offspring brain (A1) and in the hippocampal tissues from adult rats (B1) and their respective densitometric analysis in whole offspring brain (A2) and in the hippocampal homogenates from adult rats (B2)

Discussion

Recent studies have reported that new generation AEDs are less teratogenic and have less cognitive impairment than traditional AEDs [15–18]. However, there are few studies about the long-term effects of AEDs such as cognitive impairment [19, 20]. The exact mechanisms responsible for the adverse effects of AEDs on both brain maturation and long term cognitive impairment are

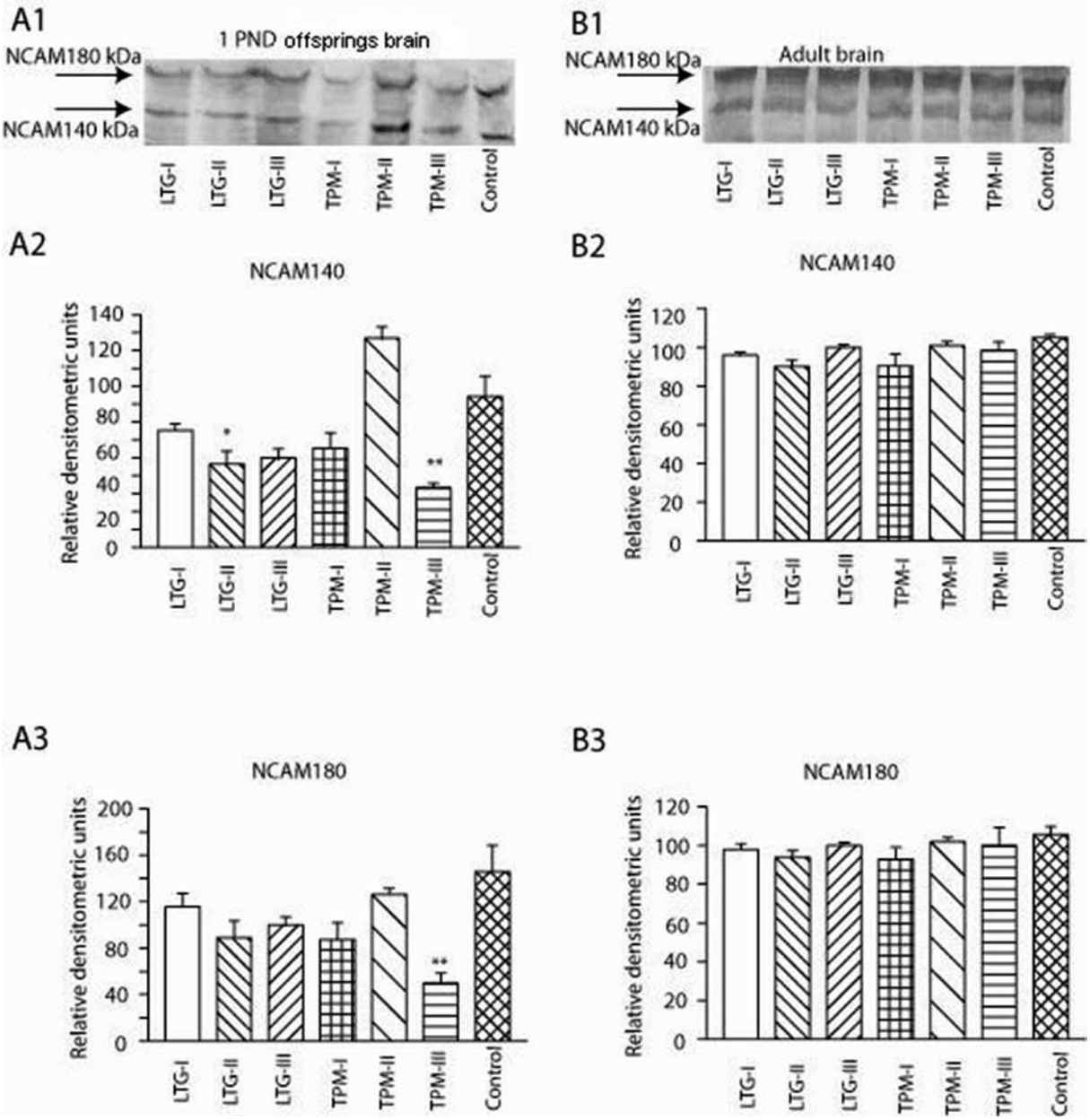

Fig. 4. Western blot analysis of NCAM 140 and NCAM180. Representative bands of NCAM 140 and NCAM180 in whole offspring brain (A1) and in the hippocampal tissues from adult rats (B1) and their respective densitometric analysis in whole offspring brain (A2 and A3 respectively) and in the hippocampal homogenates from adult rats (B2 and B3 respectively)

still not clear. Therefore, in the current study, we investigated both the brain maturation of the offspring and the cognitive status of the animals regarding spatial learning and memory.

In previous studies, high doses of TPM and LTG have been reported to be associated with brain maturation defects including cognitive impairments in some offspring of TPM- or LTG-treated mothers [3]. Impairments in cognitive functions such as learning and memory have usually been associated with congenitally occurring structural and molecular alterations in the limbic system, particularly the hippocampus of offspring [4]. However, to the best of our knowledge, there is only one study evaluating the effects of maternal exposure to TPM and LTG on cognitive behavior in offspring [19].

The elevated plus maze and Morris water maze test have been used to evaluate the cognitive status in rats [21]. Here, we performed the Morris water maze test to determine the association between maternal exposure to TPM and learning and memory retrieval deficits in their young-adult offspring. Cognitive performance was impaired in rats exposed to TPM in the first and third trimesters *in utero*, since the TPM-I and TPM-III groups

took significantly longer to find the hidden platform than the controls in the last trials of the Morris water maze test. Various mechanisms have been proposed for the teratogenicity of AEDs, including folate-related action, ischemia, neuronal suppression, reactive intermediates (free radicals) and AED-induced neuronal apoptosis. Anatomical and behavioral or cognitive teratogenesis differ in mechanisms. Because of the fetal organ development in the first trimester, AED exposure may pose the highest risk for anatomical malformations, and the above causes may have caused a difference in still births or cognitive performance in group TPM I. In the third trimester of pregnancy, the human brain develops fast structural and functional changes. During this developing process, neuronal and glial cells differentiate, mature and migrate until the term infant brain architecture is fully developed [22]. With these structural changes functional changes are occurring also. For this reason, there is a difference between groups TPM I and TPM III in cognitive performance. This data suggests that rats exposed to TPM *in utero* had a long-lasting deficit in spatial learning and memory. The learning deficit in the rats exposed to TPM appears to result from impairments in spatial learning, not from swimming ability or visual acuity because there was no significant difference between the groups in latency to reach the platform in the first trial [23].

Impairment in spatial learning in the rats exposed to AEDs *in utero* might be owing to developmental delays in brain maturation and neurogenesis during gestation, since memory impairments have been related to a decline in synaptic plasticity [4]. Therefore, the levels of glial intermediate filaments and NCAM expression were studied in the whole brain from PND1 offspring and in the hippocampus of 81 day-old young rats exposed to LTG and TPM *in utero*.

Expression levels of GFAP is commonly used as a marker for changes in astroglial cells during brain development and injury [24]. Injury of the brain resulting from trauma, disease, genetic disorders, or chemical insult causes astrocytes to become reactive, a condition characterized by an increase in GFAP [25]. In the current study, exposure to LTG and TPM *in utero* caused significant increases in GFAP in the whole brain from PND1 offspring as measured by Western blotting, indicating reactive gliosis. Although increased GFAP is commonly associated with astroglial activation, increased GFAP alone does not allow us to draw conclusions about the type of astroglial response, be it beneficial or deleterious [26].

S100β protein, a cytosolic constituent of the astrocytes, is a calcium binding protein predominantly expressed and secreted by astrocytes in the central nervous system [27]. As shown in Fig. 3A1 and A2, the amount of S100β protein in the whole offspring brain of rats from all TPM and LTG groups were significantly downregulated compared to the control. This suggest a delayed maturation of astrocytes [13]. However, expression of brain tissue S100β has been upregulated in neurodegenerative disorders, such as Alzheimer's disease [28]. The increased level of S100β in the hippocampus of 81 day-old young rats exposed to LTG and TPM *in utero* (Fig. 3B1 and B2) appearantly results from upregulation of S100β expression in astrocytes [28], which is likely to be due to neurodegeneration induced by TPM and LTG *in utero*. It has been suggested that increments of S100β may improve neurogenesis, particularly in the hippocampus [29] and also stimulate glial proliferation and neuronal survival and protect neurons against glutamate excitotoxicity [30]. NCAM is capable of incorporating long chains of α 2,8 polysialic acid (PSA) [31]. The polysialated NCAM seems to be involved in several developmental events such as neuronal migration [32], synaptogenesis [31], axonal outgrowth and fasciculation [33]. The expression of PSA-NCAM is developmentally regulated [34]; PSA-NCAM is dramatically downregulated in the brain except in areas associated with neuroplastic events such as in the olfactory bulb and hippocampus [35]. The findings in our study that animals exposed to TPM *in utero* had impaired spatial learning is meaningful, since NCAM expression in the whole brain of PND1 offspring exposed to TPM in the, particularly, third trimester *in utero* was downregulated.

In conclusion, the lower levels GFAP, S100β, and NCAM, in particular GFAP and NCAM, in the hippocampus of young rats exposed to TPM and LTG suggest that the detrimental effects of TPM and LTG appeared to be confined particularly to the early stages of brain maturation. Additionally, the early effects of TPM exposure *in utero* resulted in impairments of spatial learning. These results indicate that TPM, but not LTG, seem to have a partial role on the cognitive impairment.

Study Limitations

We are aware of the fact that there are some limitations in the present study. One of them is the small number of samples. Another is the application of uniform doses and absence of the effects of different doses. Furthermore, more realistic results could be obtained with the evaluation of serum concentration of drugs versus dose per kilogram. Therefore, additional studies with larger samples and different doses are needed.

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