

# Antiepileptic drug exposure in the juvenile period does not affect cognitive functions and histomorphology of the hippocampus in adult rats

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## Ethics Committee Approval

The study was approved by Trakya University

animal experiments local ethics committee

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## Conflict of Interest

No conflict of interest was declared by the

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## Abstract

**Background/Aim:** The impact of long-term antiepileptic drug use during childhood, particularly during critical growth and development phases, remains poorly understood, particularly in terms of its potential side effects on cognitive and locomotor functions in adulthood. This concern is further heightened for patients with a history of multiple drug use.

**Methods:** In our experimental animal study, 80 rats were divided into eight groups according to gender and the drugs used. Levetiracetam, vigabatrin, and sodium valproate were added to the drinking water from the 4th week to the 12th week postnatally (juvenile period). After the 12th week (adult period), all groups were tested in the following order: the Morris Water Maze, the Contextual Fear Conditioning Test, the Rotarod Performance Test, and a histomorphological investigation of the hippocampus.

**Results:** The Morris Water Maze Test, which evaluates learning, showed no changes after chronic usage of antiepileptic drugs during the initial 5 days of swimming tests. On the sixth day of memory retention tests, no effect was observed. Additionally, no significant impairment was noted in the Contextual Fear Conditioning Test that assesses associative learning. In the Rotarod test, which evaluates motor coordination, these drugs exhibited no effect on locomotor activity. Furthermore, the histomorphological dissection of the hippocampus revealed no signs of apoptosis or toxicity.

**Conclusion:** Consequently, the chronic use of levetiracetam, vigabatrin, and sodium valproate did not affect learning, memory, and locomotor activity. Histomorphologically, no neurodegenerative effects on the hippocampus were detected.

**Keywords:** antiepileptic drugs, cognitive functions, rats, hippocampus

## Introduction

Epilepsy is one of the most common neurological problems in childhood [1]. Antiepileptic drugs (AEDs) are the primary treatment for childhood epilepsy, but they carry the risk of central nervous system (CNS) dysfunction and other adverse effects, which families often find significantly concerning [2-4]. The treatment of drug-resistant epilepsy in patients with antiepileptic drugs beyond infancy becomes complex due to the potential long-term consequences of early drug exposure. Such exposure may disrupt brain development, leading to impaired nervous system function, cognitive difficulties, and motor deficits in adulthood. The effects of long-term use of antiepileptic drugs during childhood, particularly during the growth and development period, on cognitive and motor functions in adulthood are not well understood. The use of multiple AEDs can make it complex to identify the side effects related to the drugs, especially over the long term. Therefore, experimental animal studies with homogeneous groups are needed to assess the long-term side effects.

Human studies aimed at defining the long-term side effects of antiepileptic drugs used in childhood are limited due to ethical considerations. As a consequence, more animal-based experimental studies are required. In this regard, various methods can be leveraged to measure the cognitive (e.g., learning and memory) and locomotor (e.g., mobility, swimming speed, unit time) functions of rats. We aimed to investigate the long-term side effects of antiepileptic drugs, used in childhood, on learning, memory, and motor functions in adulthood, by conducting studies with rats.

## Materials and methods

This experimental study was conducted following the principles and procedures outlined in the National Institutes for Health Guide for the Care and Use of Laboratory Animals. Moreover, the experimental protocol received approval from the local ethics committee. The study was carried out in the laboratories of the Pharmacology Department, as well as the Histology and Embryology Department.

### Animals

In this study, a total of 80 Wistar rats (40 males and 40 females) at 24 days old were used. The offspring rats, weaned from their mothers on the 24th postnatal day, were kept four per cage under controlled conditions. These conditions included a temperature of  $22 \pm 2^\circ\text{C}$  and a 12:12 light-dark cycle, with lights turned on at 7 a.m. The rats had free access to both food and water.

### Experimental design

The rats were divided into eight groups (four groups of males and four groups of females,  $n=10$  for each group). The groups and treatments are outlined in Table 1. Rats were weighed weekly from the 4th to the 12th weeks (adult period) [5]. Levetiracetam (Keppra 100 mg/ml oral solution, UCB Pharma), sodium valproate (Depakin oral solution 200 mg/ml, Sanofi Aventis), and vigabatrin (Sabril 500 mg tablet, Sanofi Aventis) were added to the daily drinking water (Table 1). Daily water consumption and nutritional status were monitored. Dosages were determined based on the maximum therapeutic

drug doses used for children and in previous studies [6]. Drug dosing was calculated according to weekly weights, taking into account that the daily water consumption of a rat weighing 100 g is about 10–12 ml [7]. No instances of death or illness were observed during the study period.

Table 1: Groups and treatments

| Group                   | Code | Treatment                       |
|-------------------------|------|---------------------------------|
| Control male            | CM   | Free water                      |
| Control female          | CF   |                                 |
| Levetiracetam male      | LM   | 65 mg/kg/day in drinking water  |
| Levetiracetam female    | LF   |                                 |
| Sodium valproate male   | SVM  | 50 mg/kg/day in drinking water  |
| Sodium valproate female | SVF  |                                 |
| Vigabatrin male         | VM   | 100 mg/kg/day in drinking water |
| Vigabatrin female       | VF   |                                 |

## Evaluation of cognitive functions and locomotor activity in rats

**Morris Water Maze:** Since its conceptualization by Richard Morris, this test has been extensively utilized in learning and memory studies on rodents [8,9]. It constitutes a circular pool (1.5 m diameter, 45 cm high), filled with water to a depth of 30 cm. The water's temperature was uniformly maintained at  $21 \pm 1^\circ\text{C}$ . A square platform measuring  $10 \times 10$  cm was submerged 2 cm below the surface in the water tank. An inert black dye was infused into the water to render the platform invisible to the rat. For the appearance of external cues, the room walls were equipped with fixed spatial cues throughout the experiments. The pool was virtually partitioned into four equivalent quadrants. The escape platform was centrally located in the southwest quadrant. The animal's swimming behavior was observed using software (Ethovision XT 11.0, Noldus, The Netherlands) that analyzed the imagery procured from a ceiling-mounted camera overseeing the pool.

In the experiments' initial 5 days (acquisition phase), rats were placed into water from four different starting points. Each day, we randomly used each of these starting points once. We orientated the rats to face the wall of the pool at one of the starting points. If a rat failed to find the hidden platform within 60 s, we assisted the rat in locating it. Each rat was permitted to remain on the platform for 15 s. The intertrial interval (the gap between consecutive tests on the same day) was 8 min. After completing the swim tests, we dried the rats and returned them to their cages.

On the sixth day, we removed the platform from the pool and introduced the rat into the water from a previously unused starting point; it was then allowed to stay in the pool for 60 s (probe test). We then removed it from the pool, dried it, and returned it to its cage.

The parameters we recorded in the acquisition phase included the latency to find the platform, the mean distance to the platform, the duration spent in the pool's 10-cm perimeter (a thigmotaxis parameter), and swim speed. The data obtained on the same day (from four trials) was treated as that day's average (average of results from four trials on the same day). In the probe trial on the sixth day, we recorded parameters including latency to reach the target quadrant, time spent in the target quadrant, duration spent in the 10-cm perimeter of the pool, and swim speed.

**The Contextual Fear Conditioning Test:** This is a behavioral test used to evaluate fear-based (amygdala-based) memory in rats [10]. The equipment (FCS 21200-R, COMMAT,

Turkey) includes a soundproof outer cage with a test chamber made of transparent Plexiglas that contains a metal grid floor. This floor, comprised of parallel stainless-steel rods, is attached to an electric shock device. The test chamber walls also feature visual cues. The rat's freezing behavior is recorded by a connected computer through a mounted video camera located above the testing chamber. On the test's initial day, the rat is placed inside the test chamber and observed for 7 min. During this time, a foot shock (0.5 mA, 1 s) is administered at the 2nd, 4th, and 6th-minute intervals. Twenty-four hours later, the rat is placed back into the same cage for 5 min and is not subjected to any foot shocks. The freezing behavior of the rat on this second day is then automatically recorded by the software.

**Rotarod Test:** The rotarod test was utilized to assess the motor coordination of the rats. The apparatus comprised a rotating rod with a non-slippery surface, having a diameter of 3 cm and a length of 40 cm, which was placed at a height of 45 cm. The rod was divided into four equal sections (Rotamex 4/8, Columbus Instruments, USA). Each animal was positioned on the rotating rod, which operated between 4 and 20 rpm, and the duration the animal remained on the rod was recorded. This process was repeated three times for each rat. The motor coordination of the rat was evaluated based on the longest time that the animal managed to stay on the rotating mill [11].

**Histomorphological Study:** Following the completion of behavioral tests, the animals were anesthetized and decapitated. Their brain tissues were excised and preserved in 10% neutral formaldehyde for 3 days. These tissue samples were then embedded in paraffin blocks, from which sections of 5- $\mu$ m thickness were obtained. The obtained sections were finally stained with hematoxylin and eosin.

### Statistical analysis

Descriptive statistics were used to evaluate the data. In the analysis of data obtained from Morris Water Maze Test trials, we used a two-way repeated measures analysis of variance (ANOVA) and a *post-hoc* Bonferroni test. A one-way ANOVA and a *post-hoc* Bonferroni test were used to compare data obtained from the probe tests of the Morris Water Maze, the contextual fear memory test, and the Rotarod test. Analyses were performed on GraphPad Prism 6.0 for Mac OSX, Machine ID: 60B52B3D040. A *P*-value of <0.05 was considered significant.

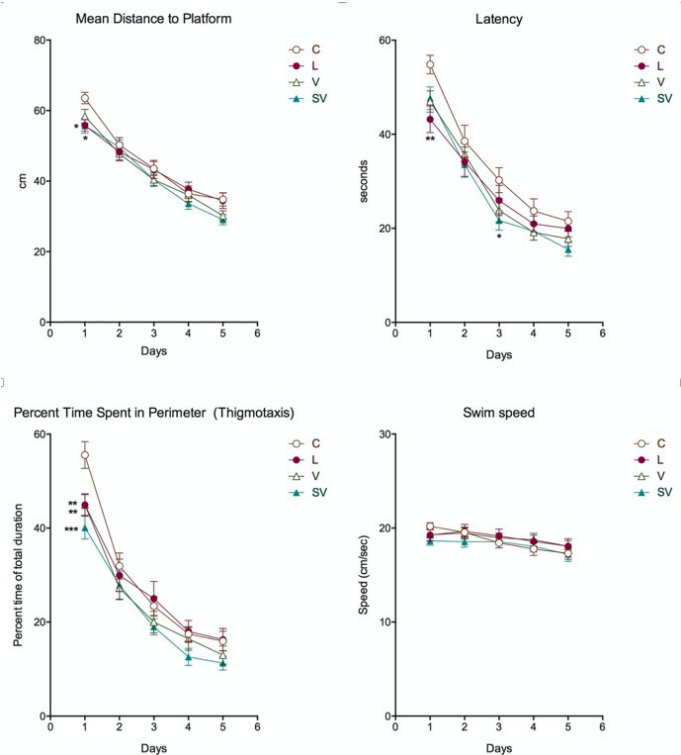
## Results

The data derived from the male and female groups were assessed both as separate entities and as combined treatment categories (e.g., merging CM and CF groups into group C, and LM and LF groups into group L).

**Data from the Morris Water Maze Test trials:** The mean distance to the platform and latency (i.e., the time taken to reach the platform) during swimming were used as learning indicators in the trials carried out over the first 5 days. A two-way repeated measures ANOVA (treatment  $\times$  day) on the mean distance to the platform showed a non-significant treatment effect ( $F [3.76]=1.68$ ;  $P=0.179$ ) and a significant day effect ( $F [4.304]=180.40$ ;  $P<0.001$ ). The interaction was not significant ( $F [12.304]=1.41$ ;  $P=0.159$ ). These results suggest that the animals learned the platform's location through consecutive learning trials, with no apparent difference in learning performance

among the groups (Figure 1). Post-hoc analysis revealed that the performance of the levetiracetam and sodium valproate groups on the first day was significantly better than that of the control group ( $P<0.05$ ); however, no significant differences were observed in the following days. A two-way repeated measures ANOVA (treatment  $\times$  day) on latency revealed a significant treatment effect ( $F [3.76]=3.30$ ;  $P=0.025$ ) and a significant day effect ( $F [4.304]=142.90$ ;  $P<0.001$ ). The interaction was non-significant ( $F [12.304]=0.91$ ;  $P=0.537$ ). A post-hoc analysis showed significantly better performances for the levetiracetam group on the first day ( $P=0.002$ ) and for the sodium valproate group on the third day ( $P=0.033$ ).

**Figure 1:** Morris water maze learning trial performances in combined groups. Each data point indicates average of the result obtained from four swimming session in the same day. Vertical bars indicate standard error of mean. Groups: C, control; L, levetiracetam; SV, sodium valproate; V, vigabatrin. Each group contains male and female rats (n=20 for each group). \*\* $P<0.05$ ; \*\*\* $P<0.001$  compared to control group on the same day

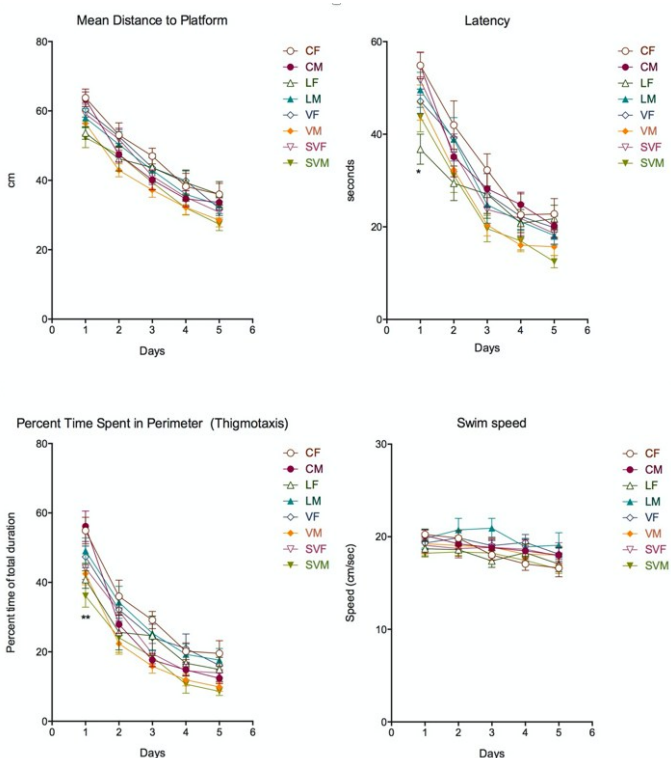


Time spent around the pool's perimeter is an indicator of thigmotaxis, a preference to stay near the pool wall, which is often a sign of an animal's anxiety. A two-way repeated measures ANOVA (treatment  $\times$  day) performed on thigmotaxis reported a non-significant treatment effect ( $F [3.76]=2.72$ ;  $P=0.051$ ) along with a significant day effect ( $F [4.304]=195.9$ ;  $P<0.001$ ). The interaction did not yield significant results ( $F [12.304]=1.74$ ;  $P=0.058$ ). In the post-hoc analysis, the thigmotaxis level on the first day in the control group was higher than that in levetiracetam ( $P=0.007$ ), vigabatrin ( $P=0.007$ ), and sodium valproate groups ( $P<0.001$ ).

A two-way repeated measures ANOVA (treatment  $\times$  day) on swim speed yielded a non-significant treatment effect ( $F [3.76]=0.48$ ;  $P=0.695$ ) and a significant day effect ( $F [4.304]=10.69$ ;  $P<0.001$ ). Although a slight decreasing trend in swim speed on consecutive days was observable, a statistically significant decrease in swim speed compared to the first day was only seen in the control group beginning on the third day ( $P<0.05$  on day 3 and  $P<0.001$  on days 4 and 5 compared to day 1). The interaction was not significant ( $F [12.304]=0.82$ ;  $P=0.625$ ).

When treatment groups were analyzed as independent units without merging the male and female groups (Figure 2), a two-way repeated measures ANOVA (treatment × day) on the average distance to the platform unveiled a significant treatment effect ( $F [7.72]=2.20; P=0.045$ ). However, post-hoc evaluations (Bonferroni test) did not highlight any significant disparities between the control group and other treatment groups on the same days. The interaction was not significant ( $F [28.288]=1.06; P=0.394$ ). The animals learned the platform's location through consecutive trials. A two-way repeated measures ANOVA (treatment × day) on latency signified a significant day effect ( $F [4.288]=144.1; P<0.001$ ) and a significant treatment effect ( $F [7.72]=2.65; P=0.017$ ). The interaction was not significant ( $F [28.288]=1.06; P=0.393$ ). Post-hoc evaluations revealed a statistically significant disparity between the control and levetiracetam female groups on the first day ( $P<0.01$ ), and this difference persisted on the second day.

**Figure 2:** Morris water maze learning trial performances in standalone groups. Each data point indicates average of the result obtained from four swimming session in the same day. Vertical bars indicate standard error of mean. Groups: C, control; L, levetiracetam; SV, sodium valproate; V, vigabatrin; F, female; M, male (n=10 for each group). \* $P<0.01$ ; \*\* $P<0.001$  compared to control group on the same day



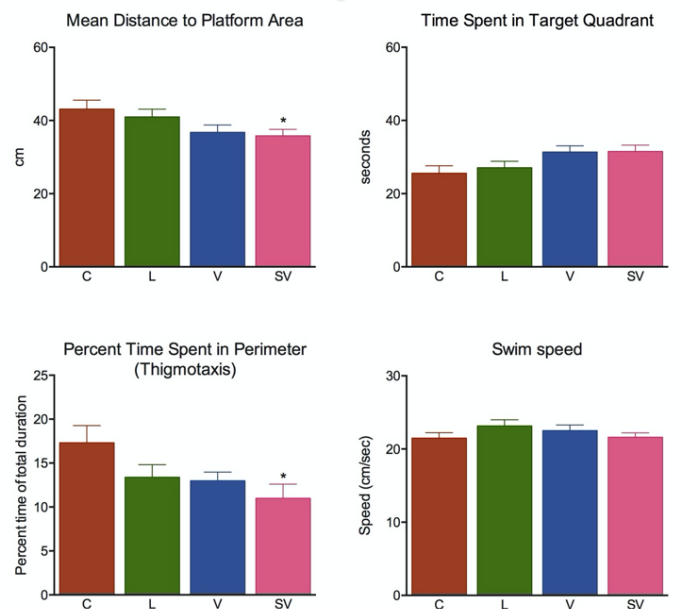
A two-way repeated measures ANOVA (treatment × day) on thigmotaxis revealed a significant treatment effect ( $F [7.72]=3.13; P=0.006$ ) and a significant day effect ( $F [4.288]=194.40; P<0.001$ ). Thigmotaxis gradually decreased on consecutive days. The interaction was not significant ( $F [28.288]=1.23; P=0.202$ ). In post-hoc analysis, the thigmotaxis level on the first day in the male group treated with sodium valproate was statistically significantly lower than that of the control male group ( $P<0.001$ ); this difference disappeared on the second day.

A two-way repeated measures ANOVA (treatment × day) on swim speed revealed a non-significant treatment effect ( $F [7.72]=0.94; P=0.481$ ) and a significant day effect ( $F [4.288]=10.60; P<0.001$ ). There was a slight decreasing trend in swim speed among the female control group on consecutive days ( $P<0.05$  on day 3,  $P<0.01$  on day 4, and  $P<0.001$  on day 5

compared to day 1); the other groups did not show such a statistically significant difference in swim speed. The interaction was not significant ( $F [28.288]=0.83; P=0.721$ ).

**Morris Water Maze Test probe data:** We used the mean distance to the platform area and time spent in the target quadrant as indicators of memory retention in probe trials performed on the sixth day. An ANOVA on the mean distance to the platform area revealed a statistically significant better performance in the sodium valproate group compared to the control group ( $P<0.05$ ). However, we did not observe such a difference in the time spent in the target quadrant. Treatment groups showed lower thigmotaxis than the control group, however, the difference was statistically significant only in the sodium valproate group ( $P=0.028$ ). There was no statistically significant difference in swim speed values among the treatment groups (Figure 3).

**Figure 3:** Morris water maze probe trial performances in combined groups. Vertical bars indicate standard error of mean. Groups: C, control; L, levetiracetam; SV, sodium valproate; V, vigabatrin. Each group contains male and female rats (n=20 for each group). \* $P<0.05$  compared to control group



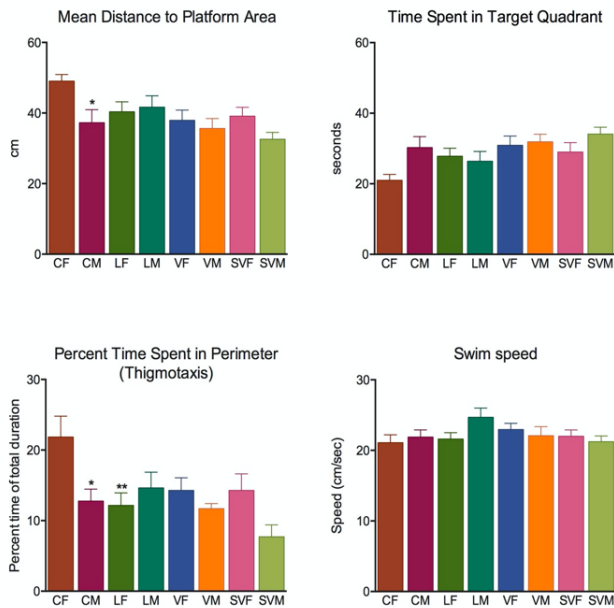
When treatment groups were analyzed independently, without combining the male and female groups (Figure 4), an ANOVA on the mean distance to the platform area revealed a statistically significant better performance in the male control group compared to the female control group ( $P=0.022$ ). However, when the parameter of time spent in the target quadrant was analyzed, no such difference was detected. Thigmotaxis was high in female control animals compared to other groups. Statistically significant differences were observed between the female control group and the male control group ( $P=0.012$ ), as well as the levetiracetam female group ( $P=0.008$ ). Regarding swim speeds, no statistically significant differences were detected among the treatment groups.

**Fear Conditioning Test:** Irrespective of whether the analyses were performed on combined male and female groups or stand-alone groups, the ANOVA analyses indicated no statistically significant difference among the groups (Figure 5).

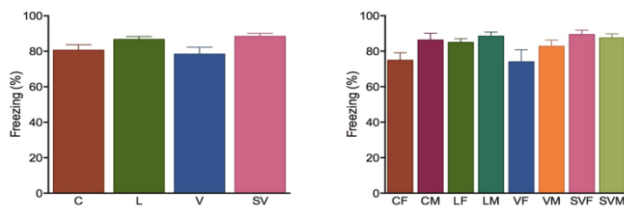
**Rotarod Test:** ANOVA analyses on combined male and female groups, as well as on stand-alone groups, revealed no statistically significant difference in the rotarod performance of animals among groups (Figure 6).



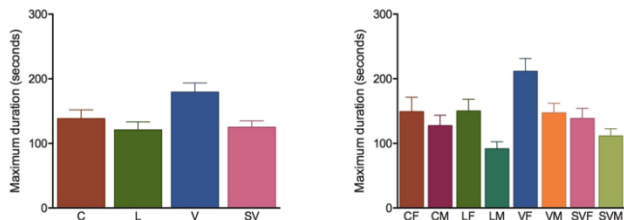
**Figure 4:** Morris water maze probe trial performances in standalone groups. Vertical bars indicate standard error of mean. Groups: C, control; L, levetiracetam; SV, sodium valproate; V, vigabatrin; F, female; M, male (n=10 for each group). \* $P < 0.05$ , \*\* $P < 0.01$  compared to control female group



**Figure 5:** Fear condition test results as combined (left) and standalone groups (right). Vertical bars indicate standard error of mean. Groups: C, control; L, levetiracetam; SV, sodium valproate; V, vigabatrin, F, female; M, male. n=20 for combined groups (10 male and 10 female); n=10 for standalone groups



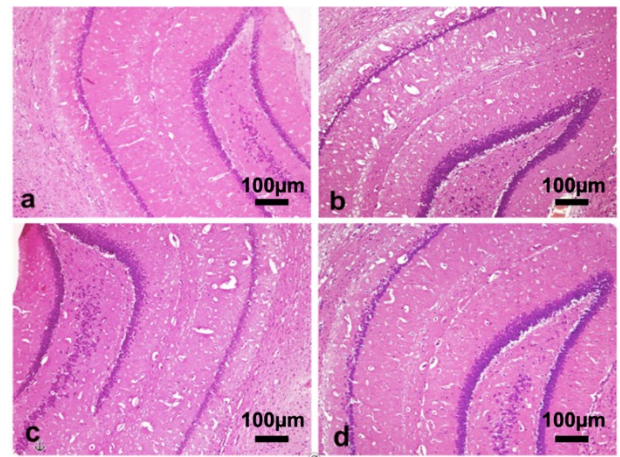
**Figure 6:** Rota-rod performances as combined (left) and standalone groups (right). Vertical bars indicate standard error of mean. Groups: C, control; L, levetiracetam; SV, sodium valproate; V, vigabatrin, F, female; M, male. n=20 for combined groups (10 male and 10 female); n=10 for standalone groups



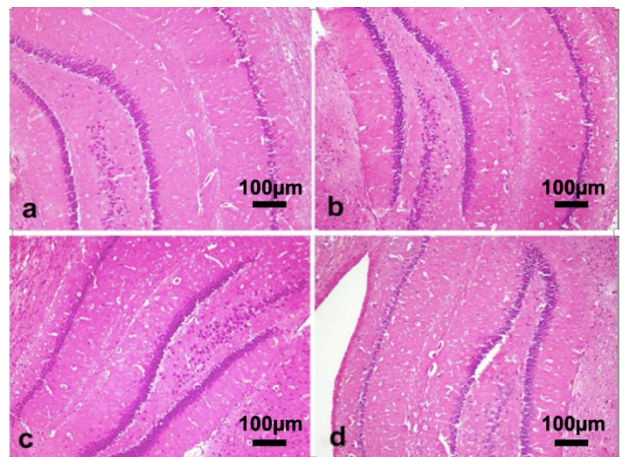
**Histology:** Evaluations of Hematoxylin and Eosin-stained coronal sections were conducted using different magnifications in a light microscope. Regarding general appearance and cellular organization, no morphological differences were observed among the control, drug, female, and male groups in the hippocampus CA1, CA2, CA3, and the dentate gyrus regions (Figure 7, Figure 8).

Upon high magnification examination (400×) of the CA1 regions, neurons close to each other in the male and female control groups were found to exhibit normal cellular morphology. Long-term exposure to drugs such as levetiracetam, sodium valproate, and vigabatrin has been linked to histopathological findings suggestive of cell toxicity or death, not only in neurons but also in other cell types. Symptoms include pronounced eosinophilia, mononuclear cell infiltration, presence of eosinophilic granules, extensive macrophage aggregation, edema, cell membrane degradation, and undetermined nuclear degradation or condensation (Figures 9 and 10).

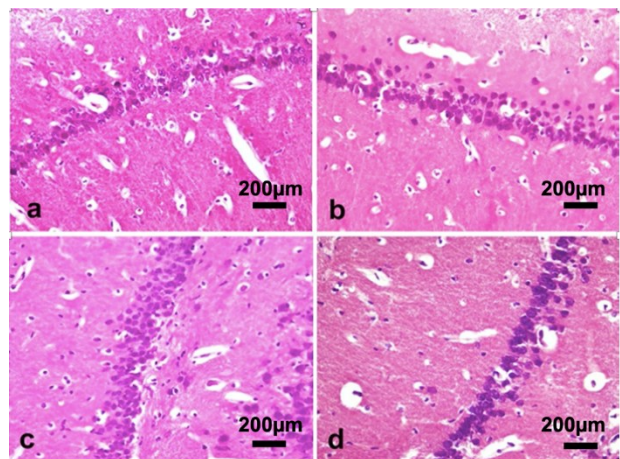
**Figure 7:** a-d. Hippocampus microphotographs of Female control (a), Levetiracetam (b), Sodium valproate (c) and Vigabatrin (d) group H+E, 100x



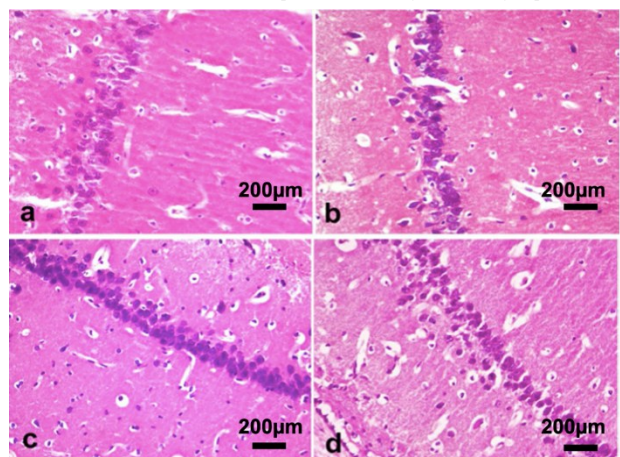
**Figure 8:** a-d. Hippocampus microphotographs of Male control (a), Levetiracetam (b), Sodium valproate (c) and Vigabatrin (d) group H+E, 100x



**Figure 9:** a-d. Histomorphological changes were not detected in the CA1 region female control (a), Levetiracetam (b), Sodium valproate (c) and Vigabatrin (d) group H+E, 400x



**Figure 10:** a-d. Histomorphological changes were not detected in the CA1 region male control (a), Levetiracetam (b), Sodium valproate (c) and Vigabatrin (d) group H+E, 400x



## Discussion

The impact of epilepsy on cognitive functions in children, whether resulting from the disease itself or the side effects of long-term antiepileptic medications, is not fully understood. Antiepileptic drugs may contribute to memory impairment, decreased vigilance, and psychomotor slowing. Current literature typically examines the effects of levetiracetam (LEV) on adult patient groups' cognitive function [12]. Levetiracetam, a newer drug, is believed to have a neuroprotective effect due to its anti-inflammatory and anti-apoptotic characteristics [13]. Side effects such as motor coordination disorders, ataxia, agitation, behavioral alterations, hyperactivity, irritability, and fatigue have been reported when levetiracetam is used [14]. However, recent studies support the safe administration of levetiracetam, even in extremely preterm infants [15]. Experimental studies have evaluated short-term drug applications, alongside cognitive and locomotor activities [16]. The Morris Water Maze, a test commonly used to identify learning and memory issues related to the hippocampus in rodents suffering from brain disorders, was used in this study [17,18]. Naive rats, administered with LEV at 65 mg/kg/day from the 4th to 12th weeks (adult period), demonstrated no difference in learning performance among groups. Our findings suggest that LEV does not impact spatial memory in rats, as they showed improved performance over successive test days, marked decreased time to find the platform and shorter travel distances. Furthermore, LEV administration from day 4 to 14 did not hinder the learning process. We concluded that long-term use of levetiracetam does not affect the memory and learning skills of rats, as evidenced by the Morris Water Maze Test in our study.

The mean distance to the platform and latency (time to reach the platform) parameters reached during swimming were utilized as indicators of learning in trials conducted during the initial five days. A non-significant treatment effect was revealed on the mean distance to the platform. Lamberty et al. [19] discovered that LEV did not obstruct learning in rats when tested in the Morris Water Maze, except for a high dose of sodium valproate (VPA) (300 mg/kg). However, rats given this high dose of VPA swam faster, possibly negatively affecting their maze performance due to increased activity levels. Interestingly, we noted a sudden drop in swimming distance on our experiment's first day. This decrease was not due to less time given to the animals but instead points towards a potential issue with the animals' movement, not a positive outcome. This problem disappeared in the subsequent days. Interpreting these results poses a challenge due to our testing method's limitations. Animals may exert varying levels of effort for the reward, and some might struggle to locate the platform. The experiment's stress could cause some animals to freeze, impeding their learning process. However, we did not observe this behavior in our study.

Previous research has shown that levetiracetam can enhance cognitive abilities such as visual and working memory, motor skills, reaction time, focus, and overall intelligence [20]. However, our study did not find any significant improvement or decline in cognitive function with levetiracetam alone. Conversely, another study reported that levetiracetam did not impact the spatial recall ability of normal mice [21]. A different

study reported cognitive issues in mice post-administration of high doses of levetiracetam (310 mg/kg p.o.) for 45 days. This negative effect was attributed to increased oxidative stress. The researchers emphasized the need for patients' close monitoring, particularly for those receiving long-term levetiracetam treatment [22]. The question of whether levetiracetam can enhance memory in individuals without epilepsy remains unanswered. Additional animal and human clinical trials, employing various cognitive tests, are needed to determine levetiracetam's impact as a supplemental treatment on cognitive function in non-epileptic individuals. Our current research established that levetiracetam alone did not noticeably affect cognition in our experimental model.

Repeated doses of levetiracetam did not significantly impair short-term memory, spatial awareness, or emotional recall in rats. However, a single, large dose of the drug resulted in difficulties with long-term memory, movement, and emotional memory. Importantly, problems with emotional memory were temporary and vanished with repeated dosing [12]. Since our study involved rats without epilepsy, it is challenging to directly apply these findings to humans with epilepsy. Further research is necessary to fully comprehend the safety of levetiracetam, particularly in animals with epilepsy.

These results corroborate previous research demonstrating VPA's adverse impact on learning and memory in rats [23]. Notably, these studies have shown that VPA can impair spatial learning and memory at doses that are comparable to, or even lower than those effective in preventing seizures in various rat epilepsy models [13,16]. In our study, VPA was administered at a dose of 50 mg/kg/day, yet it was not effective in improving the learning and memory of normal rats.

Edalatmanesh et al. [6] discovered that VPA markedly improved cognitive impairments and reduced lipid peroxidation in TMT-treated rats. VPA was identified to have anxiolytic activity both in a standard elevated plus-maze and in conflict tests in the rat [24,25]. However, detecting distinct behavioral changes induced by levetiracetam in healthy rodents can be challenging. This might clarify why levetiracetam effectively reduces anxiety in a specialized, conditioned version of the Vogel test, but not in the standard test. Recent literature has not presented the effect of vigabatrin on learning and cognitive function in naive rats. In our study, vigabatrin did not affect learning and cognitive function. This could explain the absent effects of levetiracetam in normal animals on the elevated plus-maze, perhaps due to the relatively low-stress levels associated with this test.

Long-term exposure to drugs such as levetiracetam, sodium valproate, and vigabatrin has been associated with histopathological findings indicative of potential cell toxicity or neuronal death. Preece et al. [26] reported that vigabatrin (VGB) led to cerebellar and cortical white-matter lesions. In a different study, phenytoin, levetiracetam, carbamazepine, and valproic acid were found to potentially accelerate axonal healing [27]. Our study did not identify any histopathological deterioration in rat brain tissue from exposure to levetiracetam, sodium valproate, or vigabatrin. The Fear Conditioning test, a measure of fear-based memory, yielded no different effects compared to the



control group. No studies mentioned in the existing literature examine the side effects of these antiepileptic drugs.

In our study, we found that the long-term use of levetiracetam, vigabatrin, and sodium valproate in young rats had no impact on cognitive functions in adulthood. No changes were detected in the hippocampal tissues during histomorphological examination. According to our knowledge, there is no study investigating the long-term effects of these drugs administered in young animals on the cognitive function of rats. Our results suggest the safety of long-term use of levetiracetam, vigabatrin, and sodium valproate regarding cognitive functions. However, one must be cautious when extrapolating these findings to humans. Observational studies investigating the effects of long-term use of these drugs on cognitive function should be designed and conducted. The influence of the disease itself on cognitive function should also be examined. For this purpose, by using appropriate animal epilepsy models, the effects of the disease on the cognitive functions of the animals can be assessed.

### Limitations

While we ensured homogeneity in our rat groups for this study, the number of rats could have been higher. Our limitation was that the experimenter had to handle all the rats individually, as varied handling by different people might induce behavioral differences in the rats. We also considered drug administration via oral gavage, but we were constrained by the large rat population and the potential for increased anxiety this method might cause. The complete application of such studies to clinical practices would benefit from multicenter human trials.

### Conclusion

Antiepileptic drugs may affect cognitive and locomotor functions, especially in the long term. In light of our results, levetiracetam, vigabatrin, and sodium valproate given during young period of rats had no effect on cognitive functions and on hippocampal tissues. According to our results, it can be speculated that these drugs are safe with respect to cognitive functions. Our findings need confirmation by observational studies in humans.

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