EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 4
600-608
Year: 2022

INTERFERENCE OF PENTYLENETETRAZOL-KINDLED INTERMITTENT EPILEPSY ON RATS' COGNITIVE FUNCTIONS

[1]Ebeku A.C., *[1]Olorunfemi O.J. and [2]Okoseimiema S.C.

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University Of Port Harcourt, Choba Nigeria.
[2]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University Of Port Harcourt, Choba Nigeria.

***Corresponding Author: Olorunfemi O. J.**
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba Nigeria.

Article Received on 20/02/2022 **Article Revised on 12/03/2022** **Article Accepted on 31/03/2022**

ABSTRACT
Cognitive and behavioural deficits are often associated with epilepsy and greatly reduce patients' quality of life. It is unclear whether or not intermittent epilepsy (marked by recurrent seizures) independent of its aetiology can cause or worsen these deficits in cognition, creating a need to better understand the relationship between epilepsy and its related cognitive and behavioural impairments. Hence the reason for the present efforts to contribute to the said gap in knowledge as this study is structured to study the interference of intermittent epilepsy on cognitive function in experimental animal model. This study provides experimental evidence on the interference of intermittent epilepsy on rats' cognitive function using PTZ-kindling model of epilepsy. Twenty (20) healthy Wistar rats weighting between 80-100g were used for this study. The rats were shared into four (4) groups with five rats in each group; groups 1 served as control did not receive any treatment, group 2, 3 and 4 received subconvulsive doses (35mg/kg) of Pentylenetetrazol. Thereafter cognitive assessment using the passive avoidance, Morris water maze, beam walking, inverted screen, elevated plus maze Barnes maze and handgrip tests were carried out for a period of three weeks. The results of the study showed that intermittent epilepsy significantly impaired rats' learning, short and long term memory as well as spatial reference memory in rats. Furthermore, these impairments worsened as seizure scores, measured the Racine scale, increased. It was therefore concluded that recurrent seizures are major contributors to the cognitive comorbidities observed in epilepsy.

KEYWORDS: Epilepsy,pentylenetetrazol kindling, cognitive function.

1. INTRODUCTION
Epilepsy is a prevalent and chronic neurological disorder, with high incidence of neurological disability and mortality worldwide (Fiest et al, 2017). Epilepsy is characterised by repetitive unprovoked seizures which lead to devastating results on the patients (Dhediya et al, 2016). It is one of the most common neurological clinical pathological disorders, and is estimated that around 70million people in the world have epilepsy and 90% of them reside in developing countries (Amudhan et al, 2015). In Nigeria, the estimated prevalence of epilepsy is 8 per 1000 people indicating a substantial burden of the disease in the country (Owolabi et al, 2019).

Among the co-morbidities associated with epilepsy, cognitive abnormalities are among the most common and troublesome. In people with epilepsy there is an associated high rate of cognitive difficulties that compromise educational progress and achievement throughout life (Berg et al., 2008). In addition to a higher incidence of low IQs (Farwell et al., 1985), in about half of children with epilepsy there is an identified discrepancy between IQ and achievement (Fastenau et al., 2008). Children who have poorly controlled (pharmacoresistant) seizures are more likely to have lower IQ scores than children with well controlled seizures (Berg et al., 2012). Adults with chronic epilepsy are also vulnerable for cognitive regression (Thompson and Duncan, 2005; Bell et al., 2011).

Individuals with prolonged seizures lasting 30 minutes or more, a condition termed status epilepticus (SE), or frequent intermittent epilepsy are at particular risk for brain injuries that can result in cognitive impairment. While the cause of the seizure clearly contributes to cognitive outcome, there is limited and inconsistent evidence both in humans and animal models that seizures independent of aetiology worsen cognitive outcome.Since antiepilepsy drugs show limited success in ameliorating cognitive and behavioral symptoms, there is a need to better understand the mechanisms underlying epilepsy-related cognitive, behavioural and psychomotor impairments. Hence the reason for the present efforts to contribute to the said gap in knowledge as this study is structured to study the interference of spontaneous seizures on cognitive, behavioural and psychomotor function in experimental animal model.

2. MARTERIALS AND METHOD

Experimental Animal

A total of twenty male wistar rats weighing 90-120g were obtained from the experimental animal unit of Department of human physiology, University of Port Harcourt, Rivers State. The rats were kept in clean disinfected wooden cages with saw dust as beddings in the animal house, with 12hours light/dark cycle and 50-60% humidity at a temperature of about 30^{o}C and were allowed to acclimatize to the new environment for two weeks, with free access to clean water and animal feed. The rats were weighed using an analytical weighing balance at commencement of the experiment.

Experimental Design

A total of twenty (20) albino Wistar rats were weighed and randomly divided into four groups of five rats per group.

Table 1: Experimental Design and Grouping of the Rats

Groups	**Number of animals**	**Treatment**	**Tests**
Group I (control)	5	Feed and water ad libitum	All seven (7) tests
Group II	5	Feed + water ad libitum + PTZ (35mg/kg)	Inverted screen, handgrip test and passive avoidance
Group III	5		Elevated plus maze, and beam walking test.
Group IV	5		Morris water maze and Barnes maze test.

Group I animals (control) were given clean water and feed only. No treatment was administered to the control animals. Groups II, III, and IV were treated with Pentylenetetrazol (PTZ) at a dosage of 35mg/kg. Seizure events were recorded with a video recorder and seizures were scored according to the Racine's scale.
After which experimental rats underwent a total of three (3) trials of some memory and learning tests, and neuromuscular tests which include:Passive avoidance, Morris water maze, beam walking test and hand grip tests.

Experimental Protocol for PTZ Kindling

Pentylenetetrazole (PTZ) is a GABAA receptor antagonist that is commonly used to establish tonic-clonic seizures and it is a simple and widely applicable to investigate the pathophysiology of epilepsy, which is defined as a chronic disease that involves repetitive seizures. (Shimada and Yagamata, 2018) We chose this animal model to examine the effects of intermittent epilepsy on cognitive function.

PTZ-treated rats (Group II, III, and IV) were given intraperitoneal (IP) injections of sub-convulsive doses (35mg/kg) of pentylenetetrazol (PTZ), and vehicle (saline).On the first day, one (1) 1ml of 35mg/kg IP injection of PTZ was given to the drug treated rats. Subsequently, on test days IP injections were administered to rats at 10 minute intervals until the occurrence of a convulsive seizure. This procedure was repeated on a specific days for respective experimental groups each week for three (3) weeks. The animals were observed for 30minutes after PTZ administration. Rats were observed using a video camera. The seizure activity immediately evaluated within 30 min after PTZ administration according to the modified Racine scale.A similar protocol was used by Luttjohann and colleague (2009) to test and modify the Racine's scale for PTZ-induced seizures.

Table 2: Description of Racine's scale for rodent seizure score. (Luttjohann et al, 2009)

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 on to one side position
Phase 5	generalizedclonic-tonic convulsions (or death within 30 minutes).

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

Experimental Protocols for Cognitive Function Test

Handgrip test: The Kondziela's handgrip test was designed to assess balance, coordination and muscle condition (Kondziela, 1964). These tests are based on the knowledge that mice are eager to remain hanging on a wire or grid till exhaustion. A fixed hanging limit is used of 600 sec. Rats are given three trials ensure that mice are really unable to hang and do not fall due to clumsiness.

Inverted screen: It is a test to determine the muscle strength of rats by using all four limbs (Kondziela, 1995). Most usual rats score excellently on the task performed. It is aninsensitive but fast gross screen, and the weight of the test described will provide a better measure of muscular strength.The inverted screen measured 43 cm square of mesh wire which consist of 12mm square of 1mm diameter wire. Basically it is enclosed by 4cm deep wooden beading (which occasionally prevents rats when it tries climbing to the other side.

Passive avoidance test: It is a useful task for evaluating the effects of novel chemical entities on instrumental learning and reference memory.

The testing apparatus is a trough-shaped alloy divided into two distinct compartments with an openingdoor. The white, brightly lit compartment is free of aversive stimulation whereas the black, dark compartment isequipped with shock capability. Passive shock avoidance paradigm was used based on negative reinforcement, contextual fear conditioning (Kaur et al., 2010). In this task the animal learns that a specific place should be avoidedsince it is associated with an aversive event. In Passive ShockAvoidance Paradigm, increase in number of trials required indicatesthe learning impairment and decrease in step down latency indicatesimpairment in memory.

Elevated Plus Maze: This test pits the desire to explore the novel maze against the species-typical preference to avoid open, elevated spaces as compared to enclosed spaces, and has been established as a tool for assessment of anxiety in rats. (Dawson and Tricklebank, 1995) The configuration has the shape of a plus sign having four arms (2 open, 1 semi-closed, and 1 closed), and the apparatus is elevated 32cm above the floor level.

Beam walking: The beam walking test is used to analyze gait-related behaviour and deviations in limb coordination in rodents in a testing environment that challenges their ability to maintain balance given that the animals have to cross an elevated beam with a narrow diameter. The goal of this test is for the rat to stay upright and walk across an elevated narrow beam to a safe platform (Carter et al, 2001). Performance on the beam is quantified by measuring the time it takes for the mouse to traverse the beam.

Barnes maze: The Barnes maze is a visual- spatial learning and memory task designed for rats. It consists of an elevated circular surface with holes around the edge. (Barnes, 1979).

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 an escape 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 should be recorded.

Morris Water maze: The Morris water maze is widely used to study spatial memory and learning.It is based on the rodent's (rats or mice) aversion to the water environment. Animals are placed in a pool of water that is colored opaque withpowdered non-fat milk or non-toxic tempera paint, where they must swim to a hidden escape platform. Because they are in opaque water, theanimals cannot see the platform, and cannot rely on scent to find the escape route. Instead, they must rely on external/extra-maze cues. As theanimals become more familiar with the task, they are able to find the platform more quickly. Developed by Richard G. Morris in 1984, this paradigmhas become one of the "gold standards" of behavioral neuroscience.

Data analysis

The quantitative data were represented in the charts and graphs, while qualitative data from the behavioral study was represented in tables. 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 Analysis was performed using Statistical package for Social sciences (SPSS) software version 20.

3. RESULTS AND DISSCUSSION

Table 1: Variations in seizure scores of rats in various groups according to Racine scale for a period of 3 weeks.

Grouping	Initial	Week1	Week2	Week3
Group 2	0 ± 0	2 ± 1	3 ± 1	4 ± 1
Group 3	0 ± 0	1 ± 1	3 ± 1	4 ± 2
Group 4	0 ± 0	2 ± 1	3 ± 2	4 ± 1

Values are rounded up to the nearest whole number and presented as mean ± sem. N= 5.

From table 1 above, it can be seen that there was a gradual significant increase in seizure severity score across all groups. All groups attained stage 4 Racine scale seizures by the third week.

Table 2: Variations in seizure latency for rats in various groups for a period of three weeks.

Groups	Week 1 (minutes)	Week 2 (minutes)	Week 3 (minutes)
Group 2	38.40 ± 10.58	29.00 ± 8.43	24.28 ± 5.04
Group 3	39.51 ± 15.00	26.30 ± 13.27	22.46 ± 5.00
Group 4	42.48 ± 15.20	28.55 ± 15.20	25.30 ± 8.20

Values are presented as mean ± sem. N= 5
Seizure latency significantly decreased across all groups by the week 3 compared to week1 and week 2 as seen in table 2.

Table 3: Variations in the frequency of stimulation for rats for a period three weeks.

Groups	Week 1	Week 2	Week 3
Group 2	3.5 ± 1.2	2.0 ± 0.5	2.2 ± 0.4
Group 3	3.6 ± 1.5	2.8 ± 1.0	2.5 ± 0.5
Group 4	4.0 ± 2.5	2.5 ± 0.5	2.2 ± 1.6

Values are presented as mean ± sem. N= 5
From table 3 above, it is observed that there was a decrease in frequency of stimulation to seizure across all groups in week 2 and week 3 compared to week 1.

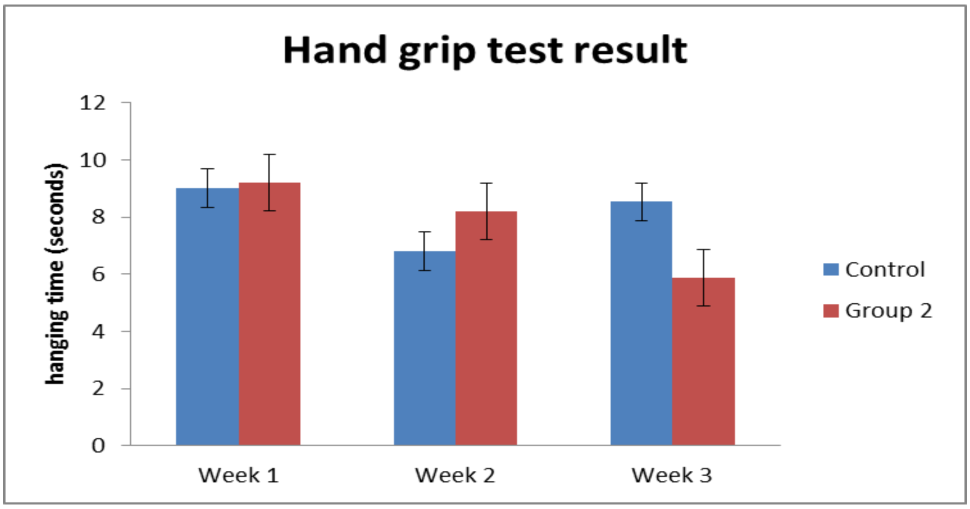

Figure 1: Hand grip test result pattern for control group and group 2 animals for three (3) weeks.

The hand grip test result, from figure 1 above, showed no statistically significant difference between the drug-treated group and the control in week 1 and week 2. However, by week 3, drug treated groups had significantly lower hanging time compared to control.

Table 4: Variations in rats' inverted screen test performance for a period of three weeks.

Groups	Week 1 (seconds)	Week 2 (seconds)	Week 3 (seconds)
Control	5.40 ± 1.09	7.27 ± 0.52	10.20 ± 1.78
Group 2	11.33 ± 3.13	8.07 ± 1.27	5.93 ± 1.46 [a]
Percentage difference	+109.81%	+11.00%	-41.86%

Values are presented as mean ± sem. N= 5, [a] is mean significant difference relative to the control groups, at $p<0.05$.

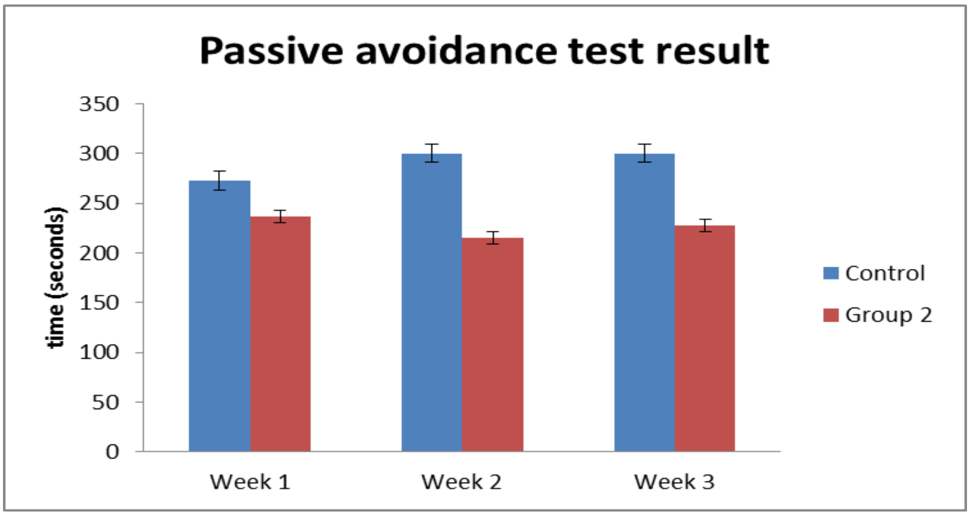

Figure 2: Passive avoidance test result pattern for control group and group 2 animals for three weeks.

In the passive avoidance test, from figure 1, PTZ-treated rats spent a significantly lower time on the task compared to control in week 2 and week 3. Control animals had successfully learned the task by week 2 and week 3, hence a perfect score of 300 seconds in both weeks unlike the PTZ-treated group which still went into the part of the box with the aversive stimuli, indicating that animals did not successfully learn the task or have poor memory retention.

Table 5: Variations in rats' performance in the 4-armed elevated plus maze test for a period of three weeks.

		Left arm (seconds)	Right arm (seconds)	Semi-close arm (seconds)	Closed arm (seconds)
Week 1	**Control**	7.73± 1.84	12.00± 4.62	13.73± 3.17	52.53± 22.83
	Group 3	2.67± 2.67	4.93± 4.63	43.67± 31.90	209.47± 56.24
Percentage difference		-65.46%	-58.92%	+218.06%	+298.76%
Week 2	**Control**	9.90± 2.00	14.83± 4.17	35.70± 7.39	24.00± 4.59
	Group 3	1.80± 1.30[a]	1.13± 1.13[a]	33.93± 22.25	128.97± 15.38[a]
Percentage difference		-81.82%	-92.38%	-4.96%	+437.38%
Week 3	**Control**	11.00± 1.71	11.73± 2.82	49.27± 19.73	36.27± 6.97
	Group 3	0.00± 0.00[a]	1.00± 1.00[a]	83.60± 58.17[a]	119.47± 58.25[a]
Percentage difference		-100.00%	-91.47%	+69.68%	+229.39%

Values are presented as mean ± sem. N= 5, [a] is mean significant difference relative to the control groups, at p<0.05.

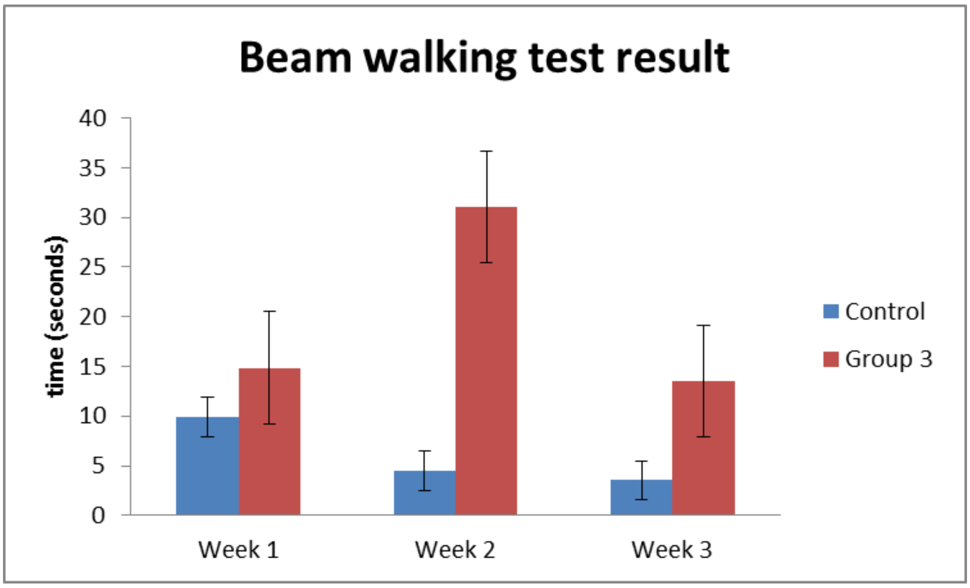

Figure 3: beam walking/climbing test result pattern for control group and group 3 animals for three weeks.

The beam walking test result from figure 3 above shows that it took the PTZ-treated rats a significantly longer time to traverse the beam compared to the control rats. The PTZ-treated rats had more slips, falls and moments of immobility compared to the control.

Table 6: Variations in rats' Barnes maze test performance for a period of three weeks.

Groups	Week 1 (seconds)	Week 2 (seconds)	Week 3 (seconds)
Control	40.53±15.36	21.47±8.52	17.73±5.35
Group 4	172.93±40.86	127.93±59.20[a]	134.53±47.43[a]
Percentage difference	+326.67%	+495.85%	+658.77%

Values are presented as mean ± sem. N= 5, [a] is mean significant difference relative to the control groups, at p<0.05.

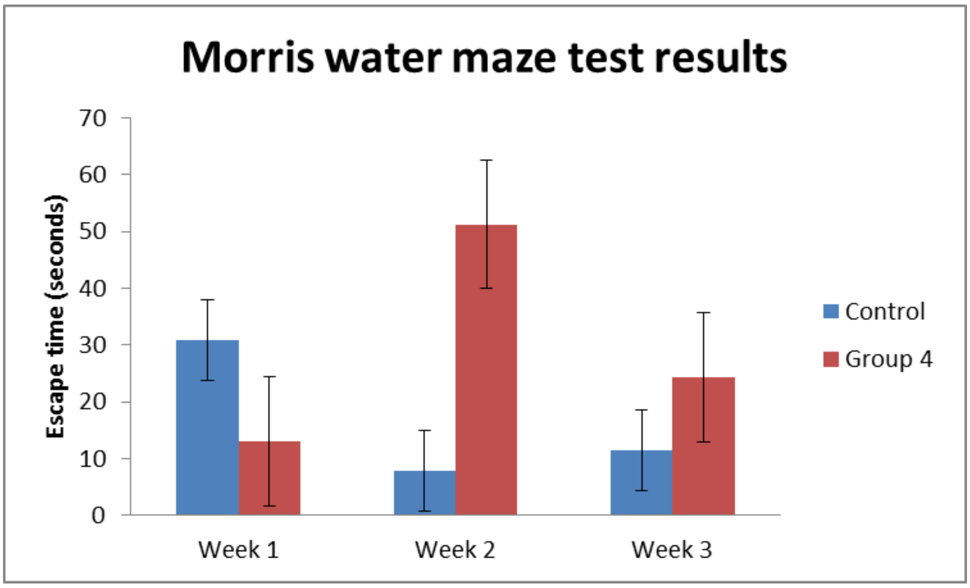

Figure 4: Morris water maze test result pattern for control group and group 4 animals for three weeks.

The Morris water maze test result from figure 2 above shows a significantly higher escape time for PTZ-treated rats compared to control in week 2 and week 3. PTZ-treated rats took a longer time locating the escape platform compared to the control groups in week 2 and week 3 as opposed to week 1, which is suggestive of regression in spatial memory.

DISCUSSION

Seizure Score and Latency

Repeated PTZ administration resulted in significant increase in seizure severity over time. By the third week 86% of rats had stage 4 seizures. This result is consistent with results from Zhu et al. (Zhu et al.2017).

Repeated PTZ administration also resulted in increased seizure susceptibility seen from the decreasing seizure latencies and number of PTZ injections to cause a seizure.

Hand Grip Test

The handgrip test is a neuromuscular assay of muscle strength. It was performed on control and group 2 animals to test for arm strength. For week 1, the hand grip test results for both control and drug treated group showed that animals in both groups had similar forelimb strength (grip strength) as animals in both groups spent a similar amount of time on the task per trial. Although in trial 3, group 2 animals spent a significantly longer time on the tasks compared to group 1 (control) animals. This showed that grip strength for animals in both groups were closely similar at the first week.

This trend was followed in week 2 – although the trend per trial were more haphazard than the preceding week – showing control animals spending longer time on the task than group 2 rats in the first trial (trial 1), whereas, group 2 animals spend a statistically significantly longer time on the task compared to control animals in trial 2 and trial 3. This indicates that the drug treated group were did not develop any neuromuscular toxicity as at this point from the PTZ treatment even though at this point, animals treated with subconvulsive dose of PTZ were already displaying level 4 seizures 10 minutes before tests were underwent.

This trend changes completely in week three (3), as results from hand grip test showed that group2 animals all spent significantly shorter time on the task in all three trials compared to the control group. This showed that the drug treated animals have diminished forelimb grip strength over the course of three (3) weeks compared to the control. Furthermore, results from the three (3) weeks of hand grip test shows the progressive reduction in muscle strength in rats.

Inverted Screen Test

The inverted screen test is a test of muscle strength, coordination and balance using all four limbs (Kondziela, 1964).

Results from the first week of testing the neuromuscular strength of rats using the inverted screen test showed that the drug tested group performed significantly better at the test compared to the control group (i.e. drug treated group animals spent significantly longer time hanging upside down on the inverted screen than control animals.

Results from the second week revealed that there was no existing statistically significant difference in the performance of the task for the drug treated groups compared to the control group – although there was a reduction in the time spent on the task in the second week for the drug treated group compared to the initial performance on the first week. This showed that PTZ treated rats had diminished muscular strength, although this change was not statistically significant.

Extrapolation from the third week showed that muscle strength in the control group here significantly increased compared to the previous week – as control animals performed much better on each trial compared to the previous weeks. On the other hand, the drug group showed diminished muscle strength, as they spent shorter time on the task compared to the control animals as well as previous week's performance. This showed that kindling seizures induced by PTZ do have a negative effect on rats' muscle strength. According to Maurissen et al (2003) this decline in muscle strength can be interpreted as an evidence of neurotoxicity.

Passive Avoidance

The passive avoidance test is a fear-aggravated test used to evaluate learning and memory in rodent models. Subjects are meant to learn and remember to avoid an environment in which an aversive stimulus (foot shock) was previously delivered.

Week 1 passive avoidance test results showed that there was impaired there was impaired acquisition/retention of fear memory and associated learning in PTZ treated rats compared to the control group. This is measured by the latency of time it takes the rats to move back into the dark compartment of the passive avoidance box after the foot-shock was administered. This result suggests impairment in short term memory as PTZ treated rats had a significantly lower latency to move back into the dark box (where the aversive stimulus was just administered) in the third trial compared to previous trial and control. This result is consistent with a finding on the status epileptcus model of epilepsy (McKay and Persinger, 2004; Kemppainen, Nissinen and Pitkanen, 2006).

Week 2 and 3 passive avoidance test results further corroborates impaired learning and memory in PTZ treated rats compared to control rats. All three trials showed markedly reduced latency to return to the dark box where the aversive stimulus was administered compared to control. Whereas, by the second and third week control animals completely refused to step in or remain in the dark box (with the aversive stimulus). These findings are consistent with the findings of Mao et al (Mao et al 2009) suggesting that brief PTZ-induced seizures impair the acquisition of contextual fear memory, as well as short and long term memory.

Elevated Plus Maze

The elevated plus maze task is widely used as an "ethologically" based animal model of anxiety (Dawson and Tricklebank. 1995). Indeed, anxiolytic drugs, especially of the benzodiazepine type, significantly increase ambulation of treated rats onto the open parts of the maze whereas control animals most often avoid the open arms. When interpreted in this context, our results do suggest that PTZ kindling does induce a significant anxiogenic response compared to the control group. This observation is contrary to that of Hoeller and colleagues who reported that PTZ kindling had no anxiogenic effects (Hoeller et al, 2017). From our results, the time spent on the open arm (as well as number of entries into the open arm) was higher for the control group through all three weeks of testing compared to the PTZ treated group. The PTZ kindled rats showed decreased time spent on the open arm of the maze and increased time spent on the semi-closed and closed arms. Our study however corroborates a similar study on mice which also showed these anxiety-like behaviour and activity in mice treated with PTZ (Anesti et al, 2020).

Furthermore, when interpreted in the context of rats' exploratory behaviour (locomotor activity) which can be measured by number of entries into each arm (data unavailable) (Prut and Belzung, 2003), PTZ kindled rats did show diminished exploratory behaviour compared to control rats. Control rats moved from one arm of the maze to the other more times compared to PTZ kindled rats that only explored the maze on the first week and subsequently preferred to stay in the closed and semi-closed arms of the maze.

Beam Walking Test

Performance on the beam is a useful measure of fine coordination and motor balance, and has been used by several researchers to detect motor deficits due to age, central nervous system lesions and pharmacological manipulations in young and older rats (Carter et al, 2001; Brooks and Dunnett, 2009), it has also been shown to offer more sensitivity in observing motor deficits compare to the Rotarod test (Stanley et al., 2005).

In this study, we evaluated the effect of PTZ kindling on rats' fine motor coordination and balance using the beam walking test. Statistical analysis showed that PTZ kindled rats took longer to traverse the bean to the finish point compared to the control rats. PTZ treated rats had few falls, more slips, and rats clung to the beam for long; which increased their time to cross. These slips and falls and hence longer beam crossing time, compared to control animals, indicates deficits in fine coordination and motor balance (Buccafusco, 2009). Our results are in harmony with previous studies showing similar motor balance and coordination deficits in PTZ kindled rats on the rotarod test (Chaudhary et al, 2011).

Barnes Maze

The Barnes maze is a dry-land based behavioral test that was originally developed by Carol Barnes to study spatial memory in rats (Barnes, 1979), and has shown to be sensitive to spatial learning related to neurodegeneration (Kennard, 2012). To determine whether we can detect deficit in learning and memory in PTZ kindled rats, we tested animals using the Barnes maze.

Our results from Barnes maze test for the first week of trial showed that there was a significantly higher latency to locate the escape box for the PTZ treated rats compared to the control group. Although, there was a recorded trial to trial improvement in task performance (reduction in latency to locate the escape box) for the first week, this was not the case for the subsequent weeks.

This significantly higher latency to locate the escape box in the PTZ kindled rats compared to the control rats indicates a marked deficit in spatial learning and memory in the kindled rats. This finding is further supported by the lack of week to week improvement in task performance compared to control. These results correlates with results from Xie et al (Xie et al, 2012) who, in a similar experiment, reported that PTZ kindling may impair spatial learning and memory in rats.

Morris Water Maze
The results obtained from the Morris Water Maze showed that there was impairment in the acquisition of spatial memory in the PTZ kindled rats compared to the control rats. Our result is supported by previous results in kindling models (McKay and Persinger, 2004) which indicated that prolonged or recurrent seizures disrupt the acquisition of spatial learning. It is also supported by results from Mao et al (Mao et al, 2009) which positing that brief seizure itself can impair the acquisition of spatial memory.

Results from the first week of testing showed that the PTZ treated group had a significantly lower latency to locate the platform compared to the control animals. There was however an improvement in trial to trial performance in these control animals (40.4, 32.4 and 19.6 for the three respective trials in week 1) which was also observed in the PTZ treated animals (although not significant).

Week 2 of Morris Water Maze testing showed significant impairment in the acquisition of spatial reference memory and learning in PTZ treated animals as latency to locate the platform significantly increased compared to the previous week and control animals. This was also the case in the third week, although there was an improvement in the trial to trial performance of the PTZ treated group. This improvement between trials in the third week does not counter the fact that there was a memory deficit in PTZ treated rats, as the first trial of the third week showed significantly higher latency in escaping to the platform compared to the control. One possible explanation to this trend observed in the third week of test is that short-term spatial memory might be spared by PTZ kindling seizures (Mao et al, 2009).

4. CONCLUSION
The study revealed that intermittent epilepsy significantly impaired cognitive function in experimental rats. These impairments in psychomotor and cognitive function progressively worsened with increased recurrence of seizures.

5. REFERENCE

1. Fiest, K.M.; Sauro, K.M.; Wiebe, S.; Patten, S.B.; Kwon, C.S.; Dykeman, J.; Pringsheim, T.; Lorenzetti, D.L.; Jette, N. (2017). Prevalence and incidence of epilepsy: A systematic review and meta-analysis of international studies. Neurology, 89: 642.
2. Dhediya, R.M.; Joshi, S.S.; Gajbhiye, S.V.; Jalgaonkar, S.V.; Biswas, M. (2016). Evaluation of antiepileptic effect of S-adenosyl methionine and its role in memory impairment in pentylenetetrazole-induced kindling model in rats. Epilepsy Behav, 61: 153–157.
3. Amudhan S, Gururaj G, and Satishchandra P. (2015) Epilepsy in India I: epidemiology and public health. Ann Indian Acad Neurol, 18(3): 263–77.
4. Owolabi LF, Owolabi SD, Taura AA, Alhaji ID, Ogunniyi A. (2019). Prevalence and burden of epilepsy in Nigeria: A systematic review and meta-analysis of community-based door-to-door surveys. Epilepsy Behav, 92: 226-234.
5. 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.
6. Farwell J.R.; Dodrill C.B.; Batzel L.W. (1985) Neuropsychological abilities of children with epilepsy. Epilepsia, 26(5): 395-400.
7. Fastenau P.S.; Shen J.; Dunn D.W.; Austin J.K. (2008) Academic underachievement among children with epilepsy: proportion exceeding psychometric criteria for learning disability and associated risk factors. Journal of learning disabilities, 41(3): 195-207.
8. Berg A.T.; Zelko F.A,; Levy S.R.; Testa F.M. (2012) Age at onset of epilepsy, pharmacoresistance, and cognitive outcomes: a prospective cohort study. Neurology, 79(13): 1384-1391.
9. Thompson P.J.; Duncan J.S. (2005) Cognitive decline in severe intractable epilepsy. Epilepsia, 46(11): 1780-1787.
10. Bell B, Lin JJ, Seidenberg M, Hermann B. (2011). The neurobiology of cognitive disorders in temporal lobe epilepsy. Nat Rev Neurol, 7: 154–164.
11. Shimada T. and Yagamata K. (2018) Pentylenetetrazole-induced kindling mouse model. Journal of visualized experiments, 136.
12. Luttjohann A.; Fabene P.F.; van Luijtelaar G. (2009) A revised Racine’s scale for PTZ-induced seizures in rats. Physiology and behavior, 98(5): 579-586.
13. Kondziela, W. Eine neue method zur messung der muskularen relaxation bei weissen mausen. *Arch. Int. Pharmacodyn,* 1964; 152: 277-84.
14. Harjeet Kaur, Damanpreet Singh, Bhupinder Singh & Rajesh K. Goel (2010) Anti-amnesic effect of Ficusreligiosa in scopolamine-induced anterograde and retrograde amnesia, Pharmaceutical Biology, 48: 2, 234-240.

15. Dawson GR, Tricklebank MD. Use of the elevated plus maze in the search for novel anxiolytic agents. Trends Pharmacol Sci, 1995 Feb; 16(2): 33-6.
16. Rebecca J. Carter, Jennifer Morton, Stephen B. Dunnett (2001) Motor Coordination and Balance in Rodents. Behavioral Neuroscience, 8(15).
17. Barnes C.A. (1979). Memory deficits associated with senescence: A neurophysiological and behavioural study in the rat. Journal of Comparative and Physiological Psychology, 93(1): 74-104.
18. Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods, 11: 47-60.
19. Zhu X.; Dong J.; Han B.; Huang R.; Zhang A.; Xia Z.; Chang H.; Chao H.; Yao H. (2017) Neuronal nitric oxide synthase contributes to PTZ kindling epilepsy-induced hippocampal endoplasmic reticulum stress and oxidative damage. Front. Cell. Neurosci, 11: 377.
20. Maurissen J.P.J.; Marable B.R.; Andrus A.K.; Stebbins K.E. (2003). Factors affecting grip strength testing. Neurotoxicology and teratology, 25(5): 543-553.
21. McKay B.E. and Persinger M.A. (2004). Normal spatial and contextual learning for ketamine-treated rats in the pilocarpine epilepsy model. Pharmacology Biochemistry and Behaviour, 78(1): 111-119.
22. Kemppainen EJ, Nissinen J, Pitkanen A. (2006) Fear conditioning is impaired in systemic kainic acid and amygdala-stimulation models of epilepsy. Epilepsia, 47: 820–9.
23. Mao RR.; Tian M.; Yang YX.; Zhou QX.; Xu L.; Cao J. (2009). Effects of pentylentetrazol-induced brief convulsive seizures on spatial memory and fear memory.
24. Hoeller AA, Cristiane RC, Pedro CF, Douglas AF, Alexandre KI, Henrique R, (2017). Behavioural and neurochemical consequences of pentylenetetrazol-induced kindling in young and middle-aged rats. Pharmaceuticals, 10: 75.
25. Anesti M.; Stavropoulou N.; Atsopardi K.; Lamari F.N.; Panagopoulos N.T.; Margarity M. (2020). Effect of rutin on anxiety-like behaviour and activity of acetylcholinesterase isoforms in specific brain regions of pentylenetetrazol-treated mice. Epilepsy and behaviour, 102: 106632.
26. Prut L. and Belzung C. (2003). Hte open field as a paradigm to measure the effects of drugs on anxiety-like behaviours: a review. Eur J Pharmacol, 463(1-3): 3-33.
27. Brooks S.P. and Dunnett S.B. (2009). Tests to assess motor phenotype in mice: a user's guide. Nature reviews neuroscience, 10: 519-529.
28. Stanley J.L.; Lincoln R.J.; Brown T.A.; McDonald L.M.; Dawson G.R.; Reynolds D.S. (2005). The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. Journal of Psychopharmacology, 19(3): 221-227.
29. Buccafusco J.J. (2009). Methods of behaviour analysis in neuroscience (second edition). Taylor and Francis Group. Page 77.
30. Chaudhary N.; Bijjem K.R.V.; Kalia A.N. (2011). Antiepileptic potential of flavanoids fraction from the leaves of Anisomeles malabarica. Journal of ethnopharmacology, 135(2): 238-242.
31. Kennard J.A. (2012). Age sensitivity of the Barnes maze and the Morris water maze: associations with cerebellar cortical Purkinje neurons. Temple university.
32. Xie T.; Wang W.; Mao Z.; Qu Z.; Luan S.; Jia L.; Kan M. (2012). Effects of epigallocatechin-3-gallate on pentylenetetrazol-induced kindling, cognitive impairment and oxidative stress in rats.