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Research Article

Stereological Examination of Parietal and Temporal Regions of the Brain in Rats Induced with Epilepsy with Pentylenetetrazole

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ABSTRACT

The aim of this study was to investigate the parietal and temporal regions of the brain stereologically after epilepsy induced by pentylenetetrazole (PTZ). 20 healthy male Wistar Albino rats, 3 months old, weighing approximately 250 gr, were chosen for the study. The rats were divided into PTZ and control groups, 10 in each group. 80 mg/kg PTZ was mixed into 0.5 ml of 0.9% physiological saline and administered intraperitoneally as a single dose injection to the PTZ group. Behavioral changes specified in six stages were determined by following the Racine scale within a 30-minute observation period. After perfusion, the rats' brains were removed. After tissue tracking, an average of 10 sections were obtained for each animal, with a thickness of 1/75 of 5µm. Sections were stained with Hematoxylin-Eosin. The preparations were photographed with a 1.25 objective. For each brain tissue, the total lobus parietalis, lobus temporalis, the grey matter volume and the total number of neurons in the lobus parietalis and lobus temporalis were calculated. Mann-Whitney U test was used for statistical analysis (IBM SPSS for Windows, ver.25) No statistically significant difference could be detected in the total lobus parietalis, lobus temporalis and the grey matter volume, volume values of the brain and the number of neurons determined between the groups ($p>0.05$). However, when evaluated according to groups, a significant difference was obtained in the volume ratio values, the grey matter volume/total lobus parietalis and the grey matter volume/total lobus temporalis ratio ($p<0.05$).

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Pentilentetrazol ile Epilepsi Oluşturulan Sıçanlarda Beynin Parietal ve Temporal Bölgelerinin Stereolojik İncelenmesi

ÖZET

Bu çalışmanın amacı, pentilentetrazol (PTZ) ile oluşturulan epilepsi sonrası beynin pariyetal ve temporal bölgelerini stereolojik olarak incelemektir. Çalışma için yaklaşık 250 gr ağırlığında, 3 aylık, 20 adet sağlıklı erkek Wistar Albino sıçan seçildi. Sıçanlar her grupta 10 adet olacak şekilde PTZ ve kontrol gruplarına ayrıldı. 80 mg/kg PTZ, 0.5 ml %0.9 fizyolojik salin içerisine karıştırıldı ve PTZ grubuna tek doz enjeksiyon halinde intraperitoneal olarak uygulandı. Altı aşamada belirlenen davranış değişiklikleri, 30 dakikalık gözlem süresi içerisinde Racine skalası takip edilerek belirlendi. Perfüzyonun ardından sıçanların beyinleri çıkarıldı. Doku takibi sonrasında her hayvan için 1/75 5 µm kalınlığında ortalama 10 adet kesit elde edildi. Kesitler Hematoksilen-Eozin ile boyanarak 1.25 objektifle fotoğraflandı. Her beyin dokusu için lobus parietalis, lobus temporalis, gri madde hacmi ve lobus parietalis ve lobus temporalis'teki toplam nöron sayısı hesaplandı. İstatistiksel analizde Mann-Whitney U testi kullanıldı (IBM SPSS for Windows, ver.25).Lobus parietalis, lobus temporalis ve beyindeki gri madde hacmi, hacim değerleri ve gruplar arasında nöron sayısı değerlerinde istatistiksel olarak anlamlı bir fark saptanmadı. ($p>0.05$). Ancak gruplara göre değerlendirildiğinde hacim oranı değerlerinde yani gri madde hacmi/toplam lobus parietalis ve gri madde hacmi/toplam lobus temporalis oranında anlamlı farklılık elde edildi ($p<0.05$).

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INTRODUCTION

The oldest known detailed source on epilepsy was found in the Mesopotamia Region as a result of archaeological excavations. Article 278 of the laws of the Babylonian King Hammurabi (1750 BC) states: "If a person buys a male or female slave and the slave develops bennu (epilepsy) disease within a month, he will return the slave to the seller and take back the money paid" was stated (Renger, 2016).

Stereology is actually a branch of science that allows interpretations to be made about the properties of three-dimensional structures with the data provided by two-dimensional sections obtained from three-dimensional structures (metallurgical, biological samples, etc.). Sections are two-dimensional examples passing through a structure. When two-dimensional sections are considered as components that shape the structure with three-dimensional intersecting planes, each building formation in these sections creates profiles (projections) with their size, length, number, area and volume ratios. These profiles are used to provide information about the components of the structure. However, the components of the structure that appear in the sections consist only of the representation of the structure to which they belong on the section plane. For this reason, making direct comments using the profiles obtained may be misleading due to the lack of real data on the three-dimensional properties of the components to which the profiles belong (Kaplan et al., 1997).

Epilepsy is one of the most common neurodegenerative diseases. It is characterized by recurrent, spontaneous seizures arising from abnormal electrical activity in the brain (McNamara, 1999). This epileptic seizure is defined by the International Association Against Epilepsy (ILAE) as "a temporary appearance of signs or symptoms due to abnormal, excessive or synchronized neural activity in the brain" (Fisher et al., 2005). Epileptic seizures are examined in three groups: partial (focal), generalized and unclassifiable. In partial epilepsy, excessive discharge of neurons occurs in a specific region of the cerebral hemisphere, whereas in generalized epilepsy, excessive discharge of neurons occurs in both hemispheres (Falco-Waltera et al., 2018). The effectiveness of anticonvulsant drug candidate chemicals, which form the basis for the history of epilepsy, was initiated by studies conducted on cats by Merrit and Putnam in the 1930s (Onat et al., 2013).

Today, there are many animal experimental models of epilepsy. The most commonly used of these is the pentylenetetrazol (PTZ) model, which enables primary generalized seizures to occur. Since the epileptic seizure patterns in this model are very similar to those in humans, the most commonly used agent is PTZ (McDonald and Barker, 1978; Paredes et al., 1989). PTZ is generally used in the dose range of 50-80mg/kg in experimental models. It shows its effectiveness with clonic convulsions occurring within 30 minutes after injection and lasting for 3-5 seconds, either in the front or back extremities alone or in the hind extremities alone (Onat et al., 2013).

The aim of this study is to calculate the total volumes of the lobus temporalis and lobus parietalis and the volumes of the grey matter regions using stereological methods and to reveal whether there is any change in the number of neurons in the grey matter.

MATERIAL AND METHODS

This study was conducted at the Experimental Medicine Application and Research Center, in accordance with the decision of the Van Yuzuncu Yil University Animal Experiments Local Ethics Committee dated 28.11.2019 and numbered 2019/11. This research was supported by Yuzuncu Yil University Scientific Research Projects Directorate as project number TDK-2020-8840. This article is summarized from the doctoral thesis "A Stereological Study on the Temporal and Parietal Region of the Brain in Male Rats with Epilepsy Applied by Pentylenetetrazole (PTZ)". Laboratory studies were carried out in the research laboratories of Van Yuzuncu Yil University, Faculty of Veterinary Medicine Department of Anatomy and Faculty of Medicine, Department of Histology and Embryology.

In this study, 3-months-old healthy twenty adult male Wistar Albino rats with an average weight of 250 g were used. Experimental animals were housed in standard cages at the Experimental Medicine Application and Research Center in an environment with 12 hours of light, 12 hours of darkness and a temperature range

of 18-24ºC. 20 male adult rats were divided into 2 groups: control and PTZ groups. The rats were divided into groups and kept for 10 days so that they could adapt to the new environment and group elements. After the waiting period, no experimental substance was given to the animals in the control group. In the PTZ group, 80 mg/kg PTZ was mixed into 0.5 ml of 0.9% physiological saline and administered as a single dose injection intraperitoneally. Observation was made for 30 minutes after the injection. It was observed that the rats, in which 6 behavioral change stages were detected according to the Racine scale, were in convulsion (Racine, 1972). Animals in both control and PTZ groups were perfused.

In perfusion application, ketas was given to the animals intraperitoneally at a dose of 50 mg/kg as an anesthetic agent. The animals were allowed to enter deep anesthesia. The skin, ribs and abdominal muscles were cut horizontally from the diaphragm border of the animals that had entered deep anesthesia. Access was made to the ventriculus sinister of the heart with a three-input and single-output cannula set. By administering 0.5cc heparin through the first cannula, the blood in the circulatory system was prevented from coagulating with the help of the heart.

After perfusion, the heads of the animals were dissected after being separated from the body, placed in 10% buffered formaldehyde and kept for 24 hours, and the calvaria, which is the dome of the fossa cranii, was removed and the cerebrum was exposed.

Cerebrum separated from meninges. The 24-hours fixing process was repeated. The lobus temporalis of the cerebrum was cut from its anterior and posterior borders. Since the lobus parietalis is located above the lobus temporalis and within the anterior and posterior borders of the lobus temporalis, the lobus parietalis was also dissected while the lobus temporalis was removed (Figure 1).

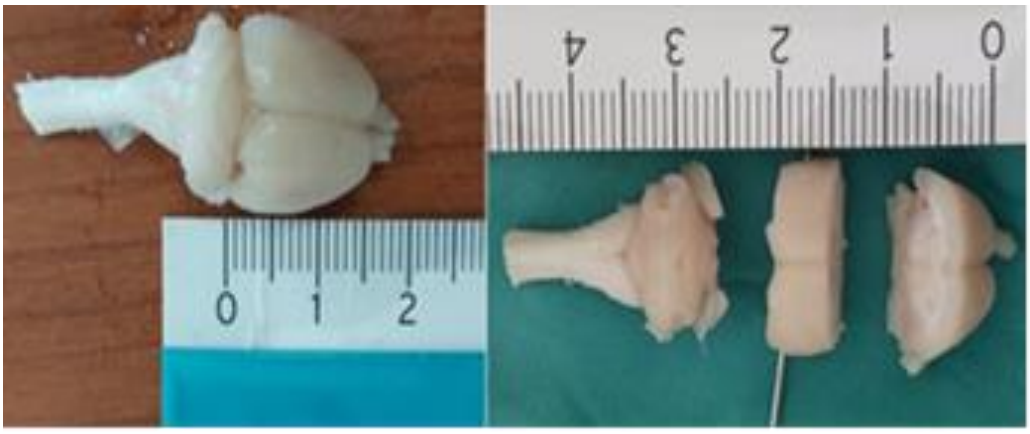

Figure 1. *Separation of lobus parietalis and lobus temporalis from the cerebrum.*

The part of the cerebrum, where both lobus temporalis and lobus parietalis are located, was divided into pieces considering the stereological fraction (f) sampling rate 1/1 (f1). Tissue tracking was applied to the dissected lobes. Then, the tissues were blocked with paraffin. Sections (f2) were taken from the obtained blocks in the form of 5µm thick parallel and serial sections at a ratio of 1/75. An average of 10 sections were obtained for each animal. Sections were stained with Hematoxylin-Eosin (Sikandar et al., 2013). It was viewed on a Zeiss 40 model light microscope. The resulting preparations were photographed under a microscope with a x1.25 microscope. The total volume of the lobus temporalis and lobus parietalis and the volume values of the grey matter regions were measured with a dotted area measurement ruler. Cell counting was performed using an unbiased counting frame on sample and observation section photographs taken at x100 objective magnification (Figure 2).

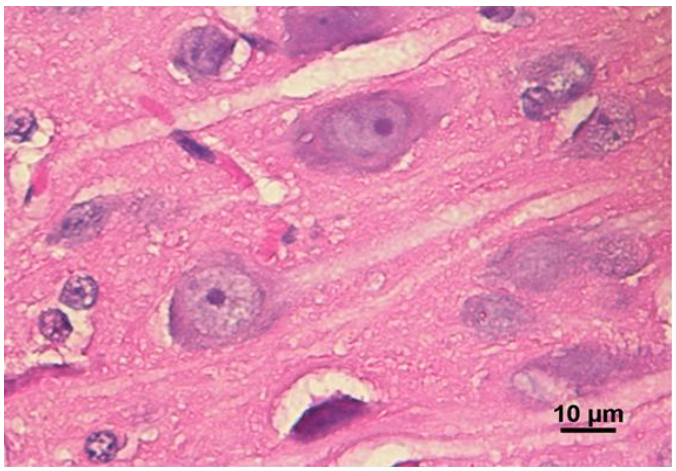

Figure 2. *View of the neuron structure under the microscope x200 objective (Hematoxylin-Eosin).*

Cavalieri's Principle was used to calculate the total volumes of lobus temporalis and lobus parietalis (Canan et al., 2002). The application of component volume ratios was used for the grey matter regions. Physical dissector Cavalieri Principle was used for cell counting (Ragbetli et al., 2010). Volume and cell count were calculated with the SHTEREOM I program and transferred to the excel environment (Oguz et al., 2007; Cakmak et al., 2019) (Figure 3, Figure 4).

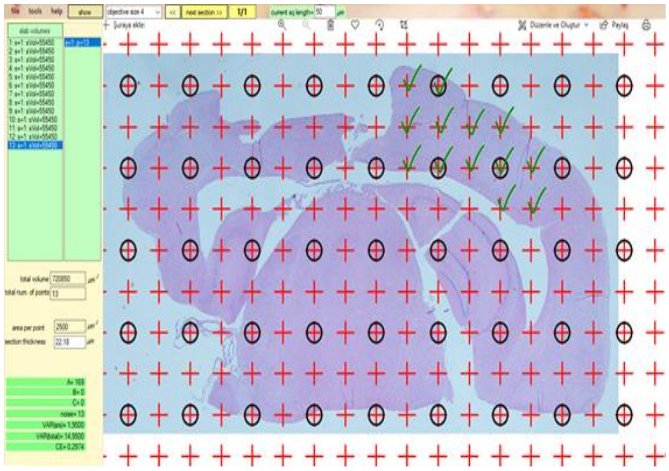

Figure 3. *Dotted area measurement ruler (Shtereom I program).*

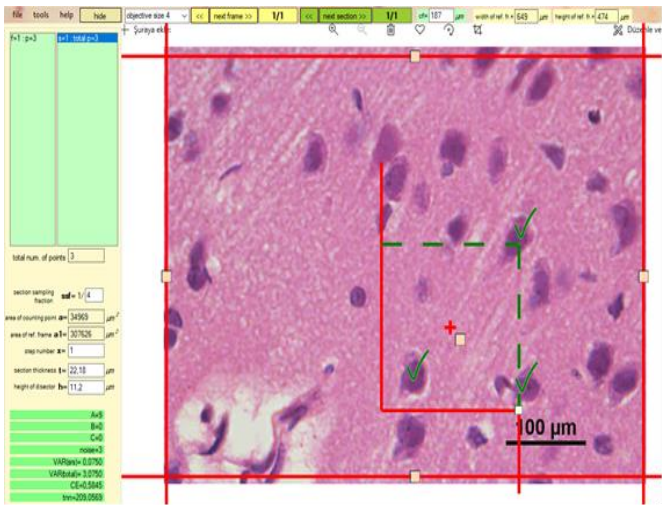

Figure 4. *Unbiased counting frame in the sample cross section.*

RESULTS AND DISCUSSION

Microanatomical Results

Cerebrum tissue taken from the control and PTZ groups was observed microscopically. In the cerebrum sections where Hematoxylin-Eosin staining was applied, the total cerebrum, grey matter region and neuron cells appeared structurally normal.

Measurement Results

Shrinkage rate of cerebrum tissue

As a result of the measurement of the cerebrum tissue on the preparation using a caliper, it was determined that the average size of the PTZ group increased by 0.02291 compared to the average size of the control group.

Comparison of volume of lobus temporalis and lobus parietalis regions, volume of the grey matter and number of neurons in control and PTZ groups

Table 1. *Comparison control and PTZ groups. of total lobus parietalis and lobus temporalis brain volume values in the*

Total lobus temporalis and lobus parietalis volume (cm^3)			
	Control Group		**PTZ Group**
C 1	0.5904	**PTZ 1**	0.5630
C 2	0.6696	**PTZ 2**	0.6486
C 3	0.7866	**PTZ 3**	0.6898
C 4	0.6638	**PTZ 4**	0.7074
C 5	0.5558	**PTZ 5**	0.5540
C 6	0.6088	**PTZ 6**	0.5360
C 7	0.6106	**PTZ 7**	0.6070
C 8	0.7086	**PTZ 8**	0.6214
C 9	0.5142	**PTZ 9**	0.7902
C 10	0.5320	**PTZ 10**	0.6660
Mean	0.6240	**Mean**	0.6383

C: Control group, PTZ: Pentylenetetrazole group.

Stereological measurements made on the sections of the control and PTZ groups, total lobus temporalis and lobus parietalis volume and grey matter volume results were obtained in cm^3. Neuron number measurement results were determined in units (Table 1).

According to these obtained values, the highest total lobus temporalis and lobus parietalis brain volume value in the control group was 0.7866 cm^3 and the lowest was 0.5142 cm^3. In the control group, the mean total lobus temporalis and lobus parietalis brain volume value was determined as 0.6240 cm^3. In the PTZ group, the highest total lobus temporalis and lobus parietalis brain volume values were 0.7902 cm^3 and the lowest were 0.536 cm^3. In the PTZ group, the mean total lobus temporalis and lobus parietalis brain volume value was calculated as 0.6383 cm^3 (Table 1).

Table 2. *Comparison of the grey matter volume values of total lobus parietalis and lobus temporalis in control and PTZ groups.*

Volume of the grey matter (cm^3)			
	Control Group		**PTZ Group**
C 1	0.1331	**PTZ 1**	0.1247
C 2	0.1510	**PTZ 2**	0.1436
C 3	0.1724	**PTZ 3**	0.1527
C 4	0.1497	**PTZ 4**	0.1566
C 5	0.1253	**PTZ 5**	0.1227
C 6	0.1373	**PTZ 6**	0.1187
C 7	0.1377	**PTZ 7**	0.1344
C 8	0.1598	**PTZ 8**	0.1376
C 9	0.1160	**PTZ 9**	0.1750
C 10	0.1360	**PTZ 10**	0.1474

Mean	0.1418	**Mean**	0.1413

C: Control group, PTZ: Pentylenetetrazole group.

When the control and PTZ groups were evaluated in terms of the mean total lobus temporalis and lobus parietalis brain volume values, it was determined that the volume value in the PTZ group increased by approximately 2.29% compared to the control group (Table 1).

The grey matter volume values of the lobus temporalis and lobus parietalis regions in the control and PTZ groups were calculated using stereological methods. According to the values given in table 2, the highest grey matter volume value in the control group was 0.1724 cm^3, the lowest was 0.116 cm^3, and the mean volume value was determined as 0.1418 cm^3. In the PTZ group, the highest grey matter volume value was determined as 0.175 cm^3 and the lowest was 0.1187 cm^3, while the mean volume value was calculated as 0.1413 cm^3. When the grey matter volume mean of the control and PTZ groups were compared, it was determined that there was an approximately 0.35% decrease in the PTZ group compared to the control group (Table 2).

Table 3. *Comparison of the number of neurons in the lobus temporalis and lobus parietalis regions in the control and PTZ groups.*

Number of Neurons			
	Control Group		**PTZ Group**
C 1	11600000	**PTZ 1**	11532000
C 2	12230000	**PTZ 2**	12396000
C 3	12798000	**PTZ 3**	12542000
C 4	12050000	**PTZ 4**	12722000
C 5	11420000	**PTZ 5**	11514000
C 6	12325000	**PTZ 6**	11364000
C 7	12418000	**PTZ 7**	12300000
C 8	12659000	**PTZ 8**	12346000
C 9	11306000	**PTZ 9**	12344000
C 10	11369000	**PTZ 10**	12432000
Mean	12017500	**Mean**	12149200

C: Control group, PTZ: Pentylenetetrazole group.

The number of neurons in the lobus temporalis and lobus parietalis regions in the control and PTZ groups was calculated using stereological methods. According to the obtained values, the highest number of neurons in the control group was 12798000 cells and the lowest was 11306000 cells, while the mean number of neurons was counted as 12017500 cells. In the PTZ group, the highest number of neurons was determined to be 12722000 and the lowest was 11364000, while the mean number was calculated to be 12149200. When the number of neurons in the control and PTZ groups was compared in terms of mean values, it was observed that there was an approximately 1.09% increase in cells in the PTZ group compared to the control group (Table 3).

Table 4. *Coefficient of Variance (CV) values of the brain volume of the total lobus parietalis and lobus temporalis regions of the groups, the grey matter volume and number of neurons.*

Groups	**Total lobus temporalis and lobus parietalis brain volume (CV)**	**Brain grey matter volume (CV)**	**Number of neurons (n) (CV)**
C	0.13	0.11	0.10
PTZ	0.12	0.12	0.11

C: Control group, PTZ: Pentylenetetrazole group.
CV= Coefficient of Variance (interindividual variation).

In the control and PTZ groups, the Coefficient of Variance (CV) value, defined as the ratio of the total lobus temporalis and lobus parietalis brain volume, brain grey matter volume and neuron number standard deviation to the arithmetic mean and multiplied by one hundred, was calculated (Table 4). Table 4 was used

in the calculation. The fact that the inter-individual variation values in both groups was between 0.10 and 0.13 showed that the number of rats used in the study was sufficient and inter-individual variation did not affect the results (Table 4).

Table 5. *Average Coefficient of Error (CE) values of the brain volume of the total lobus parietalis and lobus temporalis regions of the groups, grey matter volume and number of neurons.*

Groups	Total lobus temporalis and lobus parietalis brain volume (CE)	Brain grey matter volume (CE)	Nöron sayısı (n) (CE)
C	0.045	0.048	0.046
PTZ	0.042	0.044	0.039

C: Control group, PTZ: Pentylenetetrazole group.
CE: Coefficient of Error.

Coefficient of Error (CE), obtained by dividing the standard deviation to the arithmetic mean, was calculated separately for the total lobus temporalis and lobus parietalis brain volume, brain grey matter volume and number of neurons in the control and PTZ groups (Table 5). The results obtained showed that this value was below 5%.

Statistical Analysis

In this study, the power of each variable was determined as at least 0.80 and the type 1 error was 0.05 in calculating the sample size of the study. Descriptive statistics for continuous variables in our study are expressed as mean, standard deviation, median, minimum and maximum. Whether the measurements in the study were normally distributed or not was analyzed with Shapiro-Wilk (n<50) and Skewness-Kurtosis tests, and nonparametric tests were applied because some measurements did not show normal distribution and the number of samples was insufficient. Mann-Whitney-U Test was used to compare measurements by groups. Spearman correlation coefficients were calculated to determine the relationships between measurements, separately in the groups in the study. In the calculations, the statistical significance level (a) was taken as 5% and the Statistical Package for the Social Sciences (SPSS (IBM SPSS for Windows, ver.25)) statistical package program was used for the calculations.

Table 6. *Statistical comparison of total lobus parietalis and lobus temporalis brain volume, grey matter/volume, number of neurons and grey matter /total lobus parietalis and lobus temporalis brain volume values of the groups.*

	Groups	Mean	Std. Dev.	Median	Min.	Max.	*p.
Total lobus temporalis and lobus parietalis brain volume (cm^3)	**Control**	.6240	.0843	.6097	.5142	.7866	.650
	PTZ	.6383	.0788	.6350	.5360	.7902	
	Total	.6312	.0798	.6160	.5142	.7902	
Brain grey matter volume (cm^3)	**Control**	.1418	.0166	.1375	.1160	.1724	.940
	PTZ	.1413	.0174	.1406	.1187	.1750	
	Total	.1416	.0166	.1377	.1160	.1750	
Number of brain neurons (n)	**Control**	12017500	555774.6	12140000	11306000	12798000	.496
	PTZ	12149200	485758.2	12345000	11364000	12722000	
	Total	12083350	512493.5	12312500	11306000	12798000	
Grey matter/total lobus temporalis and lobus parietalis brain volume	**Control**	.2279	.0100	.2255	.2192	.2556	.002
	PTZ	.2214	.0001	.2214	.2213	.2215	
	Total	.2247	.0076	.2215	.2192	.2556	

* Significance levels according to Mann-Whitney-U test results.
The difference between groups receiving different names or letters is significant (P<0.05).
C: Control group, PTZ: Pentylenetetrazole group.
Std. Dev: Standard deviation, Min.: Minimum, Max.: Maximum.

In the table above, total lobus temporalis and lobus parietalis brain volume (cm^3), brain grey matter volume (cm^3), number of neurons in total lobus temporalis and lobus parietalis (n) and the grey matter/total lobus temporalis and lobus parietalis brain volume measurement values are divided into groups. Comparison results are given accordingly. Accordingly, when table 6 is examined, no statistically significant difference

was observed in total lobus temporalis and lobus parietalis brain volume values according to groups (p>0.05). In other words, total lobus temporalis and lobus parietalis brain volume measurements were found to be statistically similar in both groups. However, microanatomically, it was determined that the total lobus temporalis and lobus parietalis brain volume was higher in the PTZ group than in the control group.
Similarly, no statistically significant difference was observed between the groups in the grey matter volume measurement of the lobus temporalis and lobus parietalis regions (p>0.05) (Table 6). In other words, the grey matter volume measurement of the lobus temporalis and lobus parietalis regions was found to be similar in both groups.

No statistically significant difference was observed in the number of neurons in the lobus temporalis and lobus parietalis regions according to the groups (p>0.05) (Table 6). When these values were examined statistically, the number of neurons in the lobus temporalis and lobus parietalis regions was found to be similar in the PTZ and control groups.

On the other hand, a statistically significant difference was detected in the grey matter/total lobus temporalis and lobus parietalis brain volume values according to the groups (p <0.05) (Table 6). Thus, the grey matter/total lobus temporalis and lobus parietalis brain volume values were found to be lower in the PTZ group compared to the control group. In fact, when the volume values of the grey matter and total lobes were calculated separately and the volume values and number of neurons were evaluated statistically in the control and PTZ groups, no significant difference was detected. No statistical differences could be determined within and between groups in the grey matter/total lobus temporalis and lobus parietalis brain volume values and number of neurons for each group. However, differences were detected in grey matter/total lobus temporalis and lobus parietalis volume values in both groups. Accordingly, it has been noticed that the separate statistics are independent of the grey matter/total lobus temporalis and lobus parietalis volume values. According to scientific opinion, it can be concluded that this statistical difference is due to the cell background material in both volumes or the change in glial cells that have not been counted.

Table 7. *Correlation analysis results between the grey matter volume (cm³) and neuron number (n) measurements of lobus temporalis and lobus parietalis regions in the control group.*

		Total lobus temporalis and lobus parietalis brain volume (cm³)	Brain grey matter volume (cm³)
Brain grey matter	r	.964*	
volume (cm³)	p.	***.001***	
Number of neurons in total lobus	r	.903*	.867*
temporalis and lobus parietalis (n)	p.	***.001***	***.001***

*p<0.05 r: Coefficients of Spearman correlation.

When the inter-measurement correlation analysis results in the control group given in Table 7 were evaluated, a statistically significant relationship was found between the total lobus temporalis and lobus parietalis brain volume and the grey matter volume of the lobus temporalis and lobus parietalis regions (p<0.05). This situation was found to be 96.4% positive. In other words, as the total lobus temporalis and lobus parietalis brain volume increases, the grey matter volume of the lobus temporalis and lobus parietalis regions also increases.

Similarly, a statistically significant relationship was observed between the total lobus temporalis and lobus parietalis brain volume and the number of neurons in the lobus temporalis and lobus parietalis regions (p<0.05) (Table 7). This significant relationship was found to be 90.3% positive. In other words, as the total lobus temporalis and lobus parietalis brain volume increases, the number of neurons in the lobus temporalis and lobus parietalis regions also increases.

In the control group, a statistically significant relationship was detected between the grey matter volume of the lobus temporalis and lobus parietalis regions and the number of neurons in the lobus temporalis and lobus parietalis regions (p<0.05) (Table 7). This relationship was found to be 86.7% positive. In other words, as the number of neurons in the lobus temporalis and lobus parietalis regions increases, the grey matter volume of the lobus temporalis and lobus parietalis regions also increases. On the other hand, no statistically significant

relationship was found between the grey matter volume/total lobus temporalis and lobus parietalis brain volume and the number of neurons in the lobus temporalis and lobus parietalis regions (p>0.05) (Table 7).

Table 8. *Correlation analysis results between the grey matter volume (cm³) and neuron number (n) measurements of lobus temporalis and lobus parietalis regions in the PTZ group.*

		Total lobus parietalis and lobus temporalis brain volume (cm³)	The grey matter of the lobus temporalis and lobus parietalis regions volume (cm³)
The grey matter of the lobus temporalis and lobus parietalis regions volume (cm³)	**r**	.995*	
	p.	***.001***	
Number of neurons in lobus temporalis and lobus parietalis regions (n)	**r**	.818*	.817*
	p.	***.002***	***.004***

*p<0.05 r: Coefficients of Spearman correlation.

Table 8 shows the correlation analysis results between measurements in the PTZ group. According to the data obtained as a result of the analysis, a statistically significant relationship was detected between the total lobus temporalis and lobus parietalis brain volume and the grey matter volume of the lobus temporalis and lobus parietalis regions (p<0.05). This relationship was found to be 99.5% positive. In other words, as the total lobus temporalis and lobus parietalis brain volume increases in the group given PTZ, the grey matter volume of the lobus temporalis and lobus parietalis regions also increases.

Similarly, in the PTZ group, a statistically significant relationship was found between the total lobus temporalis and lobus parietalis brain volume and the number of neurons in the lobus temporalis and lobus parietalis regions (p<0.05) (Table 8). This value was found to be 81.8% positive. In other words, as the total lobus temporalis and lobus parietalis brain volume increases, the number of neurons in the lobus temporalis and lobus parietalis regions also increases.

In animals with PTZ, a statistically significant relationship was detected between the grey matter volume of the lobus temporalis and lobus parietalis regions and the number of neurons in the lobus temporalis and lobus parietalis regions (p<0.05) (Table 8). It was observed that this relationship was 81.7% positive. In other words, as the number of neurons in the lobus temporalis and lobus parietalis regions increases, the grey matter volume of the lobus temporalis and lobus parietalis regions also increases.

When the the grey matter/total temporal and parietal lobe brain volume and the number of neurons in the lobus temporalis and lobus parietalis regions were evaluated, a statistically significant relationship was detected between them (p<0.05) (Table 8). This situation was found to be 81.2% positive. In other words, as the brain volume ratio of the grey matter/lobus temporalis and lobus parietalis increases, the number of neurons in the lobus temporalis and lobus parietalis regions also increases.

DISCUSSION

Epilepsy is a disease that directly affects the cortex cerebri (Pardoe et al., 2017). Models of epilepsy have emerged as a result of testing molecules used to produce anticonvulsant drugs (Onat et al., 2013; Uslu and Kulaksızoğlu, 2018).

In order to examine the effect of topiramate, one of the epilepsy drugs produced in the 1990s, on neurons, Sonat and Balci (2010) created experimental epilepsy with pilocarpine. The experiment was conducted with the occurrence of numerous tonic-clonic seizures with a single intraperitoneal injection of 380 mg/kg pilocarpine, which is considered a high dose (Sonat and Balci, 2010). In our study, a dose of 80mg/kg, which is considered a high dose, was preferred in the experimental epilepsy model created using PTZ. As a result of the application of this dose, tonic-clonic contractions were observed in the animals, similar to the results obtained with pilocarpine applied at a dose of 380 mg/kg by Sonat and Balci (2010).

In a thesis study conducted in 2008, an epilepsy model (status epilepticus) was created by administering lithium (127 mg/kg) and low dose pilocarpine (50mg/kg) to rats intraperitoneally (i.p.). In this application, lithium and pilocarpine are dissolved in distilled water and administered i.p. in 0.2 ml. It was carried out by injection (Yetismis, 2008). In this study, 80 mg/kg PTZ was mixed into 0.5 ml of 0.9% physiological saline and administered as a single dose injection intraperitoneally. The study does not agree with the study conducted by Yetismis (2008) both in terms of the chemical substance used in the epilepsy model and in terms of dosage. Additionally, Yetismis (2008) used two different chemicals to be able to create epilepsy. However, in this study, the use of PTZ in a single dose was preferred. Thus, the animals used in the experimental study were kept under less stress. While Yetismis (2008) applied the status epilepticus model in his study, the generalized model was preferred in this study. Both studies also differ in the choice of creating an epilepsy model.

In an experimental study, a single dose of PTZ 35 mg/kg was given intraperitoneally to rats and the number of c-fos positive neurons in the lobus dexter cerebri was calculated by immunohistochemical method. As a result, it was determined that there was an increase in the number of c-fos positive neurons (Cetindag et al., 2021). While the chemical substance and method used in this study are similar to the study conducted by Cetindag et al., (2021), there are differences in the doses applied. In addition, although they are similar in terms of neuron count, Cetindag et al., (2021) preferred immunohistochemical staining, while the Hematoxylin-Eosin staining method was chosen in this study. However, as a result, a neuron count was made. While Cetindag et al., (2021) found an increase in the number of c-fos positive neurons in his study, no statistical difference was detected in the neuron count in this study. Similar to the study conducted by Cetindag et al., (2021), Willoughby et al., (1997) preferred the immunohistochemical method to calculate the number of c-fos positive neurons. They found that there was an increase in the number of c-fos positive neurons in both the hippocampus and cortex with the intravenous administration of 10 mg/kg kainic acid to rats. Unlike the current study, no increase in the number of neurons was found in this study.

It was examined whether there was neuronal loss in the dentate gyrus in the experimental epilepsy model created by administering kainic acid with an average dose of 32 mg/kg. Fewer neurons were observed in the subject group than in the control group (Buckmaster and Dudek, 1997). Our study differs from this study in terms of both chemical PTZ application and the subtantia grisea areas of the lobus temporalis and lobus parietalis regions. However, although we counted neurons in a similar manner, no change was detected between the groups in our study in terms of the number of neurons.

In a study, an audiogenic epilepsy model was created by exposing rats to 110–120 dB sound stimuli for 90 seconds, and the colliculus superior sinister and dexter were evaluated with the stereological method. While there was a statistically significant difference in terms of total number of neurons in the colliculus superioris dextra between the control and epilepsy groups, it was determined that there was no statistically significant difference between the control group and the epilepsy group in the colliculus superioris sinistra (Keloglan et al., 2017). Although the fact that neuron counting was performed in cortex cerebri made a difference in our study, the number of neurons was calculated in a similar way.

In the study conducted by Courchesne et al., (2000), the volume of the grey matter in the Cortex cerebri in healthy children and adults increased by 13% between early childhood (between 19-33 months, average 26 months) and late childhood (6-9 years), and then it increased by 1% every 10 years. They found that it decreased by 5% (2000). Bonilha et al., (2004) found that there was a significant decrease in cortex cerebri and the subcortical grey matter in adults with temporal lobe epilepsy In a study, no significant difference was found in cerebrum volumes in volume measurements taken from radiological images between epileptic and healthy adults and patients with partial and generalized epileptic disorder in the epilepsy group (Bonilha et al., 2004).

In another study where stereological measurements were obtained using the Cavalieri method, a total of 100 children, 50 females and 50 males, between the ages of 3-16 were used. In the study, cranial magnetic resonance images and volumetric measurements of cerebral cortex, the cerebral white matter, cerebrum, cerebellum and total brain were taken retrospectively in children with generalized and partial seizures diagnosed with idiopathic epilepsy. The brain volume values obtained between the partial and generalized seizure groups were compared and no statistically significant difference was detected. However, asymmetry

in cerebral cortex volume was detected in both seizure groups (Adanir, 2019). In this study, the physical dissector method was used and the volume measurement values on the obtained sections were carried out using the Cavalieri's Principle, one of the stereological calculation methods. This study is not similar to existing studies in obtaining measurement values. However, as stated in both literatures, no difference or change in the volume values of the lobus temporalis and lobus parietalis regions of the PTZ group was detected in this study. However, this study on the experimental epilepsy model differs from the study conducted by Adanir (2019) in terms of lobus temporalis and lobus parietalis total volume measurement and neuron count, which are targeted to be determined in this study.

In a different study on Temporal Lobe Epilepsy (TLE), the lobus temporalis/telencephalon volume ratio was examined on 30 healthy women and 30 female patients with lobus temporalis epilepsy as a control group. In this investigation, stereological measurements were made using the Cavalieri method on magnetic resonance images (MRI). In the calculation of the lobus temporalis/telencephalon volume ratio, it was determined that the patient group was significantly lower than the healthy participants. In this study, stereological measurement was obtained using the Cavalieri method, and it is similar to the methodological calculation method made by Kurkcuoglu et al., (2010) in terms of the use of the stereological method. Although the epilepsy-focused lobus temporalis/telencephalon volume ratio study of Kurkcuoglu et al., (2010) was similar to this study, it differed in terms of volume and neuron counts of the lobus parietalis region other than the lobus temporalis. Additionally, when the results were evaluated, no statistically significant difference could be detected in the grey matter/total lobus temporalis and lobus parietalis volume values between the control and PTZ groups in this study.

Bonilha et al., (2004) found that there was a significant decrease in cortex cerebri and subcortical the grey matter in adults with temporal lobe epilepsy. Epilepsy is a disease that directly affects the cortex cerebri (Bartzokis et al., 2001). Bartzokis et al., (2001). stated that there is a significant decrease in lobus frontalis and lobus temporalis the grey matter volumes with age in healthy men (Bartzokis et al., 2001). In another study conducted in healthy adults, cortex cerebri volume was found to decrease with age (Jernigan et al., 2001). In one of the studies examining the cortex cerebri volume, it was reported that the development of the cortex cerebri does not follow a constantly increasing graph, but increases until a certain age in both men and women and then begins to decrease (Giedd et al., 1999). In this study, lobus temporalis and lobus parieatalis volume and the grey matter volume were calculated, similar to the studies mentioned. Our study is exactly similar to existing studies in terms of the regions selected for volume calculations. In addition, although there is an age factor in the mentioned literature, adult rats were used in our study. Therefore, it is not possible to talk about an age-dependent increase or decrease in volume values for this study.

In a study, no significant difference was found in cerebrum volumes in volume measurements taken from radiological images between epilepsy and healthy adults and patients with partial and generalized epileptic disorder in the epilepsy group (Hagemann et al., 2002). In a different literature, in a volume study conducted on radiological imaging in healthy adults with epilepsy, no significant difference was found in cerebrum volumes between both groups (Adanir, 2019). In this study, the physical disector method was used and the volume measurement values on the obtained sections were carried out using the Cavalieri's Principle, one of the stereological calculation methods. This study is not similar to existing studies in obtaining measurement values. However, as stated in both literatures, no difference or change in the volume values of the lobus temporalis and lobus parietalis regions of the PTZ group was detected in this study.

In a thesis study, the temporal lobe epilepsy (TLE) model was created based on neuropathological findings. For creating this model, kainic acid was injected unilaterally into the dorsalis hippocampus. In this model, kainic acid (KA) caused acute status epilepticus that ended within 24 hours (Rehimli, 2013). In our study, intraperitoneally administered PTZ focused only on the lobus temporalis and lobus parietalis, and the volumetric and cellular changes in these lobes were examined. Rehimli (2013) injected kainic acid into the unilateral dorsalis hippocampus to create the TLE model and thus developed a local working method. Our study is different in this aspect. Additionally, Rehimli (2013) observed the study for 24 hours to create the acute status epilepticus model. However, this study is an acute study and the observation period was determined as 30 minutes. Unlike the acute status epilepticus model, this study is generalized.

Considering the mean values in the PTZ group compared to the control group in the study, it was determined that the number of neurons increased by approximately 1.09% and the total lobus temporalis and lobus parietalis brain volume values increased by 2.29%. However, it was determined that there was a decrease of approximately 0.35% in the grey matter mean volume value of the lobus temporalis and lobus parietalis regions and approximately 2.28% decrease in the grey matter/total lobus temporalis and lobus parietalis brain volume value.

CONCLUSION

As a result, in our study, no statistically significant change was detected in the total lobus temporalis and lobus parietalis brain volume measurement and the grey matter volume measurement of the lobus temporalis and lobus parietalis regions according to the groups. However, in the study, a statistically significant difference was observed between the control and PTZ groups in the brain volume of the grey matter/total lobus temporalis and lobus parietalis. Especially the grey matter/total lobus temporalis and lobus parietalis brain volume values were found to be lower in the PTZ group. However, this value does not change in the control group. This difference only becomes apparent when the grey matter volume values are compared to total lobus temporalis and lobus parietalis volume values. It was determined that the volume values of the lobus temporalis and lobus parietalis regions, which were statistically calculated separately, were independent of the proportional values. It was determined that this difference was not due to the change in the amount of neurons and volume. It has been revealed that the decrease in the grey matter/total lobus temporalis and lobus parietalis brain volume ratio detected in the PTZ group may be due to the decrease in the number of glial cells or the intercellular ground substance and that more detailed studies on this subject are necessary.

As a result of the literature review, it was determined that there are a limited number of anatomical and stereological studies on epilepsy. It is thought that this study may contribute to both epilepsy and stereological studies. As a result of the research, it was concluded that the relationship determined between the volumetric data calculated by stereological methods will contribute significantly to the morphology of the region. As a result, we believe that the data obtained and presented will contribute significantly to eliminating the deficiencies in these issues and can also form the basis for different stereological studies on the nervous system.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed equally.

ETHICAL APPROVAL

This study was conducted at Experimental Medicine Application and Research Center, in accordance with the decision of Van Yuzuncu Yil University Animal Experiments Local Ethics Committee dated 28.11.2019 and numbered 2019/11. There was no tampering with the data collected and this study was not sent to any other academic publication environment for evaluation.

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