

Interference of Seizure Disorders on Motor Functions, Coordination and Balance in Wistar Rats

Adesua C. Obiandu^{1*}, Koofreh G. Davies¹, Christopher E. Ekpenyong¹, Chibuike Obiandu², Karibo Okari³

¹Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria

²Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

³Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

DOI: [10.36348/sijap.2023.v06i11.002](https://doi.org/10.36348/sijap.2023.v06i11.002)

| Received: 04.10.2023 | Accepted: 09.11.2023 | Published: 11.11.2023

*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

Chronic recurrent seizures is a characteristic feature of epilepsy. It is a chronic neurological condition, usually resulting in unpredictable, unprovoked recurrent seizures that affect a variety of mental and physical functions. Some motor functions appear vulnerable to these seizure disorders. This study investigated the neurobehavioural analysis of pentylenetetrazole (PTZ) induced seizure on motor balance, coordination 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 for each phase of study. 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; groups 2, 3 and 4 were administered with subconvulsive 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 Rotarod test (RT) and Handgrip test (HT). In both tests, the time latencies were significantly ($P < 0.05$) reduced and worsened with time, when test groups were compared to control either in acute or chronic phase study. The results from the current study on the effects of the chemoculvosant seizures on motor functions, coordination and balance shows that severity and chronicity of seizures caused a deficit in motor coordination and balance. In conclusion, seizure disorder disrupts motor activities.

Keywords: Epilepsy, Pentylenetetrazole, Motor functions, Wistar rats.

Copyright © 2023 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

INTRODUCTION

Epileptic seizures are generally characterized by recurrent, unpredicted interruptions of the brain's function as a result of abnormal electrical discharge of neurons, stemming from imbalances of inhibitory (GABAergic) and excitatory (glutamatergic) neurotransmitters (Fisher *et al.*, 2005; Deme, 2016). Epidemiological surveys indicate that about 50 million persons are affected more frequently in the first two decades of life, and an incidence rate of 2.4 million people annually (Samokhina and Samokhin 2018). Epilepsy have been found to present a decrease in muscle and higher hindlimb tonus and some neurological symptoms such as limb grasping, limb clasping, tremors and trunk curling, revealing significant deficits in lower

motor neuron function. (Anabela *et al.*, 2010). The hypothesis that states that epilepsy has affected motor balance and coordination negatively has not been extensively proven. Normally, the motor cortex plays a major role in motor skill learning and excitation. Its relationship to the spinal cord is relevant for stimulating independent joint and digit movements (Alabi *et al.*, 2008). Motor cortex is the primary source of motor fiber of the pyramidal tract, that synapses directly with the brainstem and spinal cord motor neurons enabling fine motor movements (Konarski *et al.*, 2005). Even induced seizures with pentylenetetrazole consistently mirror the effects of naturally occurring pathological seizure disorders. Pentylenetetrazole (PTZ), is a GABAA receptor antagonist that is commonly used to establish

tonic-clonic seizures and it is simple to use and widely applicable in investigating the pathophysiology of epilepsy, which is a chronic disease that involves repetitive seizures (Shimada and Yagamata, 2018). However, there is paucity of data emphasizing the interference of motor functions in epileptic conditions, precisely the effect of epilepsy on motor balance and coordination. Thus, the rationale for the present study is to investigate the impact of severity and chronicity of PTZ- induced seizures on motor functions, coordination and balance in Wistar rats.

MATERIALS AND METHODS

Procurement of experimental animals

In carrying out this research study, twenty (20) Wistar rats weighing between 90-120g was used for each phase in a two phased study. The rats were purchased from the Department of Pharmacy in the University of Uyo, Uyo, Nigeria.

Acclimatization/Handling of experimental animals

The experimental animals were acclimatized for a period of two weeks before they were incorporated into any experimental procedure. They were housed in clean plastic cages with sawdust bedding and a well-ventilated standard housing condition (temp; 28-31, photoperiod; 12hours; humidity; 50-55%) at the animal house of the Department of Human Physiology, University of Uyo, Nigeria. The area was devoid of foul smells and noise. The animals were fed with standard rat chows and water *ad libitum*. Every morning, the beddings were changed while the cages, feeding and drinking trough were frequently cleaned, with new feed and water replaced. The handling of animals conformed to the guiding principles in the care and the use of laboratory animals published by the American Physiological Society (2002).

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 Pentylentetrazole (PTZ) administered intraperitoneally (i.p) every alternate day in a week for a period of 14 days.
- iii. Group 3: Received 30mg/kg body weight of Pentylentetrazole (PTZ) administered intraperitoneally (i.p) every alternate day in a week for a period of 14 days.
- iv. Group 4: Received 35mg/kg bodyweight of Pentylentetrazole (PTZ) administered intraperitoneally (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 Pentylentetrazole (PTZ) administered intraperitoneally (i.p) every alternate day in a week for a period of 28 days.
- iii. Group 3: Received 30mg/kg body weight of Pentylentetrazole (PTZ) administered intraperitoneally (i.p) every alternate day in a week for a period of 28 days.
- iv. Group 4: Received 35mg/kg bodyweight of Pentylentetrazole (PTZ) administered intraperitoneally (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. Pentylentetrazole (PTZ) (purchased from Pfizer Pharmaceuticals, New York, USA) was dissolved in normal saline (0.9% NaCl) and administered intraperitoneally.

Experimental Protocol for PTZ Kindling

The treated groups (2, 3, and 4) received 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 pentylentetrazol (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, 30 mg/kg and 35mg/kg, followed by 10mg/kg and was observed for 30minutes.

The observed seizure activity was quickly evaluated post PTZ administration according to the modified Racine scale. (Dhir, 2012).

Racine scale as follows:

- Stage 0 (no response),
- stage 1 (ear and facial twitching),
- stage 2 (myoclonic jerks without rearing)
- stage 3 (myoclonic jerks and rearing),
- stage 4 (forelimb clonus),
- stage 5 (seizures characterized by rearing, turning over into side position, generalized clonic-tonic seizures) and
- stage 6 (death).

The seizure latency was recorded as the time from injecting PTZ to the first appearance of convulsive wave through the body of the animal. The duration of the behavioral seizure activity was measured for each animal.

Experimental Protocol for Rotarod and Handgrip tests in Wistar rats (for investigating effects of PTZ-induced seizures on motor functions, coordination and balance).

Rotarod test

The Rotarod test is a widely used behavioral task used to evaluate the motor coordination and balance of rodents.

Principle:

- i. The rat was held by the back skin of the neck and placed on the rod, ensuring that the rat grasps the rod with the fore and hind paws.
- ii. The rod was rotated at an accelerating speed and the stopwatch was started.
- iii. The time taken for the animal to hold on to the rod was recorded and determined as the time latency for the procedure.
- iv. The fixed hanging limit was 300secs. Rats were given three trials to ensure that mice are really unable to hold on and do not fall due to clumsiness.
- v. Animals experiencing impaired motor coordination were unable to cope with the rotating rod and will drop off when the rotation speed exceeds their motor coordination capacity (Jones & Roberts, 1968).

Hand grip test

The Kondziela's handgrip test was designed to assess balance, coordination and muscle condition.

Principle (as adopted in present study)

- i. The rat was held by the back skin of the neck and placed on the wire, ensuring that the rat grasps the wire with only the forepaws.
- ii. The stop watch was started and time taken for the animal to hang on to the wire was recorded and determined as the time latency for the procedure.
- iii. The rat was observed while hanging till it was exhausted.
- iv. The fixed hanging limit was 300secs. Rats were given three trials to ensure that mice are really unable to hang and do not fall due to clumsiness.

STATISTICAL ANALYSIS

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 20. The mean and standard error of mean, 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.

RESULTS

The results are presented in tables 1 and 2, and figures 1 to 4.

Table 1: Variations in seizure scores in PTZ treated rats for four weeks

Groups	Initials	Week1	Week2	Week3	Week4
PTZ25mg/kg	0±0	2± 1	2 ±1	2 ±1	3 ±1
PTZ30mg/kg	0±0	3± 1	4 ±1*	3± 1	4± 1*
PTZ35mg/kg	0±0	4± 1*	5 ±2*	5±1*	5 ±2*

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

Table 2: Variations in seizure latency in PTZ treated rats for four weeks

Groups	Initials	Week1 Seizure latency(secs)	Week2 Seizure latency(secs)	Week3 Seizure latency(secs)	Week4 Seizure latency(secs)
PTZ25mg/kg	0±0	30±2.1*	23±1.2*	23±2.5*	20±1.0*
PTZ30mg/kg	0±0	25±1.3*	25±2.2*	24±2.0*	22±1.4*
PTZ35mg/kg	0±0	30±2.0*	25±2.5*	25±2.1*	24±1.4*

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

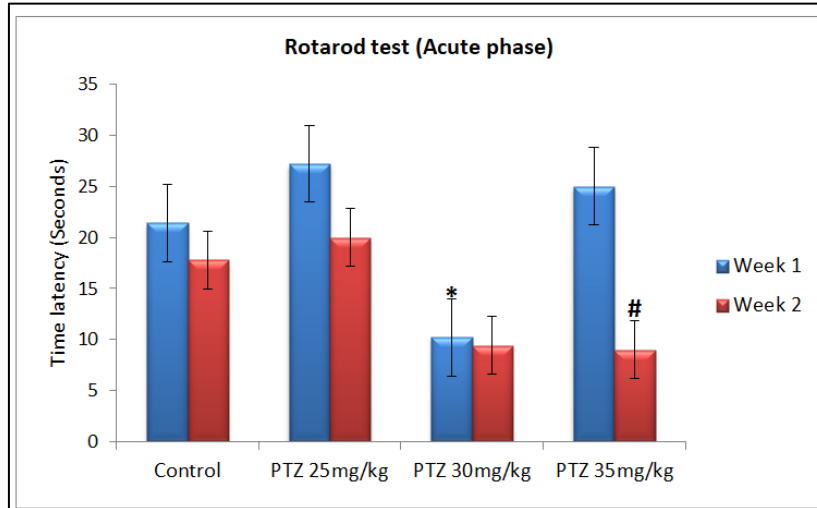


Figure 1: Effects of PTZ induced seizures on motor functions using Rotarod test in acute phase study
 *,# indicates statistically significant ($P<0.05$) changes between tests and control as well as, week 1 and week 2, respectively.

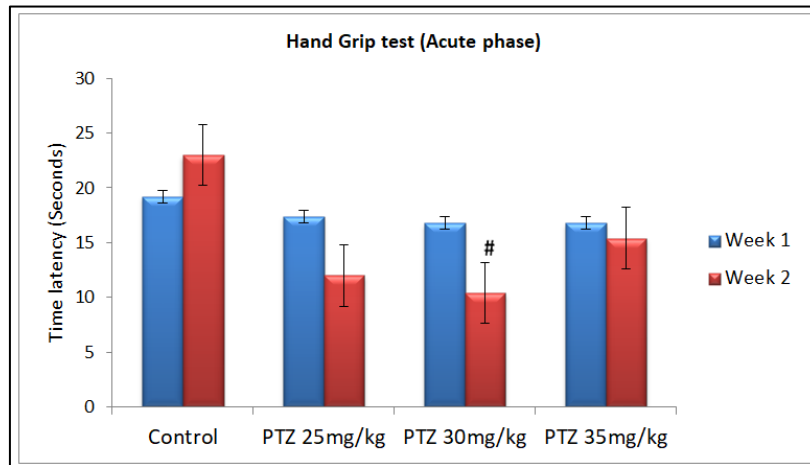


Figure 2: Effects of PTZ induced seizures on motor functions using Hand Grip test in acute phase study
 # indicates statistically significant ($P<0.05$) changes between week 1 and week 2, respectively.

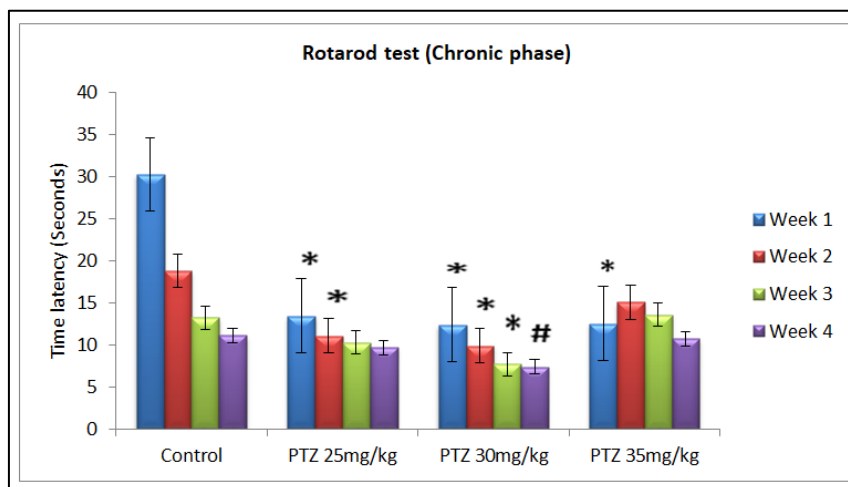


Figure 3: Effects of PTZ induced seizures on motor functions using Rotarod test in chronic phase study
 *,# indicates statistically significant ($P<0.05$) changes between tests and control as well as, week 1 and week 4, respectively.

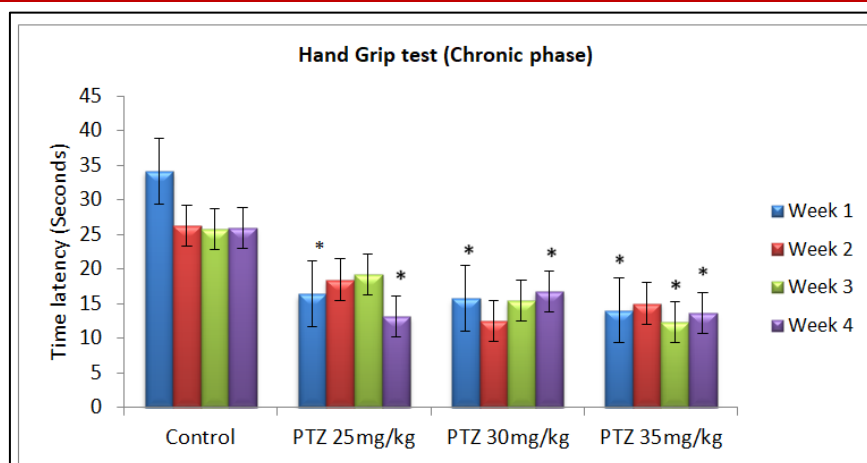


Figure 4: Effects of PTZ induced seizures on motor functions using Hand Grip test in chronic phase study

* indicates statistically significant ($P < 0.05$) changes between tests and control

DISCUSSION

In this study, there was an assessment of the effect of PTZ-induced seizure on motor coordination and balance using Rotarod test and Handgrip test. Variations in seizure scores in treated rats show that repeated administration of PTZ resulted in a gradual increase in seizure scores (according to Racine's scale) and seizure latency (Tables 1 and 2).

In the Rotarod test, the result revealed that the group that was induced with PTZ (30mg/kg) showed a significant ($P < 0.05$) decrease in time latency compared to the control group in the second week of acute phase study, while a significant ($P < 0.05$) decrease in time latency in the group that received PTZ 35mg/kg was observed when week 1 effects were compared to that of week 2.

The time it took for each rat to remain on a rod rotating at an accelerating speed was determined as the time latency. For the Rotarod test in chronic phase study, there was a significant ($P < 0.05$) decrease in time latency in week 1 for all test groups and in week 2 for rats that received PTZ 25mg/kg and PTZ 30mg/kg respectively, as well as week 3 for rats that received PTZ 30mg/kg. A significant ($P < 0.05$) decrease in time latency in the group that received PTZ 30mg/kg was observed when week 1 effects were compared to that of week 4.

In the Handgrip test, the result revealed that none of the induced animals showed a significant ($P < 0.05$) change in time latency compared to the control group in both first and second weeks of acute phase study. However, there was a significant ($P < 0.05$) decrease in time latency in the group that received PTZ 30mg/kg when week 1 effects were compared to that of week 2. In chronic phase study, there was a significant ($P < 0.05$) decrease in time latency in week 1 for all test groups and in week 3 for rats that received PTZ 35mg/kg as well as week 4 for all test groups. There was no significant ($P < 0.05$) change in time latency in all groups

when week 1 effects were compared to that of week 4. The time it took for each rat to hang on to the wire (time latency) was used as the basis for assessment of effects of seizure on motor function. The significance of time latency measurement in the animals is based on the fact that those experiencing impaired motor coordination are unable to hang on to the wire and will drop off. The result indicated that acute and chronic seizure impaired motor coordination and balance in the experimental rats. From the findings in this study, there is also an evidence to show that dysfunction in motor coordination and balance worsened over time and some dose dependent relationship exist in the decline in these functions. Thus, severity and chronicity of seizures cause a worsening of motor coordination and balance in PTZ induced seizure disorder in experimental rats. In view of this finding, there is a greater chance of epilepsy causing a negative effect on functional mobility. This finding is in consonance with the reports of Poretti and colleagues (2012). They reported that poor coordination was observed to be present as a result of post seizure activity which interrupted smooth movement of limbs, and produced pseudo ataxia in the epileptic patients. In addition, our finding corroborates with reports of (Bercem and Filiz, 2023; Martin and Salman, 2021; Gloersen *et al.*, 2000).

Therefore, it is noteworthy to suggest that epilepsy interferes with motor coordination and balance that could invariably affect activities like writing, walking, swimming and many other motor functions and this has a relationship to the severity of the seizure experience. Conclusively, in the evaluation of status epilepticus effect on motor functions, coordination and balance, using Rotarod and Handgrip test, the result suggests that epilepsy causes a deficit in motor coordination and balance.

REFERENCES

- Alaverdashvili, M., & Whishaw, I. Q. (2008). Motor cortex stroke impairs individual digit movement in

- skilled reaching by the rat. *European Journal of Neuroscience*, 28(2), 311-322.
- American Physiological Society. (2002). Guiding principles for research involving animals and human beings. *Am J physiol Regul Integr Comp Physiol*, 283, 281-283.
 - Deme, S. (2016). A study of correlation of computed tomography scan brain and electroencephalography in epilepsy. *Int Arch Integrated Med*, 3(10), 55-61.
 - Dhir, A. (2012). Pentylentetrazol (PTZ) kindling model of epilepsy. *Curr Protoc Neurosci*, 9, 9-37.
 - Erkeç, O. E., & Arihan, O. (2015). Pentylentetrazole kindling epilepsy model. *Epilepsy*, 21(1), 6-12.
 - Fisher, R. S., Boas, W. V. E., Blume, W., Elger, C., Genton, P., Lee, P., & Engel Jr, J. (2005). Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia*, 46(4), 470-472.
 - Gløersen, G., Løyning, T., & Nakken, K. O. (2000). Hidden motor dysfunctions in children with epilepsy. *Tidsskrift for den Norske Laegeforening: Tidsskrift for Praktisk Medicin, ny Raekke*, 120(18), 2115-2117.
 - Jones, B. J., & Roberts, D. J. (1968). A rotarod suitable for quantitative measurements of motor incoordination in naive mice. *Naunyn-Schmiedebergs Archiv Für Pharmakologie Und Experimentelle Pathologie*, 259, 211-211. doi: 10.1007/BF00537801.
 - Konarski, J. Z., McIntyre, R. S., Grupp, L. A., & Kennedy, S. H. (2005). Is the cerebellum relevant in the circuitry of neuropsychiatric disorders?. *Journal of Psychiatry and Neuroscience*, 30(3), 178-186.
 - Samokhina, E., & Samokhin, A. (2018). Neuropathological profile of the pentylentetrazol (PTZ) kindling model. *Int J Neurosci*, 128(11), 1-43.
 - Silva-Fernandes, A., Oliveira, P., Sousa, N., & Maciel, P. (2010). Motor and behavioural abnormalities associated with persistent spontaneous epilepsy in the fvb/n mouse strain. *Scandinavian Journal of Laboratory Animal Science*, 37(3), 213-222.
 - Sinanoglu, B., & Ozdemir, F. (2023). Evaluation of functional mobility, balance, and executive functions in children with epilepsy. *Epilepsy & Behavior*, 143, 109230. doi: 10.1016/j.yebeh.2023.
 - Martin, S., & Salman, M. S. (2021). Assessing Children with Poor Coordination Can Be Tricky—A Review on Ataxia and Ataxia Mimickers and a Study of Three Children with Severe Epilepsy. *Journal of Multidisciplinary Healthcare*, 3331-3341. doi:10.2147/JMDH.S251860.
 - Poretti, A., Benson, J. E., Huisman, T. A., & Boltshauser, E. (2012). Acute ataxia in children: approach to clinical presentation and role of additional investigations. *Neuropediatrics*, 44(3), 127-141. doi:10.1055/s-0032-1329909.