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Antiepileptic drugs and brain maturation: Fetal exposure to lamotrigine generates cortical malformations in rats

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Summary Intake of antiepileptic drugs (AEDs) during pregnancy can provoke severe and subtle fetal malformations associated with deleterious sequelae, reflecting the need for experimental investigations on the comparative teratogenic potential of these agents. We recently reported that prenatal exposure to vigabatrin and valproate, two AEDs which act through GABAergic mechanisms, induces hippocampal and cortical dysplasias in rodents. We have now investigated the effects of phenobarbital (PB, 30 mg/kg day) i.p.), a drug also endowed with GABAergic effects, and the new generation AEDs lamotrigine (LTG, 5—20 mg/kg/day i.p.), topiramate (TPM, 10 mg/kg/day i.p.), and levetiracetam (LEV, 50 mg/kg/day i.p.) on brain development. Prenatal exposure to LTG induced hippocampal and cortical malformations in a dose-dependent manner, at maternal plasma concentrations within the clinically occurring range. These abnormalities were not observed after exposure to PB, TP and LEV. These observations raise concerns about potential clinical correlates and call for detailed comparative investigations on the consequences of AED use during pregnancy.

Introduction

Managing pregnancy in women with epilepsy aims at minimizing the risks of seizures as well as the risks of adverse drug effects in the mother and the fetus. Many studies have shown that prenatal exposure to antiepileptic drugs (AEDs) may cause an increased incidence of congenital abnormalities affecting a variety of organs, including the central

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Table 1 AED dosages used in this study in comparison with other animal studies and clinically used dosages

Drug name	Dosages used in this report (mg/kg/day)	Effective doses as anticonvulsant in animal studies (mg/kg/day)	Most commonly used dosages in adults with epilepsy (mg/day)[a]
Lamotrigine	5—20	5—20[b,c]	50—500
Levetiracetam	50	10—300[d]	1000—3000
Phenobarbital	30	3—30[d]	50—200
Topiramate	10	3—30[d]	100—400

[a] Perucca, 2001.
[b] Ahmad et al., 2005.
[c] Castel-Branco et al., 2005.
[d] Shannon et al., 2005.

nervous system (Holmes, 2002; Perucca, 2005; Meador et al., 2006). The majority of the reported neurodevelopmental consequences of prenatal AED exposure consists in severe, easily detectable malformations such as spina bifida and microcephaly. However, an increased incidence of more subtle brain maturation defects cannot be excluded, and could potentially account for reports of cognitive impairments in some offspring of AED-treated mothers (Koch et al., 1999; Adab et al., 2001; Vinten et al., 2005). The occurrence of subtle abnormalities, however, has been little investigated, mainly because of the technical difficulties involved in their assessment in the clinical setting.

In a recent study, we investigated the consequences of prenatal exposure to different AEDs in rats, and reported that exposure to vigabatrin and valproate, two drugs which possess GABAergic mechanisms of action, induces hippocampal and cortical dysplasias resulting from a neuronal migration defect (Manent et al., 2007). In contrast, exposure to carbamazepine, diazepam and maternal convulsive generalized seizures did not cause statistically relevant brain abnormalities in the offspring, suggesting that only a subset of AEDs can disrupt neuronal migration. In the present report, we used the same experimental paradigm to investigate the possible impact on neuronal migration of prenatal exposure to phenobarbital (PB), a drug also endowed with GABAergic effects, and the new generation AEDs lamotrigine (LTG), topiramate (TPM), and levetiracetam (LEV). We found that exposure to LTG during the neuronal migration stage results in neocortical and hippocampal alterations in a dose-dependent manner. In contrast, the other AEDs examined did not appear to interfere with the maturation of cortical structures. Taken altogether, our earlier data and present findings raise concerns about a previously unrecognized adverse effect of some AEDs, and reinforce the need for more detailed investigations of their potential impact on cortical maturation.

Materials and methods

Treatments

Procedures were performed in agreement with European Union and French legislation concerning care and use of laboratory animals. Pregnant Wistar rats (Janvier, France) received, at intervals of about 12 h two i.p. injections per day of different AEDs from embryonic (E) day 14 (E14, vaginal smear was observed at E0) to E19, a timing which covers selectively the period of neocortical and hippocampal neuronal migration. In a first set of experiments, animals received relatively high doses of PB (Sigma—Aldrich), 30 mg/kg/day ($n = 3$); LTG (GlaxoSmithKline), 20 mg/kg/day ($n = 5$); LEV (UCB Pharma), 50 mg/kg/day ($n = 3$); or topiramate (Janssen-Cilag S.A.), 10 mg/kg/day ($n = 3$) (Table 1). In a second set of experiments, pregnant rats received lower doses of LTG, 5 ($n = 3$), 10 ($n = 3$), and 15 ($n = 3$) mg/kg/day, which are within the range of doses used in humans (Stephen and Brodie, 2002). Control animals ($n = 5$) were injected with vehicle only (DMSO 2% in PBS). Volumes injected were approximately 0.5 ml/injection.

Postnatal (P) 30 rats born to treated animals were anaesthetized with chloral hydrate (Sigma) and perfused intracardially with 4% paraformaldehyde—0.5% glutaraldehyde in PBS. Their brains were then cut coronally (60 μm thickness) with a vibratome (Leica, Nussloch, Germany).

Some pregnant animals (five control animals and three animals treated with LTG 15 mg/kg/day) also received a single i.p. injection with the S-phase marker 5-bromo-2′-deoxyuridine (BrdU, 50 mg/kg, dissolved in PBS—NaOH 0.007N, Sigma) at E15, to identify at P30 the final positioning of cells generated at E15.

In vivo experiments: plasma concentration of LTG

Plasma LTG concentrations were evaluated in separate groups of pregnant animals (six animals per group and sampling time) treated with LTG as described before at a dosage of 5 mg/kg/day and 20 mg/kg/day (i.e.; two daily injections of 2.5 mg/kg or 10 mg/kg), respectively. Treatments were initiated at E14 and animals were sacrificed at E17, under deep chloral hydrate anesthesia, 2 h or 10 h after the last injection, which correspond to the expected times of peak and trough plasma LTG concentrations, respectively (Castel-Branco et al., 2005). Blood samples were collected from each animal, and the plasma was separated and frozen at −20 °C until assay. Plasma LTG concentrations were determined with an HPLC—UV assay according to Bartoli et al. (1997) with minor modifications, including the use of metoclopramide instead of nortripyline as internal standard.

Immunohistochemistry

Brain sections were permeabilized for 10 min at room temperature (RT) in PBS—Triton X-100 (0.1%)—goat serum (5%). For BrdU staining, the permeabilization step was followed by a 20 min incubation in PBS—HCl 2N at 45 °C. Slices were then washed three times in PBS and incubated overnight at RT with primary antibodies diluted in PBS—Triton X-100 (0.1%)—goat serum (5%). The slices were rinsed three times in PBS and incubated for 2 h at RT with appropriate secondary antibodies, used separately for double immunolabeling. After three final washes in PBS, slices were mounted on glass slides and cover-slipped in Gel Mount (Biomedia). The primary antibodies were mouse anti-NeuN (1:1000, Chemicon) and rat anti-BrdU (1:50,

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Harlan Sera-Lab). The secondary antibodies were donkey anti-rat conjugated with FITC (1:200) and goat anti-mouse conjugated with Texas Red (1:200), both from Jackson ImmunoResearch.

Sections were examined under a fluorescence microscope (Nikon Eclipse E800 equipped with a digital camera) or a Zeiss LSM510 confocal microscope using 10× and 20× objectives, and images were digitized using the built-in software. Digitized images were exported as images, stacks of images, Z-projections or orthogonal views in tiff format for quantitative analysis. Some figures given in this report were developed as black and white negatives.

Quantitative analyzes

Two different experimenters, blind to the nature of the treatment received by the animal, performed the examination of randomly selected sections (nine sections per animal, three animals per litter, each section being separated from the others by three sections to minimize the risk of counting the same alterations). Coronal sections were located from 2.92 mm to −4.56 mm from bregma accordingly to the atlas of Paxinos (1998). These involve fronto-parietal somatosensory cortex and dorsal hippocampus. Images were analyzed with ImageJ 1.33d (Wayne Rasband, NIH).

For each section the number of individual alterations was evaluated. These included layer disruptions or gaps (areas depleted of neurons) greater than 30 μm in diameter and layer dispersions (neurons occurring in a dispersed pattern, enlarging the borders of the respective layers). Additional measurements included the mean diameter of layer disruptions; the mean thickness of CA1 pyramidal cell layer and cortical II–IV layers and the maximum and minimum thickness of these layers. The normality of data distribution was checked using SigmaStat (Systat Software), and the effects of treatments were compared using ANOVA or Kruskal–Wallis one-way analysis of variance on ranks and Mann–Whitney tests.

Results

High doses of LTG caused reduction of maternal weight and litter size

PB, TPM and LEV had no effect on maternal weight gain and number of pups born. In contrast, high doses of LTG (20 mg/kg/day, LTG20) reduced maternal weight gain by about 20% as compared to controls (Fig. 1). Litter size was also significantly reduced after exposure to LTG20 (12 ± 1 pups in controls versus 4 ± 5 in LTG20; $p < 0.001$). Of 25 pups exposed prenatally to LTG20, eight died during the first 24 h, while no pup died in the control litters and in litters born to PB-, TPM- and LEV-treated animals. Treatment of pregnant rats with lower doses of LTG (5, 10 and 15 mg/kg, LTG5, LTG10, and LTG15) did not affect either maternal weight gain or litter size.

Prenatal exposure to LTG caused hippocampal and neocortical malformations

Prenatal exposure to PB, TPM, and LEV did not appear to affect cortical or hippocampal maturation, and brain sections from adult animals exposed to these AEDs showed a normal laminar organization as compared to controls (not illustrated). In contrast, treatment with a high dose of LTG (20 mg/kg/day, LTG20) resulted in clear morphological alterations in the parietal cortex (Fig. 2) and in the CA1 and

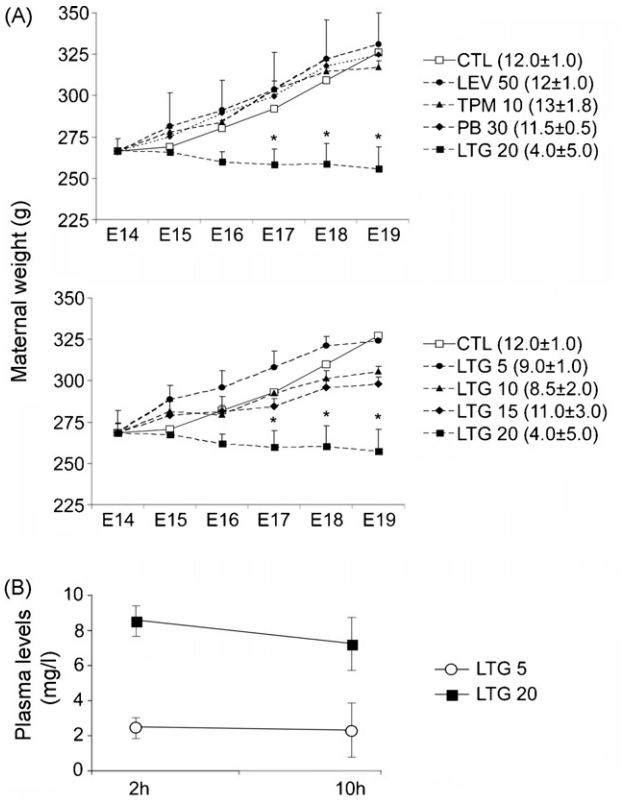

Figure 1 Impact of AED treatments on pregnant rats. (A) Maternal weight gain and litter size after AED exposure. Graphs illustrate maternal weight, expressed (±S.E.M.) as a function of the mean weight of vehicle-treated control animals (CTL), during gestation (from E14 to E19) in rats exposed to different AEDs. Litter size at birth (±S.E.M., given in parentheses) is also shown. Note the lower body weight gain and the smaller litter size after exposure to LTG 20 mg/kg/day i.p. as compared to controls or rats treated with lower LTG doses or other AEDs. (B) Plasma concentrations of LTG. Pregnant rats ($n = 6$ per group and sampling time) were exposed to either 5 mg/kg/day or 20 mg/kg/day of LTG i.p. for 4 days and sacrificed 2 h or 10 h after the last injection. Plasma LTG concentrations were determined as specified in Materials and Methods. The concentrations observed are within the range encountered in patients treated with therapeutic doses. CTL: control, LEV50: levetiracetam 50 mg/kg/day, TPM10: topiramate 10 mg/kg/day, PB30: phenobarbital 30 mg/kg/day, LTG5–20: lamotrigine 5–20 mg/kg/day.

CA3 hippocampal fields (Fig. 3), mainly characterized by the presence of:

(1) *Areas of neuronal depletion* (*or neuronal gaps*): These were clear-cut interruptions in the continuity of the hippocampal pyramidal cell layer or superficial cortical (II–IV) layers. These areas were deprived of neuronal cells or contained only a few scattered neurons. Their size was variable but always larger than 30 μm in diameter (mean diameter: 91.5 ± 3 μm in the neocortex and 51 ± 5 μm in the hippocampus). While neuronal gaps were rarely observed in control sections (0.04 ± 0.04 neuronal gaps/neocortical hemisection and 0.076 ± 0.03 neuronal gaps/hippocampal

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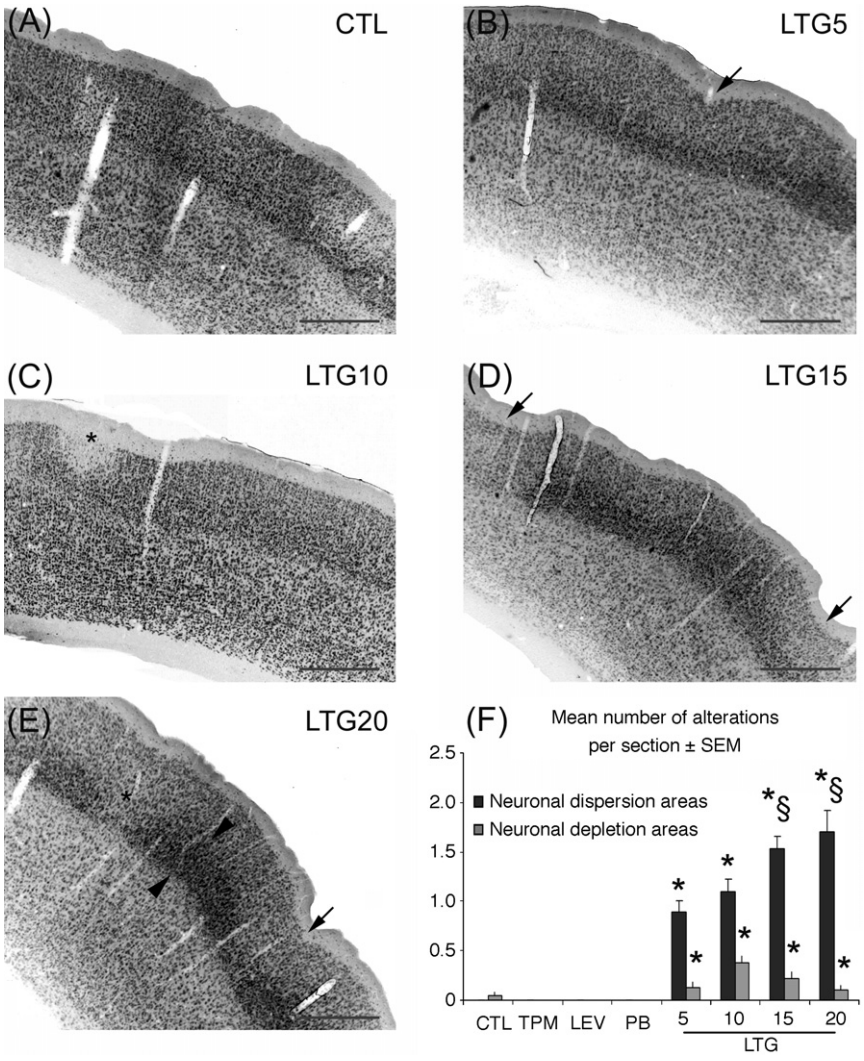

Figure 2 Fetal exposure to LTG causes cortical malformations. (A)—(E) NeuN immunostainings of neocortical sections from P30 rats born to vehicle-treated animals (A) or animals exposed during gestation to LTG 5 mg/kg/day (B), 10 mg/kg/day (C), 15 mg/kg/day (D) or 20 mg/kg/day (E) i.p.. Asterisks indicate the presence of a neuronal depletion area. Arrowheads indicate loss of compaction of the neuronal layers (neuronal dispersion area). Arrows indicate the presence of cortical undulations. Scale bars, 200 μm. (F) Histogram illustrating the mean number of alterations (±S.E.M.) observed per neocortical hemi-section as a function of treatments received by pregnant animals. CTL: control, TPM: topiramate, LEV: levetiracetam, PB: Phenobarbital, LTG5—20: lamotrigine 5—20 mg/kg/day. (*) indicates $p < 0.001$ as compared to CTL; (§) indicates $p < 0.001$ as compared to LTG5 or LTG10 (Mann—Whitney test).

section, $n = 10$ animals, Figs. 2F and 3E), their occurrence was significantly increased in brain sections from LTG20-treated animals (0.10 ± 0.05 neuronal gaps/neocortical hemi-section and 2.84 ± 1.30 neuronal gaps/hippocampal section, $n = 7$ animals, $p < 0.001$, Figs. 2F and 3E).

(2) *Areas of neuronal dispersion*: These were characterized by loss of compaction of the layers, resulting in fluctuations in layer thickness (or undulations). In the hippocampus, dispersions affected the pyramidal cell layer and also resulted in neurons concentrating outside the main pyramidal layer, giving rise in some cases to the formation of a double layer composed mainly of pyramidal neurons located ectopically in the stratum oriens. Alterations in thickness in the neocortex involved essentially layers II—IV. While areas of neuronal dispersion were never observed in neocortical or hippocampal sections from control animals, they were relatively common in brain sections from LTG20-treated animals (0.12 ± 0.05 neuronal dispersion areas/hippocampal section, $p < 0.001$ and 1.7 ± 0.22 neuronal dispersion areas/neocortical hemi-section, $p < 0.001$, Figs. 2F and 3E). Areas of neuronal dispersion alternated with unaltered areas within the same section, so that the mean thickness of layers showed greater variation as compared to controls (Fig. 4A—D). These variations produced the appearance of abnormal undulations: for example, while the mean thickness of the CA1 pyramidal cell layer in control animals ranged from 37 ± 5 μm (smallest diameter) to 54 ± 10 μm (largest diameter), the thickness of this layer in LTG20-exposed animals ranged between 36.63 ± 1.70 μm and 110.70 ± 1.92 μm. Similarly, the thickness of layers II—IV in the parietal

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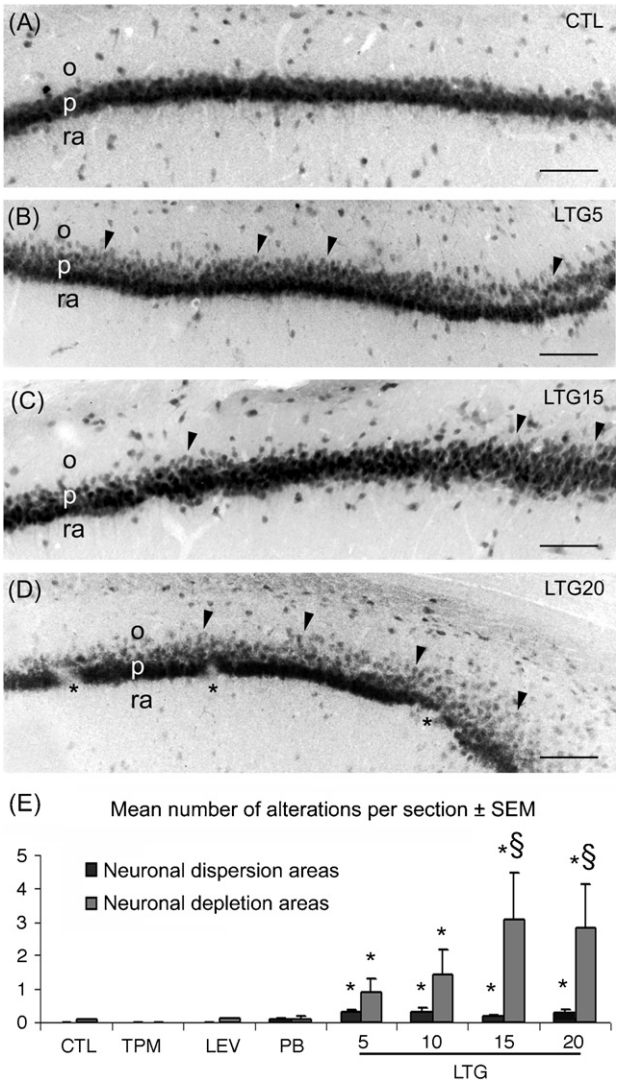

Figure 3 Fetal exposure to LTG causes hippocampal malformations. (A)—(D) NeuN immunostainings of hippocampal sections (CA1 field) from P30 rats born to vehicle-treated animals (A) or animals exposed during gestation to LTG 5 mg/kg/day (B), 15 mg/kg/day (C) or 20 mg/kg/day (D) i.p.. Asterisks indicate the presence of a neuronal depletion area. Arrowheads indicate loss of compaction of the neuronal layers (neuronal dispersion area). Scale bars, 200 μm. (F) Histogram illustrating the mean number of alterations (±S.E.M.) observed per hippocampal section as a function of treatments received by pregnant animals. CTL: control, TPM: topiramate, LEV: levetiracetam, PB: phenobarbital, LTG5—20: lamotrigine 5—20 mg/kg/day. (*) indicates $p < 0.001$ as compared to CTL; (§) indicates $p < 0.001$ as compared to LTG5 or LTG10 (Mann—Whitney test).

cortex ranged from 466 ± 6 μm to 490 ± 10 μm in control sections and from 476 ± 10 μm to 555 ± 13 μm in sections from LTG20-exposed rats. The differences in largest diameter between control and LTG20 animals were statistically significant ($p < 0.001$). When fluctuations in layer thickness were quantified (Fig. 4B and D), an increased variability in the thickness of the layers was found in neocortical or hippocampal sections from LTG-treated animals as compared to control sections.

In view of these findings, lower doses of LTG (5, 10 or 15 mg/kg/day, henceforth referred to as LTG5, LTG10 and LTG15, respectively) were also tested to determine whether they produced similar alterations. Morphological analysis of adult brains showed that cortical abnormalities were also present in rats exposed prenatally to LTG5 (0.13 ± 0.05 neuronal depletion areas/cortical hemisection, $p < 0.014$ versus controls; 0.71 ± 0.07 neuronal dispersion areas/hippocampal section in the CA1 field and 0.89 ± 0.12 neuronal dispersion areas/neocortical hemisection, $p < 0.001$ versus controls, Figs. 2F and 3E). In addition, a dose-dependent response was observed, with the highest number of abnormalities being observed in animals exposed to the highest doses (LTG15 and LTG20; Figs. 2F and 3E). The most dramatic increase was in the number of dispersion areas in the somatosensory cortex, which reached 1.7 ± 0.22 neuronal dispersion areas/neocortical hemi-section in animals exposed to LTG20 ($p < 0.001$, Fig. 2F).

No apparent alteration was observed in other areas of the telencephalon, including the diencephalon, the pyriform cortex and the amygdala complex (not illustrated).

Does LTG disrupt neuronal migration?

Alterations in neuronal proliferation and migration may lead to a variety of developmental disorders. Our previous report suggested that certain AEDs, such as vigabatrin and valproic acid, may disrupt neuronal migration (Manent et al., 2007). Based on these findings, we investigated the distribution of cells labeled with BrdU at E15 in animals exposed to LTG15. The distribution of NeuN and BrdU double-labeled cells was analyzed at P30. This protocol allows to investigate the final location (after migration from the germinative layer) of neurons generated at E15. In control animals, these neurons accumulated, as expected, mainly in the stratum pyramidale of the hippocampal complex and in the superficial cortical layers (II—IV). In animals exposed to LTG, no BrdU positive cells were observed within the areas of cell depletion and layer disruption, in both the neocortex and the hippocampus (Fig. 5). The absence of E15-generated neurons within the areas of neuronal depletion is likely to result from a cell proliferation—migration defect, although these data do not allow to exclude the possibility that, in LTG-exposed animals, normally migrated neurons degenerated within their destination layer by the end of the gestation. However, investigation of neuronal degeneration with Fluoro-Jade or TUNEL staining of P0 pup brains did not reveal major changes in the number of degenerative cells in these cortical fields (not shown).

Plasma concentrations of LTG

Plasma LTG concentrations were measured in rats treated with 5 mg/kg/day or 20 mg/kg/day (2 injections per day of 2.5 mg/kg and 10 mg/kg, respectively) from E14 and sacrificed at E17, 2 h or 10 h after the last injection (Fig. 1B) (six

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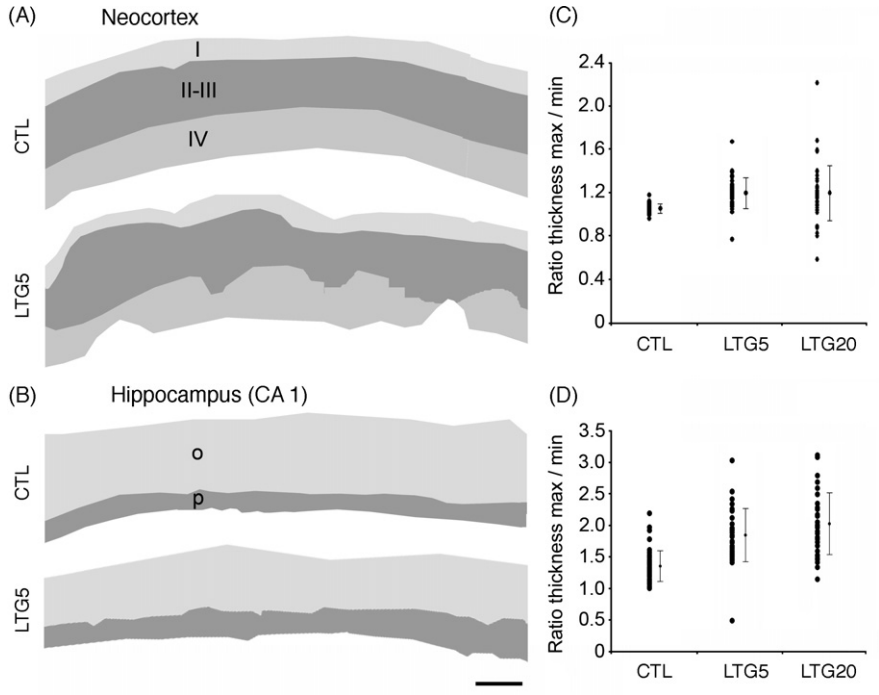

Figure 4 Fetal exposure to LTG causes fluctuations of the neocortical and hippocampal layers thickness. Reconstructions of neocortical (A) and hippocampal (B) sections from P30 rats born to vehicle-treated or LTG-treated animals, illustrating the fluctuations in layers thickness in the neocortex (layers II and III) and in the hippocampus (pyramidal cell layer in the CA1 field). o: stratum oriens, p: stratum pyramidale. Scale bar, 200 μm. Graphs illustrating the variability in neocortical (C) and hippocampal (D) layers thickness in LTG-treated animals vs. controls. Black dots represent ratios between the maximum and minimum layer thicknesses measured (±S.E.M.) per individual neocortical and hippocampal sections, the mean ratios being represented by black dots with error bars.

animals per group). Treatment with 5 mg/kg/day resulted in LTG concentrations of 2.65 ± 0.59 mg/l and 2.49 ± 0.87 mg/l at 2 h and 10 h after the last injection, respectively. In rats exposed to 20 mg/kg/day, plasma LTG concentrations were 8.63 ± 1.5 mg/l and 7.36 ± 5.3 mg/l at 2 h and 10 h, respectively. The modest difference in concentration between 2 h and 10 h is consistent with previous data in rats (Castel-Branco et al., 2005). These values are within the range associated with anticonvulsant effects in rats (Castel-Branco et al., 2005), and are also within the range encountered in patients with epilepsy treated with therapeutic doses of LTG (Perucca, 2000).

Discussion

The present study shows that prenatal exposure to LTG induces hippocampal and cortical malformations in a dose-dependent manner. Moreover, these effects were found at plasma LTG concentrations that are within the range encountered in patients receiving therapeutic doses of the drug (Perucca, 2000). While at the highest dose tested (20 mg/kg/day) signs of systemic maternal toxicity were observed, clear-cut abnormalities in brain development were also present at doses that had no effect on either maternal body weight or viability of the litters.

These effects of LTG may result from a cell proliferation–migration defect and/or cell death. An earlier study showed that postnatal exposure to phenytoin, phenobarbital and valproate triggers apoptosis of developing neurons (Bittigau et al., 2002), and we reported recently that fetal exposure to valproate and vigabatrin increases the number of apoptotic neurons in the hippocampus at birth (Manent et al., 2007). However, in the present study the investigation of newborn rats with markers of cell death (TUNEL and Fluoro-Jade) failed to reveal any evidence of significantly increased neuronal death in LTG-exposed animals (data not shown). These findings suggest that disruption of neuronal proliferation–migration may play a key role in the adverse action of LTG on brain development. Indeed, maternal LTG treatment covered the period of neuronal proliferation and migration of cortical (superficial layers) and hippocampal pyramidal neurons in the fetus. There was a clear disorganization of these layers, characterized mainly by neuronal dispersions, and BrdU-marked neurons were absent from the depletion areas and layer disruptions. Prenatal exposure to other AEDs, namely vigabatrin and valproate, also generates cortical and hippocampal malformations linked to cell migration defects (Manent et al., 2007), indicating that this may be a common mechanism for the deleterious effects of AEDs on fetal brain development. Not all AEDs, however, appear to cause these defects, since in our previous study carbamazepine (Manent et al., 2007) and in the present study PB, TPM and LEV did not adversely affect brain development. At least for carbamazepine, TPM and LEV, however, these negative findings should be interpreted cautiously since the doses

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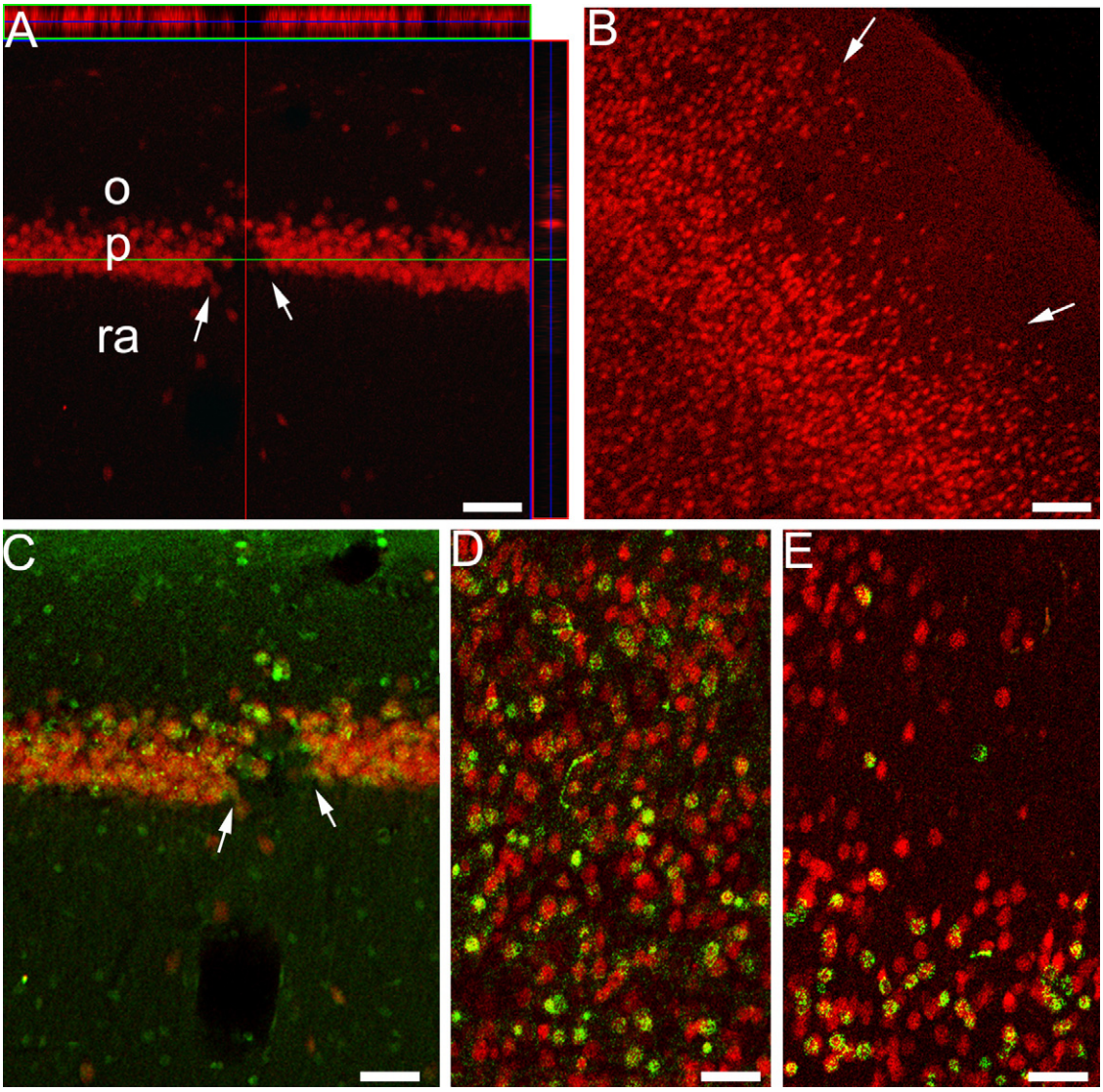

Figure 5 Layer disruptions are linked to migration defects. Confocal images of hippocampal (A and C) and cortical (B, D, E) sections stained with antibodies against NeuN (red) and BrdU (green); scale bars: 100 μm in (A) and (B) and 50 μm in (C) and (E). (A) Orthogonal view (image stack thickness: 30 μm) of a pyramidal cell layer disruption (arrows) in the hippocampus of a P30 rat exposed prenatally to LTG (15 mg/kg/day). A higher magnification of this disruption is shown in (C). (B) Z-projection of three focal planes from a P30 rat exposed prenatally to LTG (15 mg/kg/day). Arrows indicate the borders of a large neuronal depletion area of layers II and III. Part of this field is enlarged in (E). (C) Z-projection of three focal planes of the alteration indicated by arrows in (A). The section was stained with NeuN (red) and BrdU (green). The majority of double-labeled cells localize within the stratum pyramidale with the exception of the depletion field. Note a few double-labeled cells in the stratum oriens aligned with the depletion field, suggesting a failure of migration. (D) Double NeuN and BrdU staining of layers II and III from a control animal. (E) Double NeuN—BrdU staining of layers II and III from an animal exposed prenatally to LTG. The figure corresponds to a higher magnification of the depletion field illustrated in (B). Note the absence of BrdU cells within this field.

tested were not far in excess of the highest doses used in humans.

In our previous study, we hypothesized that the deleterious effects of valproate and vigabatrin in this paradigm could be mediated by GABAergic actions of these drugs. However, high doses of PB, which is also considered to have some GABAergic actions (Czapinski et al., 2005), had no deleterious effects in the present study, whereas LTG, which has no known major GABAergic effects, did disrupt neuronal development. LTG is considered to act primarily by reducing the release of glutamate through inhibition of voltage-dependent sodium channels, although a variety of effects on other ion channels and transmitters have also been reported (Leach et al., 2002). Fetal brain neurons are responsive to ambient glutamate, which modulates their proliferation (LoTurco et al., 1995) and migration (Komuro and Rakic, 1993; Manent et al., 2005, 2006), although proliferating and migrating neurons do not appear

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to functionally express the sodium channels on which LTG is supposed to act (Manent et al., 2005). Neuronal migration is known to be an active process strongly modulated by neuronal activity and by neurotransmitters, including not only GABA and glutamate but also a variety of voltage-gated channels (Manent and Represa, 2007). LTG could exert its deleterious actions on brain development through some of these targets. In particular, LTG inhibits A-type potassium currents that are present at an early stage in fetal development (Danielsson et al., 2005), and it may also modify brain maturation indirectly by an action on various hormones and trophic factors, e.g. through inhibition of corticotropin releasing hormone (Tringali et al., 2006).

The functional consequences of the brain alterations identified in the present study remain to be investigated. Migration defects, including minor defects such as subtle discontinuous subcortical heterotopia (des Portes et al., 2002) can play a key role in seizure generation and other neurological disorders. Since prenatal LTG exposure affects primarily cortical structures, cognitive functions might also be impaired. Clinical reports have suggested that some AEDs taken during pregnancy may result in lower IQ and cognitive delay in the offspring (Koch et al., 1999; Adab et al., 2001; Vinten et al., 2005), but to date these defects have not been linked specifically to LTG exposure. While the potential clinical implications of LTG-induced alterations in brain maturation are unclear, our findings are a cause for concern. Further investigations are clearly warranted to assess in experimental models whether LTG-induced cortical and hippocampal malformations result in postnatal cognitive deficits in offspring, and to evaluate whether any evidence for a clinical correlate of these animal findings can be identified in humans.

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