OPEN ACCESS
Saudi Journal of Biomedical Research
Abbreviated Key Title: Saudi J Biomed Res
ISSN 2518-3214 (Print) |ISSN 2518-3222 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Acute and Chronic Study of PTZ –Induced Seizure Effects on Visuo-Spatial Functions in Wistar Rats

Adesua C. Obiandu[1*], Koofreh G. Davies[1], Christopher E. Ekpenyong[1], Chibuike Obiandu[2], Chimzi Asabah[3]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria
[2]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria
[3]Senior Secondary Schools Board, Port Harcourt, Rivers State, Nigeria

DOI: 10.36348/sjbr.2023.v08i10.001 | **Received:** 29.08.2023 | **Accepted:** 02.10.2023 | **Published:** 11.10.2023

***Corresponding author:** Adesua C. Obiandu
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria

Abstract

Epilepsy is one of the chronic neurological conditions affecting people of all ages globally. Educational progress have been affected negatively by some abnormalities associated with epilepsy. This study employed neurobehavioural analysis of pentylenetetrazole (PTZ) induced seizure on some visual spatial functions in Wistar rats. The study was done in two phases: acute (14days) and chronic (28days). A total of twenty adult Wistar rats weighing between 90-120g were used. The rats were divided into four (4) groups (1-4, n=5). PTZ was administered intraperitoneally to the PTZ treated groups one (1) week before the neurobehavioral experiments commenced. Group 1 (control) received distilled water, group 2, 3and 4 were administered with doses of PTZ (25mg/kg, 30mg/kg and 35mg/kg) respectively at alternate days (48 hourly) until kindling was achieved. The study evaluated neurobehavioural parameters using Barnes maze (BM), navigational box test (NBT). The results from neurobehavioural evaluations on effects of the chemo-convulsant seizures on visual spatial functions, suggests that severity of seizures caused a deficit in learning and visual spatial memory which was worsened by chronicity. In conclusion, PTZ-induced seizure negatively interferes with visual spatial functions.

Keywords: Epilepsy, Pentylenetetrazole, Visual spatial functions, Wistar rats.

1. INTRODUCTION

Epilepsy is a chronic neurological condition globally affecting people of all age brackets, with incidence peak rates in adults and children. About 50million people across the world have epilepsy with 80% living in middle and low income countries. Above 5million new cases are diagnosed yearly in the United States (WHO, 2019).

In Nigeria, the estimated prevalence of epilepsy is 8 persons in every 1000 people showing a substantial burden of the disease in the country (Owolabi *et al.*, 2019). Impairments of certain cognitive functions has been identified as common co-morbidity of epilepsy and these impairments significantly add to the disability experienced by epileptic patients (Abrahams *et al.*, 1999), visual spatial memory is an essential domain in cognition, as this memory expresses the ability to visualize and appreciate the activities, roles and co-ordinates in an environment.

With an increase in availability of anti-epileptic drug therapy and abundance of natural resources, evidences still show that the prevalence of epileptic seizures in Nigeria is irrefutable. Cognitive abnormalities have been thought to negatively affect educational progress and achievement (Berg *et al.*, 2008). Individuals with prolonged seizures lasting more than 30 minutes, a condition termed status epilepticus (SE), or frequent recurrent seizures are at a particular risk for brain injuries that can result in cognitive impairment (Kleen *et al.*, 2012). The postictal period is usually associated with reduced cognitive functions. As the postictal state of lethargy and confusion improve, impairment in cognition may still persist for a variable period of time ranging from several minutes to several days, depending on the nature of the seizure (Lin *et al.*, 2009). Although, the exact cause of postictal impairment is not yet known, findings in some experimental animal studies has indicated that, seizures occurring over a longer period may cause affectation of long term

potentiation thereby leading to a loss of accuracy and stability of the hippocampal place cells (Nickels & Wirrell, 2017). The long term potentiation is a mirroring of sustained increase in synaptic activity which is essential for adjustments in learning, memory and functional improvement of vision (Cooke & Bliss, 2006). Seizure latency, frequency and severity may have significantly negative impact on cognition and the visual spatial function. There are inconsistent and controversial evidences both in human and animal models that show that seizures independent of aetiology worsen cognitive outcome, but there is a need to understand the effects that severity of seizure may have on the visuo-spatial functions and also, to better understand the impact of chronicity of seizures on epilepsy related cognitive, visual spatial memory. Thus, this study aims to determine the interference of different degrees of epileptic seizure on visual spatial functions in experimental animal models.

2. MATERIALS AND METHODS

Procurement of Experimental Animals

In this study, twenty (20) Wistar rats weighing between 90g and 120g were used. The rats were purchased from the animal house of the Department of Pharmacy, University of Uyo, Nigeria.

Acclimatization and Handling of Experimental Animals

The experimental animals were acclimatized for a period of two weeks before commencement of the experiment. They were housed in well-ventilated standard housing conditions involving clean plastic cages with sawdust bedding (temp; 28 - 31, photoperiod; 12hours; humidity; 50-55%) at the animal house of Department of Human Physiology, University of Uyo, Nigeria. The area was devoid of foul smells and noise. Every morning, the cages, feeding and drinking trough were frequently cleaned, with new feed and water replaced.

Experimental Design

The experimental animals were divided into the acute and chronic phases respectively. Each phase consisted of four (4) groups of five (5) rats each.

(a) The Acute phase

i. Group 1: Negative control.
ii. Group 2: Received 25 mg\kg body weight of Pentylenetetrazole (PTZ) administered intra-peritoneally (i.p) every alternate day in a week for a period of 14 days.
iii. Group 3: Received 30mg\kg body weight of Pentylenetetrazole (PTZ) administered intra-peritoneally (i.p) every alternate day in a week for a period of 14 days.
iv. Group 4: Received 35mg\kg bodyweight of Pentylenetetrazole (PTZ) administered intra-peritoneally (i.p) every alternate day in a week for a period of 14 days.

(b) The Chronic phase:

i. Group 1: Negative control
ii. Group 2: Received 25 mg\kg body weight of Pentylenetetrazole (PTZ) administered intra-peritoneally (i.p) every alternate day in a week for a period of 28 days.
iii. Group 3: Received 30mg\kg body weight of Pentylenetetrazole (PTZ) administered intra-peritoneally (i.p) every alternate day in a week for a period of 28 days.
iv. Group 4: Received 35mg\kg bodyweight of Pentylenetetrazole (PTZ) administered intra-peritoneally (i.p) every alternate day in a week for a period of 28 days.

Drugs and Chemicals

All the drug solutions were freshly prepared before use. Pentylenetetrazole (PTZ) was purchased from Pfizer Pharmaceuticals New York, USA. PTZ was dissolved in normal saline (0.9% Nacl) and administered intraperitoneally. The reagents used in this study were of analytical grade.

Experimental Protocol for PTZ Kindling

PTZ-treated groups (2, 3, and 4) were given intraperitoneal (i.p) injections with a 1-mL syringe attached to a 27- gauge needle of sub-convulsive doses (25mg/kg, 30 mg/kg and 35mg/kg) of pentylenetetrazol (PTZ), and vehicle (saline) respectively on the first day. Subsequently on test days, status epilepticus was induced by repeated i.p. injection of PTZ. Rats were given initial doses of 25mg/kg, 30mg/kg and 35mg/kg, followed by 10mg/kg every 10 minutes. Status epilepticus was defined for this study to be seizure lasting at least ten (10) minutes and comprising of prolonged episodes of seizures interrupted by post-ictal depression phases with no return to quadruped posture. The aforementioned procedure was repeatedly done on particular days for respective testing groups each week for a period of fourteen (acute) and twenty eight (chronic) days respectively. The experimental animals were keenly observed for thirty (30) minutes after PTZ administration. The observed seizure activity was quickly evaluated post PTZ administration according to the modified Racine scale.

Racine scale Description
Phase 0; No response (inactive)
Phase 1: Ear and facial twitching
Phase 2: Myoclonic body jerks (convulsive wave through the body)
Phase 3: Clonic forelimb convulsions (myoclonic jerks and rearing)
Phase 4: Generalized clonic convulsions, turning onto one side position
Phase 5: Generalized clonic-tonic convulsions/Status epilepticus (SE)
Phase 6: or death (within 30 minutes).

Seizure latency was determined as the time from injecting PTZ to the first appearance of convulsive wave through the body. The duration of the behavioral seizure activity was measured for each animal. [Racine's scale description for rodent seizure score. (Lüttjohann *et al*., 2009)]

Experimental Protocol for Barnes-Maze and Navigational Box Task in Wistar Rats (For Investigating Effects of PTZ-Induced Seizures on Visual Spatial Functions)

Barnes Maze

A visual- spatial learning and memory task designed for rats. It consists of an elevated circular surface with holes around the edge.

Principles

It is a dry-land based rodent's behavioural paradigm for assessing spatial learning and memory. The rats use extra-maze visual cues to locate a hole that allows them to escape from open space and bright light into a dark box beneath the maze. The time it takes to locate the escape hole into the dark box beneath the maze is recorded. (Barnes, 1979).

i. The animals were placed on the center of the elevated circular surface of the maze.
ii. The circular surface was mechanically spanned in a clockwise direction at a moderate speed.
iii. After the circular surface spanned came to a halt, the time at which the animal was able to escape from the open space and bright light into the dark box beneath the maze was recorded at a maximum of 300 seconds, beyond which the task is considered incomplete.

Navigational Box Task

It is widely used in behavioural neuroscience to study spatial learning and memory. It is used to measure the effect of neurocognitive disorder on spatial learning and possible neural treatments, to test the effects of lesions to the brain in area concerned with memory.

Principle

It is basically used to test mnemonic function in rats. These tasks are designed in such a way that the rats has to use either spatial or cue information to solve them. The animals find their way through the environment without getting lost, which require memory for locations and routes.

i. The animals were placed in the box through the entrance door and immediately the stop watch was started.
ii. The animals were allowed to find their way through the environment at a maximum time of 300 seconds (5mins).
iii. The duration of time it took for the animal to move from the starting point to the stopping point (Arrival time) was recorded. When the animal took significantly longer time to arrive at the stopping point it was recorded as an incomplete task.
iv. The arrival time was determined by when the mice reaches the stop point and then stayed there for at least 10 sec.

Statistical Analysis

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 20. The variation and the statistical significance of the differences between the groups were determined by Analysis of Variance (ANOVA) and Turkey post Hoc test. The result was presented in charts and tables.

3. RESULTS

Table 1: Variations in seizure scores of rats in PTZ treated groups according to Racine's scale for two weeks.

Groups	Initials	Week1	Week2
PTZ 25mg\kg	0±0	2±1	3±1
PTZ 30mg\kg	0± 0	3±1	4±1*
PTZ 35mg\kg	0± 0	4± 1*	5± 2*

Values are presented as Mean±SEM. *statistically significant (P<0.05) when compared to initials.

Table 2: Variations in seizure latency for rats in PTZ treated groups for a period of two weeks.

Groups	Initials	Week1 Seizure latency (secs)	Week2 Seizure latency (secs)
PTZ 25mg\kg	00secs	30±2.2	25±2.5*
PTZ 30mg\kg	00secs	26±1.5	20±1.0*
PTZ 35mg\kg	00secs	30±1.0	25±1.0*

Values are presented as Mean±SEM. *statistically significant (P<0.05) when compared to initials.

Source: Laboratory data, 2021.

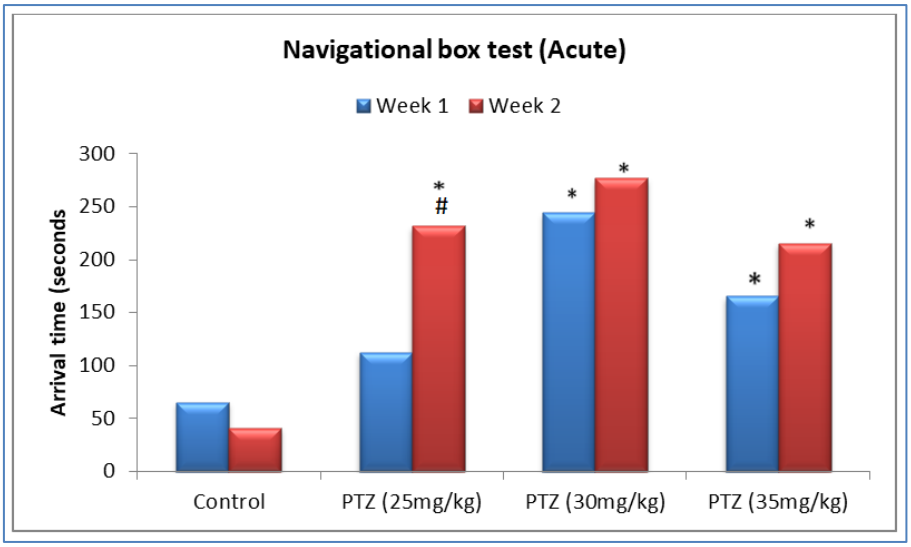

Figure 1: Effects of PTZ on visual spatial functions using navigational box test in acute phase study.*,# indicates statistically significant (P<0.05) changes between tests and control as well as, week 1 and week 2, respectively.
Source: Laboratory data, 2021.

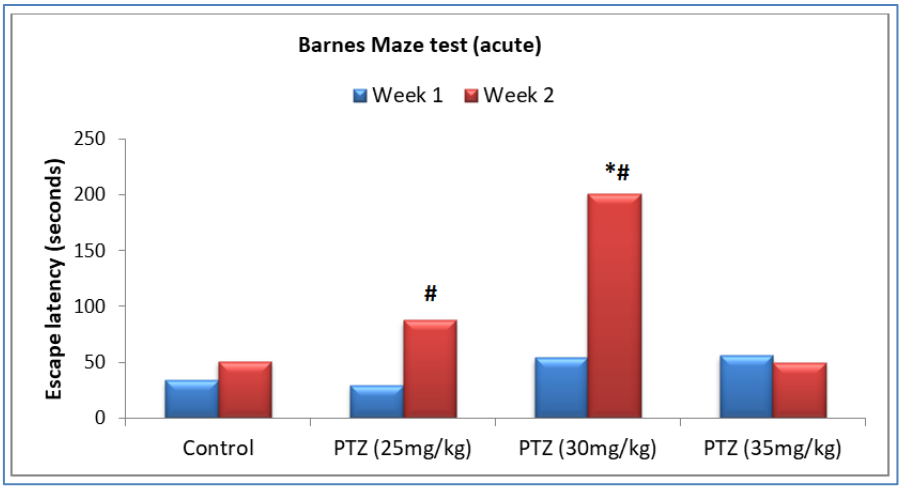

Figure 2: Effects of PTZ on visual spatial functions using Barnes Maze test in acute phase study.*, # indicates statistically significant (P<0.05) changes between tests and control as well as, week 1 and week 2, respectively.

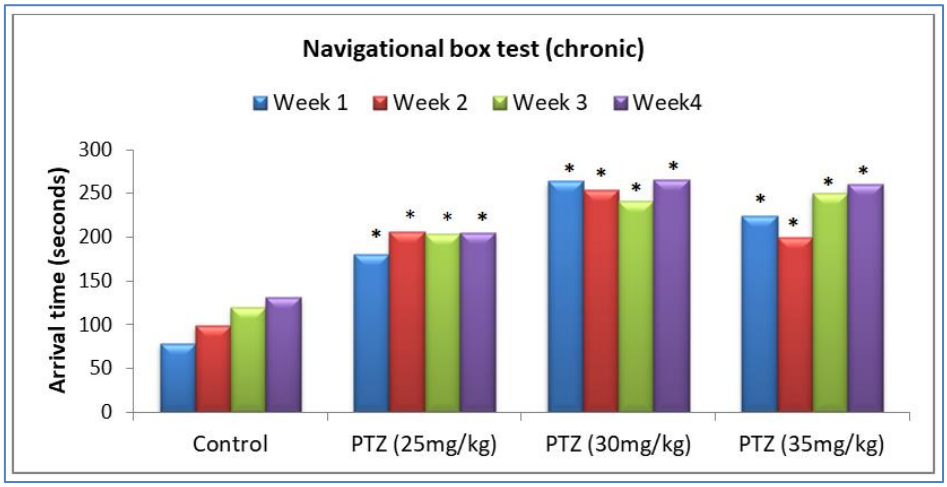

Figure 3: Effects of PTZ on visual spatial functions using navigational box test in chronic phase study.*indicates statistically significant (P<0.05) changes between tests and control.

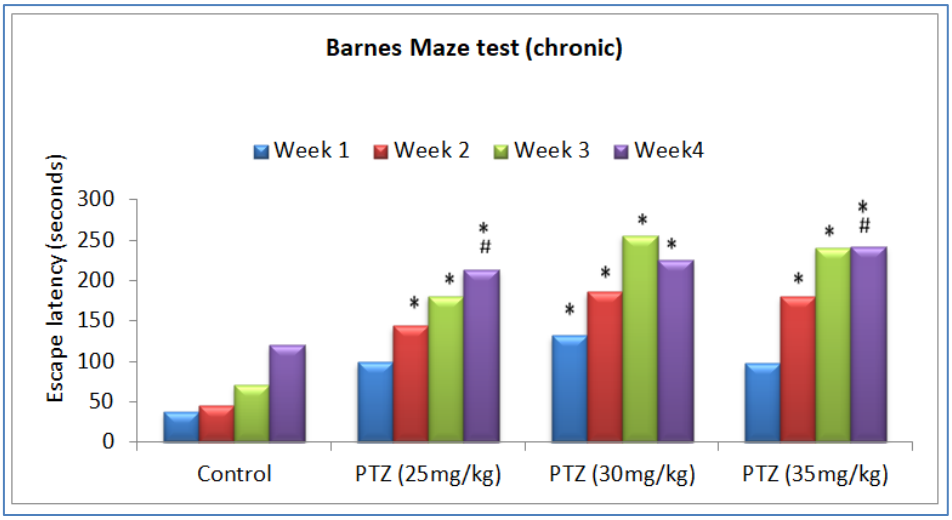

Figure 4: Effects of PTZ on visual spatial functions using Barnes Maze test in chronic phase study.*, # indicates statistically significant (P<0.05) changes between tests and control as well as, week 1 and week 2, respectively.

4. DISCUSSION

An assessment of visual spatial memory using a navigational box task (NBT) and Barnes maze (BM) were carried out in this study. In the acute phase study, the results showed that the animals which received 30mg/kg and 35mg/kg doses of PTZ showed a significant increase in arrival time in week 1 while all test groups caused an increase in arrival time in week 2, compared to the control group. Also, in the animals that received the lower dose (25mg/kg) of PTZ, there was a significant (P<0.05) increase in arrival when effects in week 2 was compared to week 1. Interestingly, this finding could be a resultant effect of acute seizures on the animal's ability to use visual cues intelligently in assessing its environment thereby causing a delay and leading to an increase in the arrival time of the animal. In the Barnes Maze test, the PTZ treated groups (30mg/kg) showed a significant (P<0.05) increase in escape latency when compared to the control group. The escape latency was significantly higher (P<0.05) in week 2 for the animals that took the lower dose (25mg/kg) and medium dose (30mg/kg) of PTZ, compared to week 1. Interestingly, the two paradigms used to evaluate the effect of PTZ- induced seizures clearly reveal impairment in the visual spatial domain of cognition.

In the chronic phase study, all the test groups showed significant increase in arrival time in week1, week2, week3 and week4 compared to the control group. However, there was no significant (P<0.05) changes in arrival time when effects in week 4 was compared to week 1. For the Barnes Maze test, the group that received 30mg/kg PTZ showed a significant (P<0.05) increase in escape latency in week1 to week4 while those that received 25mg/kg and 35mg/kg caused significant (P<0.05) increase in escape latency from week2 to week4 when compared to the control group. The escape latency was significantly higher (P<0.05) in week 4 for the animals that took the lower dose (25mg/kg) and higher dose (35mg/kg) of PTZ, compared to week 1. The animal's inability to use visual cues intelligently was worsened in the chronic phase of study revealing a deepened impairment in the visual spatial domain of cognition over time.

This finding suggests that the severity of the seizure in the rats affected the visual spatial functions and the outcomes of the results in the two phases were similarly negative. This corroborates with the reports from a study on the impaired visual spatial memory functions found in temporal lobe epileptic patients (Schmidbauer *et al*., 2022) as well as in recent evidences revealing visual spatial memory impairment in epileptic patients (Francesca *et al*., 2021). However, the difference between the previous studies and this study is that generalized epilepsy was the focus of this study. This findings are in agreement with reports of Tallarita *et al*., (2019) and Elena *et al*., (2022). In their study on the relationship between visual perceptual functions and parietal abnormalities in temporal lobe epilepsy, poor visual spatial performances was also observed in temporal lobe epileptic patients. Summarily, Findings from the neurobehavioural evaluations of effects of epileptic seizures on visual spatial functions using Barnes maze and Navigational box, suggests that epilepsy causes a deficit in learning and visual spatial memory. This study showed that epilepsy impaired both short and long term visual spatial memory and learning.

5. CONCLUSION

The findings from the neurobehavioral evaluations of effects of epileptic seizures on visual spatial functions using Navigational box and Barnes Maze tests, suggests that epileptic seizures causes a deficit in learning and visual spatial memory. These changes become worse with chronic seizures.

REFERENCES

- Barnes, C. A. (1979). Memory deficits associated with senescence: a neurophysiological and

behavioral study in the rat. *Journal of comparative and physiological psychology*, *93*(1), 74. doi:10.1037/h0077579.

- Berg, A. T., Langfitt, J. T., Testa, F. M., Levy, S. R., DiMario, F., Westerveld, M., & Kulas, J. (2008). Global cognitive function in children with epilepsy: a community-based study. *Epilepsia*, *49*(4), 608-614.
- Cooke, S. F., & Bliss, T. V. (2006). Plasticity in the human central nervous system. *Brain*, *129*(7), 1659-1673.
- Kleen, J. K., Scott, R. C., Lenck-Santini, P. P., Holmes, G. L. (2012). Cognitive and behavioural co- morbidities of epilepsy. https://www.ncbi.nih.gov/books/NBK98139/
- Lin, H., Holmes, G. L., Kubie, J. L., & Muller, R. U. (2009). Recurrent seizures induce a reversible impairment in a spatial hidden goal task. *Hippocampus*, *19*(9), 817-827.
- Lüttjohann, A., Fabene, P. F., & van Luijtelaar, G. (2009). A revised Racine's scale for PTZ-induced seizures in rats. *Physiology & behavior*, *98*(5), 579-586. doi:10.1016/j.
- Nickels, K. C., Wirrell, E. C. (2017). Cognitive and Social Outcomes of Epileptic Encephalopathies. *Semin. Pediatr. Neurol*, 24, 264–275.
- Owolabi, L. F., Owolabi, S. D., Taura, A. A., Alhaji, I. D., & Ogunniyi, A. (2019). Prevalence and burden of epilepsy in Nigeria: A systematic review and meta-analysis of community-based door-to-door surveys. *Epilepsy & Behavior*, *92*, 226-234.
- Schmidbauer, V., Nenning, K. H., Schwarz, M., Foesleitner, O., Mayr-Geisl, G., Yildirim, M. S., ... & Bonelli, S. (2022). Imaging visuospatial memory in temporal lobe epilepsy—Results of an fMRI study. *Plos one*, *17*(2), e0264349.
- Tallarita, G. M., Parente, A., & Giovagnoli, A. R. (2019). The visuospatial pattern of temporal lobe epilepsy. *Epilepsy & Behavior*, *101*, 106582.
- World Health Organization. (2019) Epilepsy: www.who.int/mediacentre/factsheets/fs999/en. Accessed June7.