ORIGINAL ARTICLE

Pharmacological Effects on Brain Morphology and Cognitive Functions in Idiopathic Generalized Epilepsy

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Abstract

Purpose: Sodium Valproate (VPA) is one of the first-generation Antiepileptic Drugs (AEDs) which mediates epileptic activities by releasing stimulators of Gamma-Aminobutyric Acid (GABA) and is greatly used to treat partial and generalized seizures. Lamotrigine (LTG) is commonly used as AEDs that are widely utilized in first-line monotherapy or in combination with other AEDs. Our specific goal was to compare these two AEDs with different molecular mechanisms on both cortical and subcortical brain structures, along with cognitive performance and dysfunctions.

Materials and Methods: We conducted a retrospective study comparing LTG with sodium VPA, both administrated as monotherapy. Twenty patients with a confirmed generalized-epilepsy tonic-clonic seizure who had been treated at least 6 months with LTG (n=8) and Sodium VPA (n=12) were retrospectively recruited. We also included 12 age, gender, and education-matched Healthy Controls (HC). We evaluated cognitive performances. All participants underwent a Magnetic Resonance Imaging (MRI) scanner and T1-weighted MR images were acquired. Voxel-based morphometric alterations in the brain cortex, as well as subcortical structures, were inspected using Statistical Parametric Mapping (SPM) software.

Results: The cognitive performance was revealed inferior in patients on Sodium VPA. Poor performance was associated with significant volume reduction in insula bilaterally, and subcortical structures of thalamus, cerebellum, and hippocampus compared to the HC. Comparing patients on LTG to HC revealed significant volume reduction in the anterior cingulate cortex in concordance with slight cognitive dysfunctions.

Conclusion: These findings suggested that different molecular mechanisms of Antiepileptic Drugs (AEDs) may affect brain structures and cognition with different severity levels, presumably with more adverse effects induced by GABA mediations from sodium VPA.

Keywords: Sodium Valproate; Lamotrigine; Antiepileptic Drugs; Idiopathic Generalized Epilepsy; Cognitive Performance; Voxel Based Morphometry.

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1. Introduction

Lamotrigine (LTG) and sodium Valproate (VPA) are among Antiepileptic Drugs (AEDs), both with established effectiveness in patients with epilepsy [1]. LTG is rarely associated with cognitive deficits in epileptic patients [1, 2]. VPA, on the other hand, is a first-line AED for adults and children with epilepsy [3, 4] and is believed to associate with reversible atrophy in brain structures and cognition [5-7]. The administration AEDs can alter the excitatory and inhibitory neurotransmitter release [8], and specific to the type of neural system and its behavior, when exposed to the external factors, the final excitatory and inhibitory consequences are determined [9]. Previous studies have widely revealed the inhibitory role of gamma-aminobutyric acid (GABA) in mediating the antiepileptic process in some AEDs [10] such as VPA [11]. However, some AEDs such as LTG control seizures through sodium-channel blocking [12]. LTG inhibits releasing of glutamate which is an excitatory neurotransmitter, most likely by blocking voltage-gated sodium channels, and is antagonistic to excitatory N-methyl-D-aspartate receptors [13-15]. It can also attenuate voltage-sensitive calcium channels [16-19].

Much information has frequently been received from structural neuroimaging studies reporting the relationship between cognition and microstructural alterations [20-23]. Magnetic Resonance Imaging (MRI) has been one of the important instruments which can distinguish microstructural and functional changes manifested on the brain of patients with epilepsy [24, 25]. Voxel-Based Morphometry (VBM) is a neuroimaging technique for MRI analyses being increasingly exerted for exploring focal Gray-Matter (GM) alterations between patients and Healthy Controls (HC) [26-28]. The effects of AEDs on alterations in the brain structure and cognitive functions have frequently been investigated. Recently, Xiao and colleagues assessed the brain cognitive performance in patients with focal epilepsy and administration of LTG as monotherapy compared to the patients on Carbamazepine (CBZ): they found that the patients who are on CBZ have low activation in frontal compared to the patients on LTG [12]. A previous study examined grey matter volume, cortical thickness, surface area, and subcortical volume in Juvenile Absence Epilepsy (JAE) patients taking VPA as an AED. They reported volume reductions in several brain regions compared to HC [29]. In another study [30], researchers reported that a small dose of VPA or Levetiracetam (LEV) on rhesus monkeys may induce brain alterations in mainly GM and White Matter (WM) volumes, respectively.

VPA [31, 32] and LTG are commonly used AEDs that are widely utilized in the first-line monotherapy or in combination with other AEDs [33]. Notably, alterations in the brain structure have been evaluated through VPA/LEV administration as a monotherapy treatment in rhesus monkeys [30]. Also, the association between AEDs such as VPA and LTG [34]; and VPA and LEV as monotherapy treatments with the resting-state functional MRI (rs-fMRI) has been assessed [35]. Moreover, some studies have assessed the effect of AEDs on both brain structure and cognition [12, 36, 37]. Although it is generally agreed that AEDs can induce alterations in the brain structure and cognition, because of the clinical huge impact, the necessity of reassessment of different aspects of changes cannot be overstated. Our specific goal was to compare these two AEDs with different molecular mechanisms on both cortical and subcortical brain structures, along with cognitive performance and dysfunctions.

2. Materials and Methods

The human ethic committee of the Iran University of Medical Science approved this study and written informed consent was obtained from all participants.

2.1. Subjects

We conducted a retrospective study on 20 patients with generalized-epilepsy with tonic-clonic seizures (GE-TCS), of which 8 patients administrated LTG as monotherapy and 12 were admitted VPA immediately after diagnosis. The exclusion criteria were: an age of under 20 or over 60 years, the standard education of less than twelve years, taking any drugs, alcohol or substance, drug comorbidities, renal and liver dysfunction, depression, diabetes, cardiovascular disease, status epilepticus or cluster, and other major neurological or psychological disorders. The confirmed diagnosis of GE-TCS was based on the patient's clinical history and Electroencephalogram (EEG) findings and was approved by an experienced neurologist. Twelve right-handed volunteers were recruited as HC with age, gender, and education level matched with the patients. They had no family history of epilepsy, neurological disease and seizure, psychiatric disorders, or history of neurosurgery.

Table 1. Demographic and clinical information in participants

Clinical Data	HC (n=12)	Patients on LTG (n=8)	Patients on VPA (n=12)	p-Value
Sex (Male/Female), n	7/5	5/3	7/5	0.97
Age, (y)	38.58± 7.50	36.38± 11.48	34.67± 8.02	0.52
Level of education, (y)	18.42± 4.31	19.38± 2.32	18.67± 3.70	0.83
Seizure onset age, median, (y)	-	31.13± 10.43	28.33± 6.98	0.84
Epilepsy duration, (y)	-	5.25± 2.31	6.33± 3.31	0.45
Seizure frequency, (y)	-	1.50± 0.75	1.75± 0.86	0.52

Table note: HC= Healthy Controls, LTG= Lamotrigine, VPA= Valporic Acid, y=year, n= number. One- way ANOVA and Tukey were used (p-value < .05).

Clinical and demographic information for all subjects is summarized in Table 1.

2.2. Cognitive Assessments

An hour before MRI acquisition, all subjects underwent cognitive assessments using standard neuropsychological tests. Forward Digit Span Task (FDST) evaluated short-term memory, where a sequence of digits appeared in each line, the length of which increased by one in the following consecutive lines. The sequence of digits should be recalled by the participants in a forward manner after appearing in each line. Backward Digit Span Task (BDST) evaluated the working memory in a similar manner to FDST, but in BDST the participants should recall the digits in a backward manner. Moreover, we evaluated the inhibitory response/attention/processing speed by the Stroop test in three states: neutral, congruent, and incongruent, in the neutral state, the patient read colored squares in maximum speed and congruent state, where patient read colors' names that their names are congruent with their colors in maximum speed, and incongruent state, where patient read colors' names which their names are incongruent with their colors in maximum speed where the mean reaction time and task accuracy for each patient were recorded. Furthermore, we assessed the semantic and phonetic memory by Verbal Fluency Test-Semantic (VFT-S) and Verbal Fluency Test-Phonetic (VFT-P), where the subjects were asked to recall the object names for each category and words for each letter. These assessments demonstrate memory capacity and inhibitory response functions as essential parts of the executive function. The study was analyzed by the one-way Analysis of Variance (ANOVA) and Tukey Test in SPSS.

2.3. MRI Acquisition

Healthy subjects and all patients after one year of administrating of AEDs underwent high-resolution brain MRI on a Siemens Trio 1.5T scanner (Erlangen, Germany).

One patient in the LTG group refused to undergo an MRI scanner after neuropsychological assessment. For the identification of the brain structural abnormalities, a gadolinium-enhanced axial T1-weighted image was also acquired. The MR images of all participants were reviewed by a neuroradiologist for the identification of abnormalities. For VBM analysis, high-resolution three-dimensional (3D) magnetization prepared rapid gradient echo (MPRAGE) sequence was acquired for each subject using the following parameters: Repetition Time (TR) = 4.52 msec, Echo Time (TE) =2.38 msec, voxel size = 1 × 1 × 1 mm^3, matrix size =259 × 259, and slice thickness=1 mm. During scanning, all participants were asked to relax and keep their eyes closed while staying awake. None of them used a sedative drug before the exam, and neither reported falling asleep or experiencing seizures during the scan.

2.4. VBM Preprocessing

Details of our VBM method were published previously [38]. As a summary, at the first step, the quality of the T1-weighted images was checked by an expert to guarantee the correct orientation and matrix size, a proper signal to noise ratio, and perfect absence of imaging artifacts using the Display toolbox, SPM12 (Welcome Department of Imaging Neuroscience: http://www.fil.ion.ucl.ac.uk/spm, in MATLAB). This quality check did not result in the exclusion of any of the MRI scans. Next, VBM analysis was performed in the following steps: Using the Segment toolbox, the scans were segmented into GM, WM, and

Cerebrospinal Fluid (CSF) maps, which were created in the native space, along with Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) imported outputs [28]. Using the "Run DARTEL: create template" toolbox and its default settings, the accuracy of inter-subject alignment was enhanced by iteratively averaging the DARTEL-imported data of the GM and WM maps to generate population-specific templates. Using the generated templates and through the "Normalize to Montreal Neurological Institute (MNI) space" toolbox, all the GM and WM maps were normalized to the MNI standard space. Finally, all images were smoothed using a Gaussian kernel (10mm Full Width at Half Maximum (FWHM)) before conducting the statistical analysis.

2.5. Statistical Analysis

The total cerebral volume of all participants was estimated by adding the probability estimates of the GM and WM maps, then multiplying the resulting values by the volume of each voxel (3.375 mm^3) before the final statistical inferences. T1-weighted scans of the cohorts were compared in a voxel-wise manner, using two-tailed two-sample independent t-test analysis with the significance level set at the corrected p-value < 0.05. The p-value was corrected for multiple comparisons using the family-wise error correction. Implicit masking of the final images was performed using a relative threshold to eliminate any effect of edge differences between the two cohorts. Moreover, subcortical brain areas were parcellated on T1-weighted images using SPM software.

We analyzed volumetrics of seven subcortical structures which are proven to be affected by epilepsy, including the caudate nucleus, putamen, globus pallidus, thalamus, nucleus accumbens, hippocampus, and amygdala [39], and compared across epilepsy medication cohorts and HC cohort. Additionally, we calculated the Pearson correlations between subcortical gray matter volumes and cognitive performances for each patient compared them among the cohorts.

3. Results

3.1. Demographic and Clinical Characteristic

As summarized in Table 1, there is no significant difference in seizure onset and seizure duration between the patient cohorts, neither in demographic variables such as age, sex, and education. All of the patients were seizure-free after antiepileptic remediation.

3.2. Neuropsychological Assessments

Table 2 shows cognitive function scores in the HC cohort, as well as the patient cohorts of LTG and VPA.

The mean reaction time for neutral, congruent, incongruent statues of the Stroop test was significantly higher in the VPA cohort compared to the HC (p-value < 0.05), also they showed a significantly lower performance (measured by correct percentage) and in short-term memory assessed by FDST compared to the HC (p-value < 0.01), and in both phonetic and semantic verbal fluency evaluated by VFT-P (p-value < 0.01) and VFT-S (p-value < 0.001), respectively.

The mean reaction time in the incongruent state in the Stroop test in the LTG administrated cohort was significantly higher than the HC (p-value < 0.05). A significantly lower correct percentage rate in a neutral condition of the Stroop test (p-value < 0.01) and also, a significantly poorer performance rate in VFT-S (p-value < 0.05) were detected in the LTG cohort compared to the HC. Based on our findings, the difference in the cognitive functions was not statistically significant between the two medication cohorts (Table 2).

3.3. Cortical Alterations

The LTG group showed significant GM volume reductions in the Anterior Cingulate Cortex (ACC) (LTG < control group; Figure 1.A; Table 3; p-value < 0.001). The GM volume in the VPA group was significantly decreased in the left and right insulae (VPA group < control group; Figures 1.B, C, Table 3; p-value < 0.001). VPA group revealed a significant GM volume reduction in the paracentral lobule and right superior temporal gyrus compared to the LTG group (VPA group < LTG group; Figures 1.D, E, Table 3; p-value < 0.001). Compared to the HC, GM volume in patients (both groups) showed significant atrophy in left precuneus and right insula (patients < controls; Figures 1.F, G, Table 3; p-value < 0.001). There were no regions of enlarged GM volume in patients compared to the control subjects.

3.4. Subcortical Alterations

Subcortical segmentation in a patient in the VPA group and in the LTG group was shown (Figure 2.A, B). Compared to the HC, LTG cohorts revealed asymmetry in

cerebellum (p-value < 0.05, Figure 3.A). The VPA group showed a significant and severe decrease in the total thalamus and right thalamus (p-value < 0.01), total cerebellum, left cerebellum, left hippocampus, right cerebellum, caudate asymmetry, and total hippocampus (p-value < 0.05) compared to HC (Figure 3.B).

The VPA group underwent extensive volume atrophy in the right cerebellum (p-value < 0.01) as well as total cerebellum, lateral ventricles asymmetry, and cerebellum asymmetry (p-value < 0.05) compared to the LTG group (Figure 3.C; Table 4). In plus, we performed individual analysis through a scatter plot and assessed alterations in subcortical structures between two groups with epilepsy disease and healthy control subjects. This result appeared a severe significant difference in right thalamus volume between VPA patients and healthy

Table 2. Cognitive test scores in participants

Brain Functions	Cognitive Tests	Measure	LTG vs. HC			VPA vs. HC			VPA vs. LTG		
			LTG M(SD)	HC M(SD)	Pvalue	VPA M (SD)	HC M(SD)	Pvalue	VPA M (SD)	LTG M(SD)	Pvalue
Short Term Memory	FDST	Correct percentage (%)	9.13(2.41)	10.25(1.86)	0.19	7.83(2.48)	10.25(1.86)	0.002**	7.83(2.48)	9.13(2.41)	0.13
Working Memory	BDST	Correct percentage (%)	6.5(2.2)	7.33(3.05)	0.53	5.33(2.42)	7.33(3.05)	0.103	5.33(2.42)	6.5(2.2)	0.21
Verbal Fluency	Phonetic	Correct percentage (%)	38.75(16.87)	49.08(10.94)	0.17	31.83(12.02)	49.08(10.94)	0.003**	31.83(12.02)	38.75(16.87)	0.26
	Semantic	Correct percentage (%)	56(9.78)	67.25(10.03)	0.02*	45.83(13.78)	67.25(10.03)	).001***	45.83(13.78)	56(9.78)	0.08
Inhibitory Response/ Attention Stroop test	Neutral	Correct percentage (%)	99.13(0.83)	99.92(0.28)	0.01**	99.33(1.49)	99.92(0.28)	0.24	99.33(1.49)	99.13(0.83)	0.18
		Mean Reaction Time	122.25(23.63)	104.08(8.45)	0.058	128.58(36.84	104.08(8.45)	0.03*	128.58(36.84)	122.25(23.63)	0.72
	Congruent	Correct percentage (%)	100	100	1.00	100	100	1.00	100	100	1.00
		Mean Reaction Time	99.63(25.3)	83.58(7.93)	0.13	108.42(37.35)	83.58(7.93)	0.03*	108.42(37.35)	99.63(25.3)	0.72
	Incongruent	Correct percentage (%)	96.75(5.36)	99.50(0.67)	0.11	99.17 (0.83)	99.50(0.67)	0.27	99.17 (0.83)	96.75(5.36)	0.32
		Mean Reaction Time	216.50(51.28)	169.25(25.82)	0.04*	201.17(54.67)	169.25(25.82)	0.04*	201.17(54.67)	216.50(51.28)	0.48
	Incongruent Mean Reaction Time— congruent Mean Reaction Time	Interference time	116.38(37.86)	84.83(24.70)	0.054	92.17(25.92)	84.83(24.70)	0.58	92.17(25.92)	116.38(37.86)	0.15

Table note: LTG= lamotrigine; HC= healthy control; VPA= valproate acid, FDST= forward digit span task; BDST= backward digit span task; VFT= verbal fluency test; M= mean; SD= standard deviation. The study was analyzed via one-way ANOVA and Tukey Test in SPSS.

*P< 0.05, **P< 0.01, ***P< 0.001

control subjects (p-value < 0.01). The Right cerebellum volume shows a severe significant difference between the two patient groups (p-value < 0.01), but there is a slightly significant difference between VPA/HC subjects (p-value < 0.05). Total cerebellum volume shows a slightly significant difference between VPA/HC and VPA/LTG groups (p-value < 0.05). Cerebellum asymmetry was shown a significant difference in the LTG group compared with the HC group (p-value < 0.05) and also it was revealed in the VPA group compared with the LTG group (p-value < 0.05). In plus, patients on VPA show a vast reduction in total thalamus volume with significant differences compared with the HC group (p-value < 0.01).

Also, patients on VPA showed a significant difference in the left cerebellum compared with HC subjects (p-value < 0.05).

There is a slightly significant difference in lateral ventricles asymmetry between the two patient groups (p-value < 0.05, Figure 3.D).

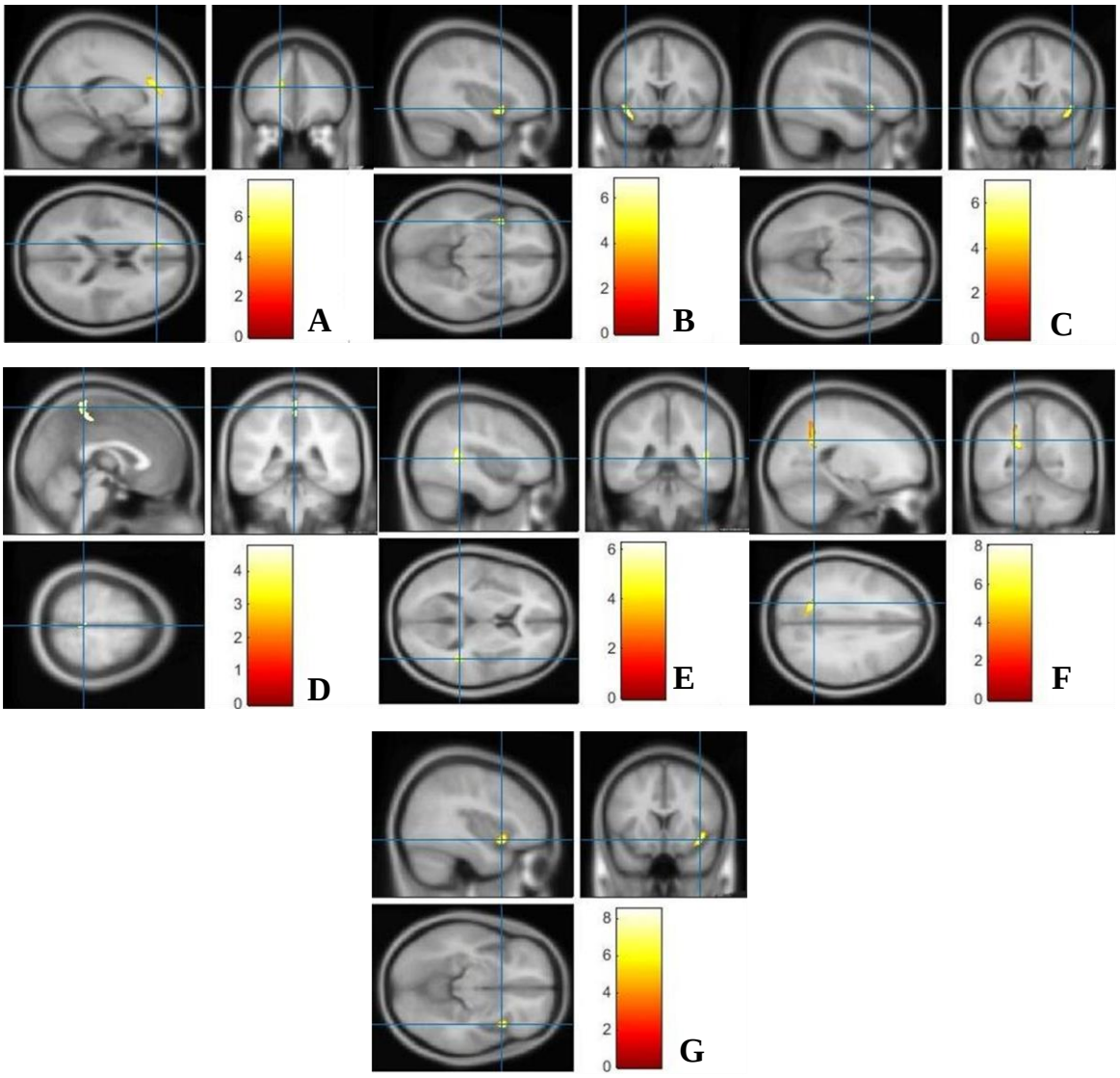


Figure 1. Statistical brain map reveals GM volume reductions in the brain of epileptic patients with GE-TCS compared with the healthy control (HC) group. Compared to the HC, GM volume in the LTG group underwent significant decrease in ACC (A). Moreover, the VPA group caused a GM volume reduction in left and right insula (B, C). The VPA group experienced GM volume reductions in paracentral lobule and right superior temporal gyrus

3.5. Correlation Analyses

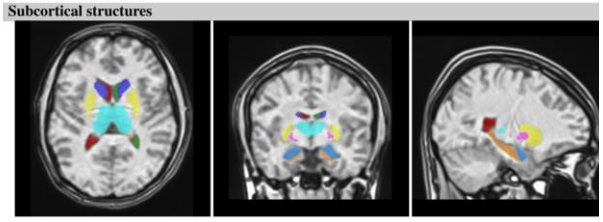
We calculated paired bivariate correlations between subcortical structures and cognitive performances for the patient groups (Figure 4).

Significant positive correlations were shown for VFT-S vs. total thalamus (r = 0.475, p-value = 0.047) and VFT-S vs. right thalamus (r = 0.511, p-value = 0.030); and significant negative correlation for VFT-S vs. hippocampus asymmetry

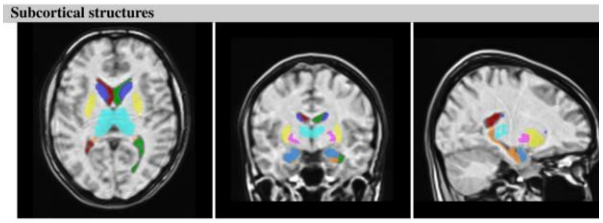
Table 3. Brain regions with significant correlations in participants

Region value	MNI Coordinates (X, Y, Z)	z score	p value
LTG group < Control group			
ACC	-13.50, 33, 25.5	6.59	< 0.001
VPA group < Control group			
Insula- L	-37.5, 13.5, -9	5.17	<0.001
Insula- R	43.5, 13.5, -7.5	4.8	<0.001
VPA group <LTG group			
paracentral lobule	0.00, -39, 64.5	4.2	<0.001
STG- R	42, - 39, 7.5	5.33	<0.001
Patients group <Control group			
precuneus - L	-21, -54, 30	5.65	< 0.001
Insula - R	39, 13.5, -10.5	6.59	< 0.001

Table note: MNI= Montreal Neurological Inistitute, LTG= Lamotrigine, ACC= Anterior Cingulate Cortex, VPA= Valproate Acide L= Left, R= Right, STG= Superior Temporal Gyrus, z-score of the effect, and the p-value of the comparisons are also provided



A. VPA group



B. LTG group

Figure 2. Subcortical segmentation in a patient in VPA group (A) and a patient in LTG group (B)

(r = -0.474, p-value = 0.047), when the two patient groups are lumped together. The same correlations were also found for each of the LTG and VPA groups, but not statistically significant. Therefore weak discrimination was possible due to the small sample size of the groups.

Significant positive correlation was found for Stroop test (neutral) correct percentage vs. right globus pallidus (r = 0.599, p-value = 0.009) by considering the two patient groups together. However, the LTG group alone revealed significant positive correlation (r = 0.907, p-value = 0.005) between Stroop test (neutral) correct percentage and right globus pallidus, which was not the case for the VPA group (r = 0.540, p-value = 0.087).

We also found significant negative correlations for Stroop test (neutral) mean reaction time (s) vs. right globus pallidus (r = -0.477, p-value = 0.045) when the two patient groups are lumped together. VPA showed significant negative correlation between these markers (r = -0.696, p-value = 0.017), which was not significant for the LTG group and there is no correlation in the LTG group for Stroop test (neutral) mean reaction time (s) vs. right globus pallidus (r = -0.096, p-value = 0.837). Significant positive correlations for Stroop test (Incongruent) correct percentage vs. total caudate (r = 0.473, p-value = 0.048) and for Stroop test (Incongruent) correct percentage vs. left caudate (r = 0.515, p-value = 0.029) were discovered. The LTG group alone also revealed significant positive correlation for Stroop test (Incongruent) correct percentage and left caudate (r = 0.800, p-value = 0.031), which was not the case for the VPA group and there is no correlation in the VPA group for Stroop test (Incongruent) correct percentage vs. left caudate (r = 0.028, p-value = 0.935), also there is no correlation in the VPA group for Stroop test (Incongruent) correct percentage vs. total caudate (r = 0.026, p-value = 0.940).

4. Discussion

In this study, we explored the relation between GM volume alterations in the cortical and subcortical structure and neurocognitive functioning on VPA and LTG administration in idiopathic GE-TCS. We found that both

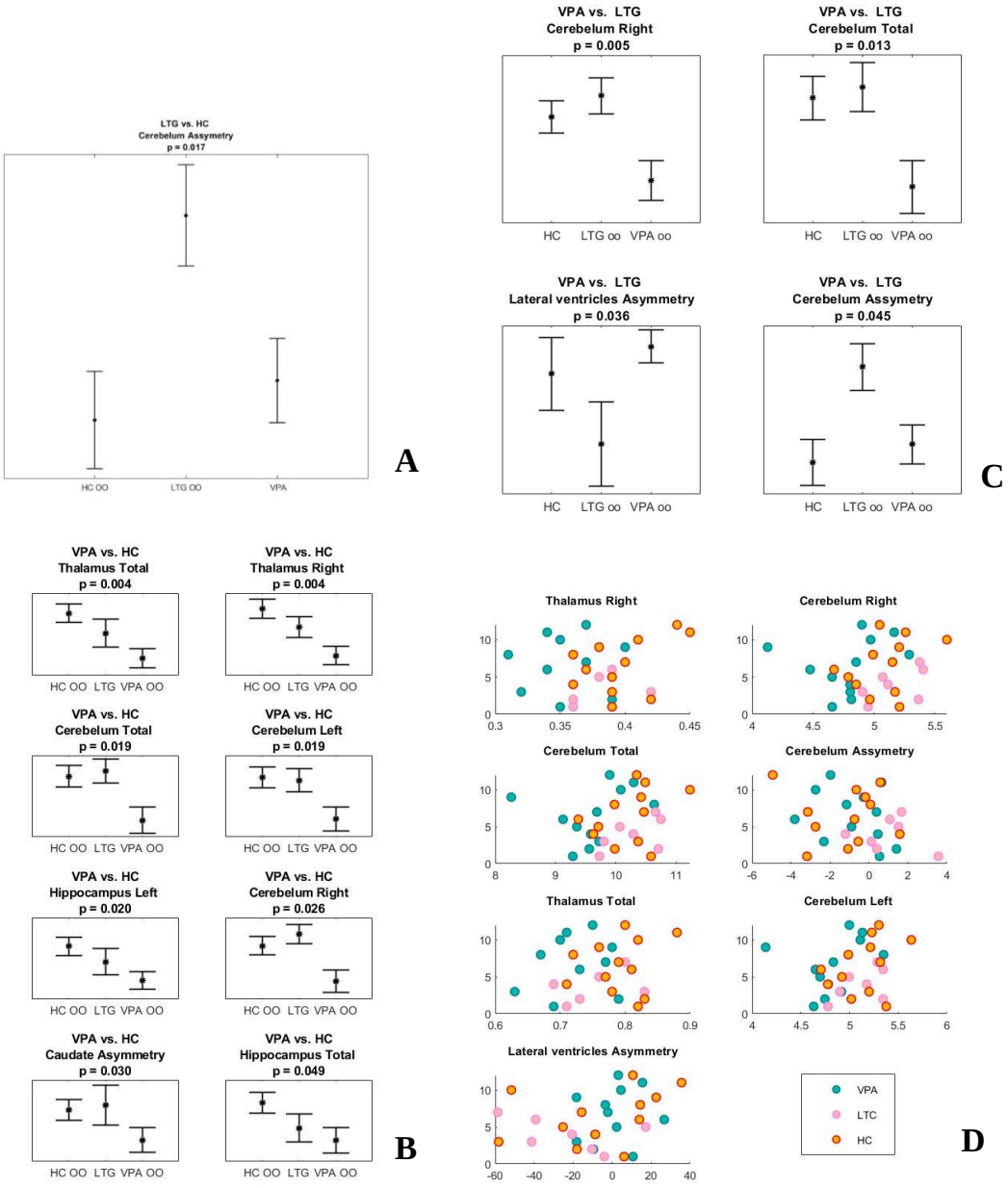


Figure 3. Standard errors are revealing volume differences in subcortical structures between LTG, VPA, and healthy control (HC) groups (a, b, c), under each figure comparison between two groups shown by "oo" sign. Scatter plots compare volume differences in subcortical structures in individuals between three groups (d)

VPA and LTG drugs affect the GM volume and cognition. VPA resulted in an extensive reduction in brain volume in the cortical area (bilateral insula) and the subcortical regions (thalamus, cerebellum, hippocampus, caudate asymmetry), and also LTG resulted in an intensive decrease in the cortical area (ACC) and caused asymmetry in the cerebellum compared with HC. Compared to the LTG group, the VPA group revealed a more pronounced GM volume reduction in the paracentral lobule, right superior temporal gyrus, cerebellum, and caused asymmetry in lateral ventricles and cerebellum. Besides, the cognitive assessment revealed extensive poor performance in VFT-S, VFT-P, and FDST in patients on VPA regimen compared to HC. Likewise, compared to the control group, this group showed weak accomplishment in the Stroop test (mean reaction time), however, the patients treated with LTG showed poor performance in the Stroop test (neutral- correct percentage), Stroop test (Incongruent- mean reaction time), and VFT-S.

Furthermore, we investigated the correlations between subcortical structure alterations and cognitive functions in two patients groups. Three subcortical structures, including thalamus, caudate, and globus pallidus showed positive significant correlations with VFT-S and Stroop test (correct percentage) in the two patients groups. Also, we found

negative correlations between hippocampus asymmetry and globus pallidus with cognitive performances measured by VFT-S and Stroop tests (mean reaction time).

Based on these results, it seems that a causal link exists between the type of administrated drugs depending on their distinctive molecular mechanisms with the indices of cognitive performances, including reaction time, correct response number, and gray matter alterations.

VPA is one of the fulcrums for the treatment of different types of epilepsy in adults and children [40-43]. It is a potent anticonvulsant drug and a long-standing usage [36, 41]. The current study investigating the VPA effect on cortical and subcortical structures indicated a reduction in the bilateral insula, right thalamus, cerebellum, left hippocampus, and caudate asymmetry compared to the HC. VPA utilizing in Alzheimer's disease revealed brain volume reduction in the hippocampus, ventricular enlargement, and vast cognitive impairment [44, 45], and cortical thinning in visual cortex and occipital regions were shown in patients on VPA administration [36]. A previous study on rhesus monkeys on VPA showed increased GM volume in the right geniculate nucleus and the right pulvinar regions. MRI data has shown that little dose of VPA and LEV can induce brain alterations in GM and WM volumes, respectively [30].

Table 4. Group comparison of subcortical volume reductions among

Brain regions	P value
LTG vs. HC	
Cerebellum asymmetry	0.017
VPA vs. HC	
Thalamus (total)	0.004
Thalamus (R)	0.004
Cerebellum (total)	0.019
Cerebellum (L)	0.019
Hippocampus (L)	0.020
Cerebellum (R)	0.026
Caudate asymmetry	0.030
Hippocampus (total)	0.049
VPA vs. LTG	
Cerebellum: (R)	0.005
Cerebellum: (total) 0.013	0.013
Lateral Ventricles asymmetry	0.036
Cerebellum asymmetry	0.045

VPA controls seizure via the inhibitory role of the GABAergic system [10, 46]. In the cerebellum [47] and the thalamic nuclei, the highest concentration of GABA receptors was detected [48]. In the hippocampus, two kinds of GABAergic receptors have been identified; one stimulated by the vesicular release of GABA, and another one activated by a low concentration of GABA neurotransmitters [49]. The VPA administration regulating the concentration of GABA to control the seizure likely imposes alterations on the morphology and volume of the underlying structures. Moreover, it seems that inhibitory interneurons such as GABA may induce asymmetries in such structures [50].

A Previous study has shown that the patients on the VPA regimen have visual cortical thinning, left hippocampus and right pallidum reduction, and ventricular enlargement vs. HC. Another study on VPA administration did not show these results though. This discrepancy may suggest that the VPA might have transient brain alterations [36].

In this study, the patients on a VPA regimen showed vast thalamus reduction and poor cognitive function in VFT compared to HC. Sodium VPA induces GABA to synthesize and release in different brain regions [11]. Previous neuro-pharmacological studies supported that VPA is strongly associated with alterations in GM volume in the thalamus and parietal lobe thickness [51, 52]. This presumably suggests that the VPA mechanism and its inhibitory effect on both cortex and thalamus may cause these alterations [35]. Tondeli *et al.* (2016) reported that VPA consumption altered GM volume, cortical thickness, surface area, and subcortical volume in JAE [29]. As the finding of our study, VPA has shown adverse effects on the thalamus volume and verbal fluency, more severe than other parts of subcortical structures and cognitive performances. It assumes that the thalamus efficiently takes part in verbal fluency tasks.

In the current study, the LTG group showed cerebellum asymmetry and ACC volume reduction compared to the HC. Following LTG treatment in bipolar disorder volume reduction was identified in the amygdala, cerebellum, and nucleus accumbens [53]. LTG controls seizures via inhibiting the release of glutamate neurotransmitters. It also blocks voltage-gated sodium channels and exerts aspartate receptors' antagonist [16, 17]. Aspartate receptors concentrate on cerebellar granule cell layers, and thalamic nuclei [54]. Cerebellar Granule

Neuron Progenitors (CGNPs) after developing alter to the cerebellum [55]. As a result, the effect of LTG on the cerebellum with a high concentration of aspartate sites to control seizure presumably induces alterations on these structures, also imposes asymmetry in the contralateral of the cerebellum associating with the weak modulating

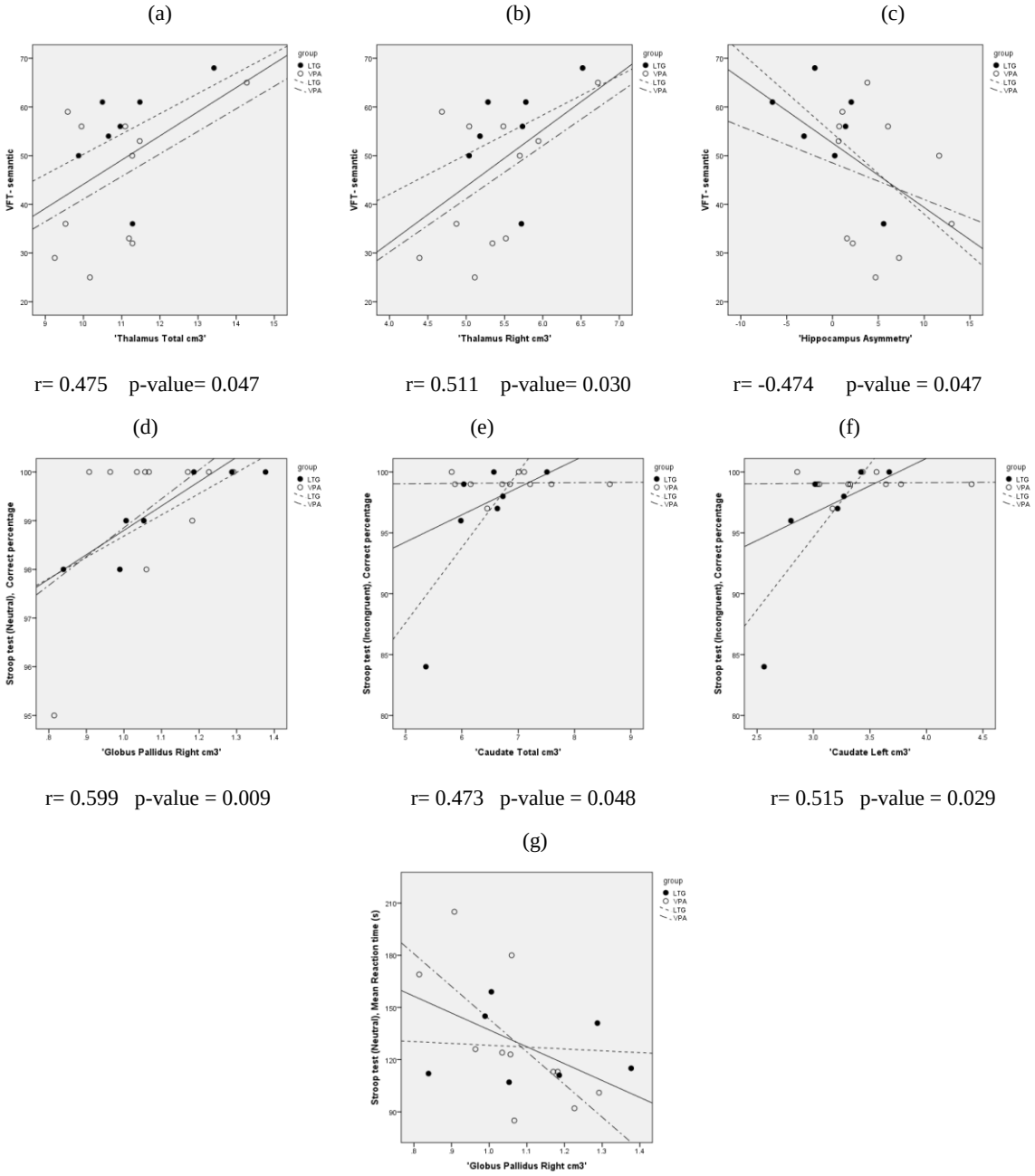


Figure 4. The correlation between cognitive functions and subcortical structures in two group patients. (a) VFT-semantic showed a significant positive correlation with the thalamus (total) in two patient groups (LTG and VPA groups) (r= 0.475, p-value = 0.047). (b) VFT-semantic showed a significant positive correlation with the thalamus (right) in two groups (r= 0.511, p-value = 0.030). (c) VFT-semantic showed a significant negative correlation with the hippocampus asymmetry in two group patients (r= -0.474, p-value = 0.047). (d) Stroop test (neutral) correct percentage showed a significant positive correlation with globus pallidus (right) in two group patients (r= 0.599, p-value = 0.009). (e) Stroop test (Incongruent)correct percentage showed a significant positive relation with the caudate (total) in two group patients (r= 0.473, p-value = 0.048). (f) Stroop test (Incongruent)correct percentage showed a significant positive relation with the caudate (left) in two group patients (r= 0.515, p-value = 0.029). (g) Stroop test (neutral) mean reaction time showed a significant negative correlation with the globus pallidus (right) in two groups (r= 0.477, p-value = 0.045).
Bivariate significant correlations between subcortical structures and cognitive functions in LTG and VPA group patients analyzed with SPSS. LTG: lamotrigine, VPA: valproate

influence of the frontal cortex on the metabolism of the cerebellum [56]. Our study revealed that brain structural alterations are linked with cognitive performance. In the current study, patients on LTG revealed cortical volume reduction in ACC, poor performance in the Stroop test, also revealed weak accomplishment on VFT-S compared to the HC. This atrophy may be relevant to poor efficiency in processing speed [57], attention [58], and verbal fluency [59] according to previous cognitive studies [60]. Previous studies have consistently indicated a significant alteration in the structures within the medial frontal cortex, ACC, and medial temporal lobe in JAE. These alterations may affect awareness, attention, and memory [29]. LTG treatment has a better performance in VFT-P compared to the patients on CBZ [61]. Yet, another study showed that LTG and CBZ did not affect the cognitive functions and quality of life in patients [62, 63].

In the current study, patients on LTG have significantly shown a positive correlation in right globus pallidus and left caudate nucleus volume with Stroop test (correct percentage), whereas the patients on VPA was shown a significant negative correlation in right globus pallidus volume and Stroop test (neutral- mean reaction time). Therefore, Stroop test (neutral) mean reaction time (s) and right globus pallidus together can discriminate the two LTG and VPA groups, in plus Stroop test (Incongruent) correct percentage and left caudate can discriminate these two groups. The previous study demonstrated abnormal anatomical measures in the globus pallidus and caudate nucleus correlate with inhibitory response/attention in Attention Deficit Hyperactive Disorder (ADHD) patients [64, 65]. In this study, both LTG and VPA treatment indicated their significant correlation in attention /inhibitory response with basal ganglia volume (globus pallidus and caudate nucleus). There are a few limitations in this study. The sample size was relatively small, which may affect the reliability of the results. We will pursue longitudinal cohort studies in a near future.

5. Conclusion

Different pharmacomolecular mechanisms of AEDs such as LTG and VPA with different intensities can affect brain structure and cognition. LTG did not show any reduction in subcortical structures and indicated slight cognitive dysfunction. It is a relatively newer AED than VPA and blocks the sodium channels and inhibits the release of glutamate as an excitatory neurotransmitter [66]. The alterations in VPA administration were greater than the alterations in LTG treatment. The observed alterations might be depending on the pharmaco-molecular mechanism that is presumably linked to the GABAergic system involving in VPA administration.

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