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INTERFERENCE OF ELECTROSHOCK ON BRAIN ACETYL CHOLINESTERASE PATTERN AND SPATIAL LEARNING ACTIVITIES IN RATS

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ABSTRACT
Exposure to high voltage electricity (greater than 500 volts) has the potential to result in serious tissue damage including the human brain. Serious electrical shock injuries usually have an entrance and exit site on the body because the individual becomes part of the electrical circuit. The study was designed to investigate the brain Acetyl cholinesterase and spatial learning activities in rats exposed to electroshock. Twenty five wistar rats were arranged into five groups: Group 1(negative control) was given saline water and feed without electroshock, group 2 was exposed to 2mA shock using electroconvulsive therapy (ECT), group 3 was exposed to 3mA shock using ECT, group 4 was exposed to 5mA shock using ECT, group 5(positive control) was given 0.1ml/100g epinephrine. All procedures of treatment lasted throughout the period of study. They were all made to undergo some set of neurobehavioral tests that included a total of nine (9) trials spanning three weeks of spatial learning and memory tests which include: Navigational maze, Passive avoidance test, and Barnes maze test. The results obtained were analyzed using SPSS version 20.0 statistical package. Neurobehavioral observations showed that quality of adaptive locomotion improved significantly ($p<0.05$) the test groups exposed (especially at 5mA) to shock from week 1 to week 3 and thus up regulated cognition aspect of motor function when compared to the control groups while the degree of awareness declined significantly at week 3 with 5mA though observations from spatial learning poor quality from 5mA but was enhanced at lower shock exposure with significance. This study was also designed to investigate the effect of different shock levels on brain Acetyl cholinesterase. With electroshock of 2mA, 3mA, there was an increase in AchE which caused destruction in Ach, then there was a reverse at 5mA as there was a decrease in AchE significantly though this observation did not influence any visible decrement in behavioral response during the period of study. From result obtained, it can be seen that ECT can cause elevation response in Brain Acetyl cholinesterase level alongside improved spatial learning activities with a delayed onset of cognitive decline in Rats.

KEYWORDS: Electroshock, wistar rat, Acetyl cholinesterase, Navigation maze, Barnes maze.

INTRODUCTION
From administering a painless electrical current to deliberately triggering a seizure, brain stimulation therapy has a long and diverse history, the first cases of brain stimulation being used to treat mental health problems are thought to have occurred in Italy around the start of the 19th century. Brain stimulation therapy involves sending pulses of electricity to the brain to stimulate neural activity for the treatment of conditions such as depression, anxiety and obsessive-compulsive disorder. The therapy typically involves placing electrodes on the outside of the scalp, although it can also require surgery to insert a device beneath the skin in more invasive treatment methods.[1] Brain stimulation techniques are often used to treat mental health conditions when antidepressant drugs and talking therapies such as CBT (cognitive behavioural therapy) have failed. Nowadays, such treatments can even be administered at home using simple devices including the Flow headset. But the history of brain stimulation dates back hundreds of years to a time when medical technology was far more basic.[1]

Electroconvulsive therapy (ECT), in the past known as electroshock therapy, is a mental treatment where seizures are electrically prompted in patients to give help from mental disorders. Typically, 70 to 120 volts are applied remotely to the patient's head bringing about

roughly 800 milliamperes of direct flow went through the brain, for 100 milliseconds to 6 seconds length, either from temple to temple (two-sided ECT) or from front to back of one side of the head (one-sided ECT).[2] The ECT methodology was first led in 1938 by Italian therapist UgoCerletti,.[3] and quickly supplanted less protected and compelling types of organic medicines being used at that point. ECT is frequently utilized with educated consent as a sheltered and successful intervention for significant depressive disorder, mania, and catatonia.[4] ECT machines were initially positioned in the Class III classification by the United States Food and Drug Administration (FDA) since 1976 They were renamed as Class II gadgets, for treatment of mental shock, significant depressive issue, and bipolar issue, in 2018.[5,6,7] As far back as the 1700s, TES (transcranial electrical stimulation) machines for brain stimulation were available to members of the public. During Victorian and Edwardian times in the UK — the 1800s and early 1900s — these machines used battery, friction or static to generate a low electrical current. The rudimentary technique could be self-administered, and physicians, therapists and patients alike all claimed it could generate feelings of euphoria and improve mental performance.

However, its use was unregulated, and is thought to have produced side-effects including headaches, dizziness, and nausea.[1] While there are reports that there are not any absolute contraindications for ECT, contradicting reports states that Cognitive impairment is typically noticed after ECT. While it has been claimed by some non-medical authors that amnesia occurs to some extent within the majority of patients receiving ECT, most experts consider this adverse effect relatively uncommon. The American Psychiatric Association (APA) report in 2001 acknowledges: "In some patients the recovery from amnesia are going to be incomplete, and evidence has shown that ECT may end in persistent or permanent memory loss". It is the purported effects of ECT on memory that make so much of the priority surrounding its use. Therefore this study was designed to checkmate the effects of ECT on the activities of Acetyl cholinesterase and memory-related activities in rats.

MATERIALS AND METHOD

Experimental Animal

A total of twenty five male wistar rats weighing 180-200g were obtained from animal house. 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 five albino wistar rats were weighed and randomly divided into five groups of five rats per group

Table 1: Experimental Design and Grouping of the Rats.

Groups	Number of animals	Treatment:
Group I (Negative control)	5	Feed + Water ad libitum
Group II	5	Feed + Water ad libitum + ECT (2mA)
Group III	5	Feed + Water ad libitum + ECT (3mA)
Group IV	5	Feed + Water ad libitum + ECT (5mA)
Group V (Positive control)	5	Drug Epinephrine-treated (0.1ml/100g)

Group I animals (control) were given clean water and feed only, without any of treatment, Groups II animas were exposed to 2mA shock only, Group III animals were exposed to 3mA shock and group IV was exposed to 5mA shock while group V was the positive drug group treated with epinephrine. After which they underwent a total of nine (5) trials of selected memory and learning tests which include: Passive avoidance task, Barnes maze, and Navigational task. The animals were sacrificed after four[4] weeks of treatment.

Experimental protocol – Investigating the Effects of ECT on the cognitive activities (learning, memory & perception) using Barnes-maze, passive –avoidance test and Navigational task in wistar rats.

a. Barnes maze - it is a visual- spatial learning and memory task designed for rats. It consists of an elevated circular surface with holes around the edge. Modified method of Barnes was adopted.[8]

Principles – it is a dry-land based rodent's behavioral 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 took to locate the escape hole into the dark box beneath the maze should was recorded.

Procedure and precautions

- The maze was assembled in an isolated room away from any extraneous interference of noise.
- The maze was cleaned with 70% ethanol before starting the test in order to remove any odor cue and possible dirt accumulated on it.
- The animals were placed on the center of the elevated circular surface of the maze.
- The circular surface was mechanically spin at clockwise direction at a moderate speed.
- After the circular surface spinned 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 although at a maximum of 300 seconds.
- The procedure was repeated for all the animals and the test were performed for 3 consecutive days with 3 trials carried out on each of the day.
- The apparatus was cleaned with 70% ethanol to remove residual smell and fecal boli from the first rat.

b. Passive –avoidance test – (Modified method of Kameyama et al, was employed.[9] it is a useful task for evaluating the effects of novel chemical entities on learning and memory as well as studying the mechanism involved in cognition.

Principle – The testing apparatus is a trough-shaped alloy divided into two distinct compartment with an opening door. The white, brightly lit compartment is free of aversive stimulation whereas the black, dark compartment is equipped with shock capability. Its measures the basic ability to learn and remember the presence and place of a shock stimulation. In accordance with the guidelines of the American psychological association, the shock intensity used in this task should be the minimal amount needed to motivate the animal. However, no aversive stimulus applied to animals upon re-entry into the dark compartment during testing.

Procedures
- The animals were placed on the white, brightly lit compartment facing the door such that it was allowed access into the dark compartment through the door.
- When the animal steps into the dark, black compartment with all four paws, a 1-2 seconds foot shock was delivered (0.2 – 0.5 mA shock, minimum required to elicit flinching, running, jumping and /or vocalization).
- After the termination of the aversive stimulus in the dark compartment, some animals ran out of the dark compartment into the bright lit compartment.
- The latency to re-enter the dark compartment was recorded for a maximum time of 300 seconds. However there is no aversive stimulus applied to animal upon re-entry into the dark compartment during testing,
- The procedure was repeated for all the animals and the test were performed for 3 consecutive days with 3trials carried out on each of the days.

Precautions
- The apparatus was cleaned with 70% ethanol to remove residual smell and fecalboli from first rat.
- No aversive stimulus applied to animals upon re-entry into the dark compartment during testing.

c. Navigational task – (Modified Method of Gillner & Mallot (10). 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.

Procedure and precautions taken
- The Navigational box was cleaned with 70% ethanol before starting the test in order to remove any dirt accumulated on it.
- Animals were placed in the box through the entrance door and immediately the stop watch was start.
- Animals placed in the box were allowed to find their way through the environment at a maximum time of 300 seconds (5mins)
- The procedure was repeated for all the animals and the test were performed for 3 consecutive days with 3trials carried out on each of the days.
- After each trials, the apparatus was cleaned to remove residual smell and fecalboli from first rat.

Biochemical assay for Brain-derived Neurotropic Factor (BDNF)

Brain specimens were harvested from the animal at the end of study, crushed, homogenized and centrifuged under a negative degree and assayed for BDNF using standard laboratory procedure to determine the pattern of activities of the factor under various treatment in the study.

Data Analysis

The data from the study and 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.

RESULTS

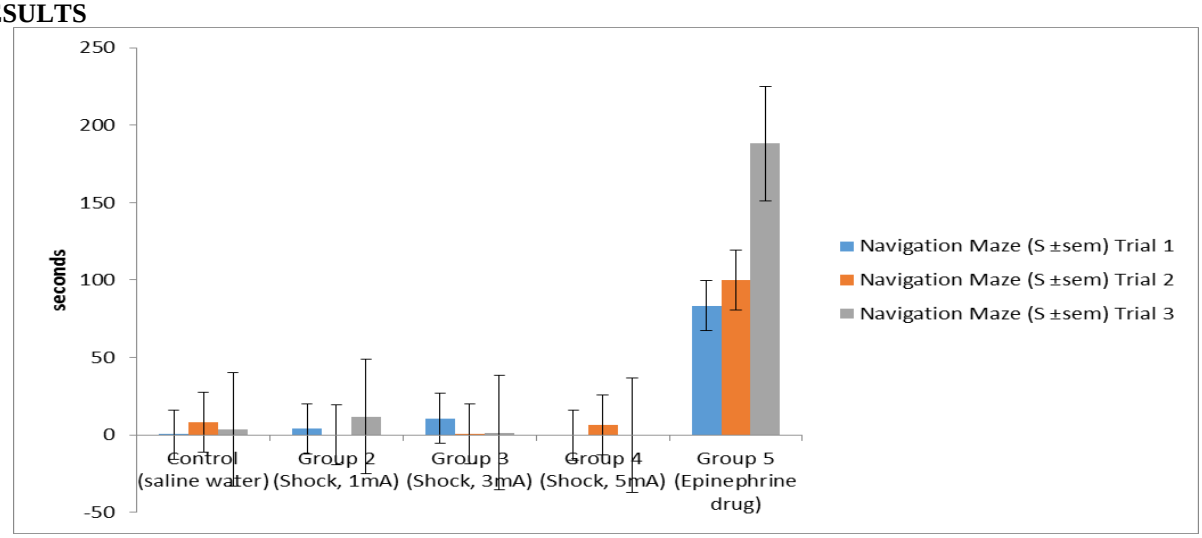

Figure 1: Pattern of adaptive locomotion using Navigation Maze test observed in the test groups and the control during week 1 of study.

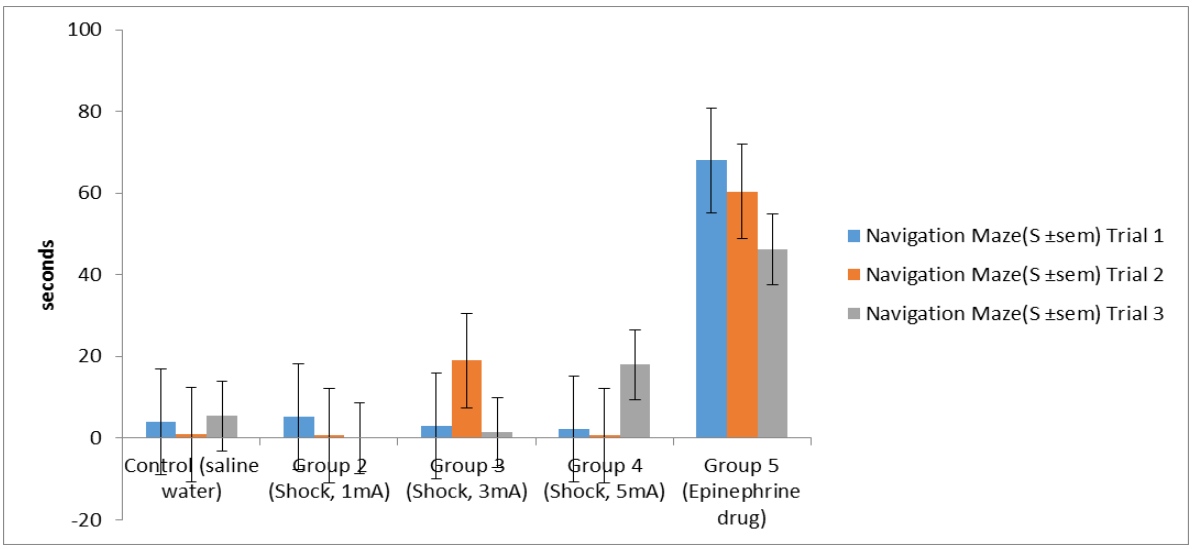

Figure 2: Pattern of adaptive locomotion using Navigation Maze test observed in the test groups and the control during week 2 of study.

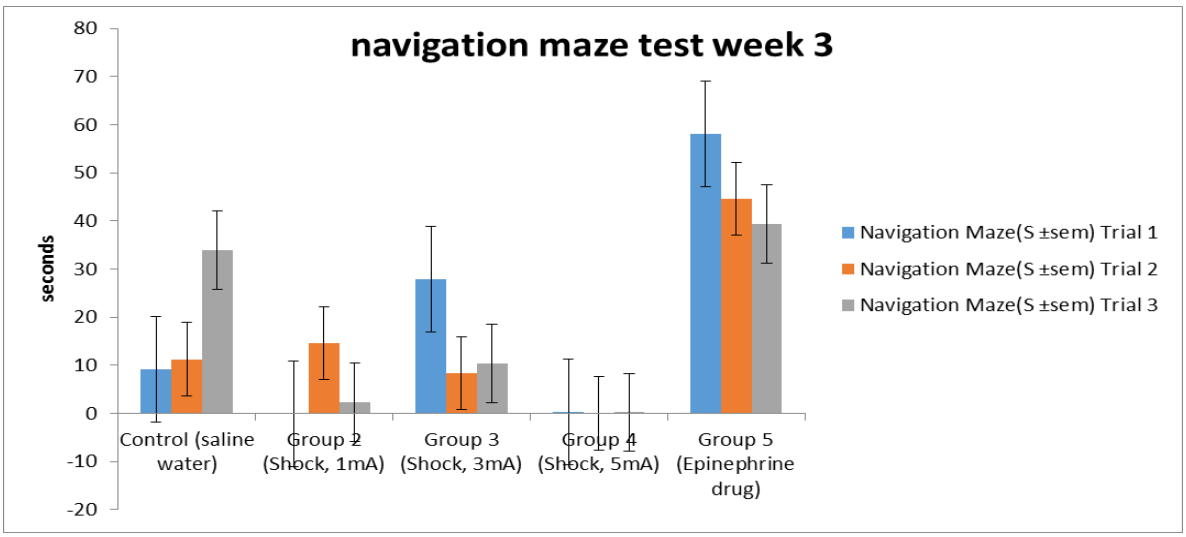

Figure 3: Pattern of adaptive locomotion using Navigation Maze test observed in the test groups and the control during week 3 of study.

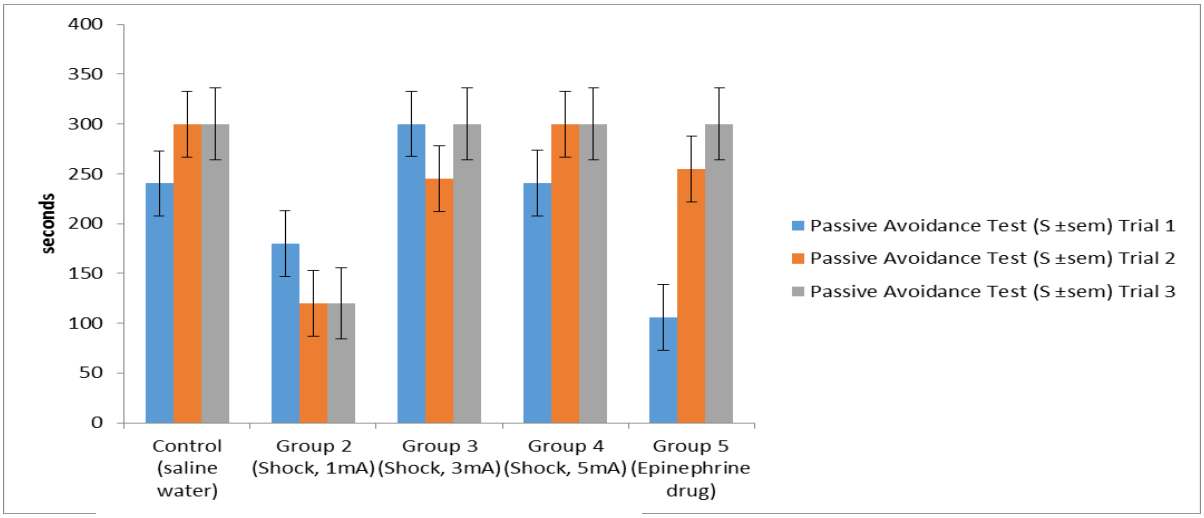

Figure 4: Pattern of step-down latency and escape latency in the test and control groups using PAS in week 1.

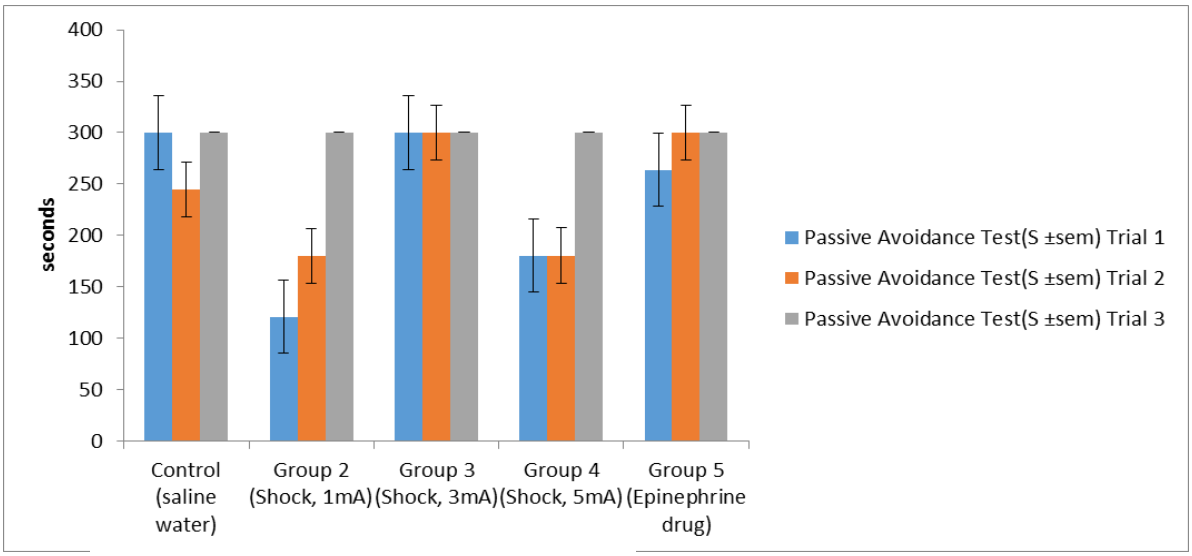

Figure 5: Pattern of step-down latency and escape latency in the test and control groups using PAS in week 2.

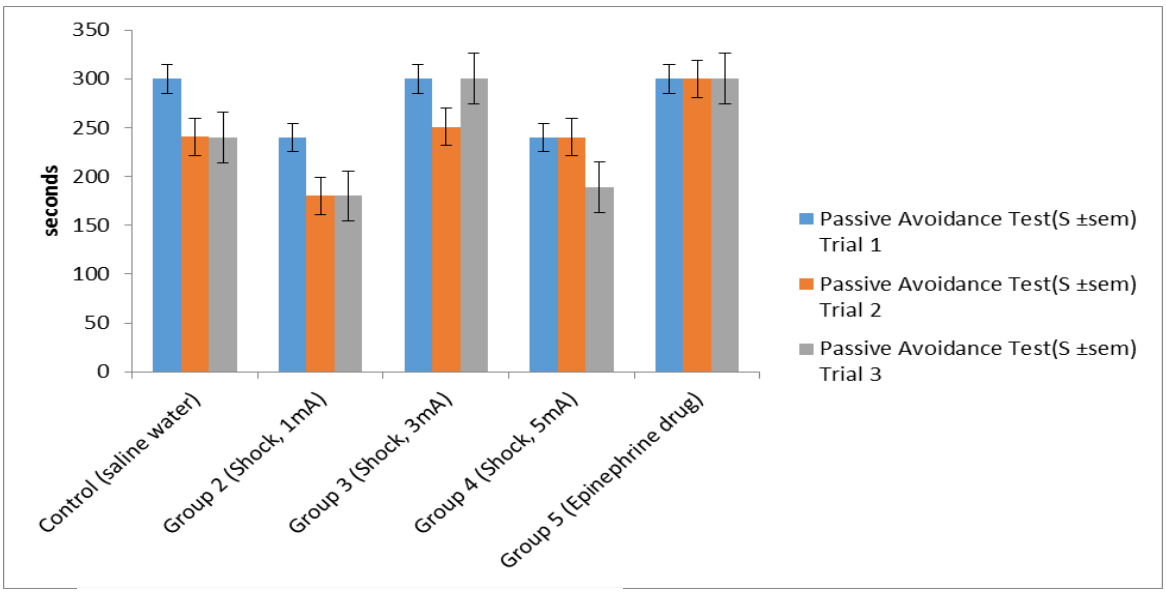

Figure 6: Pattern of step-down latency and escape latency in the test and control groups using PAS in week 3.

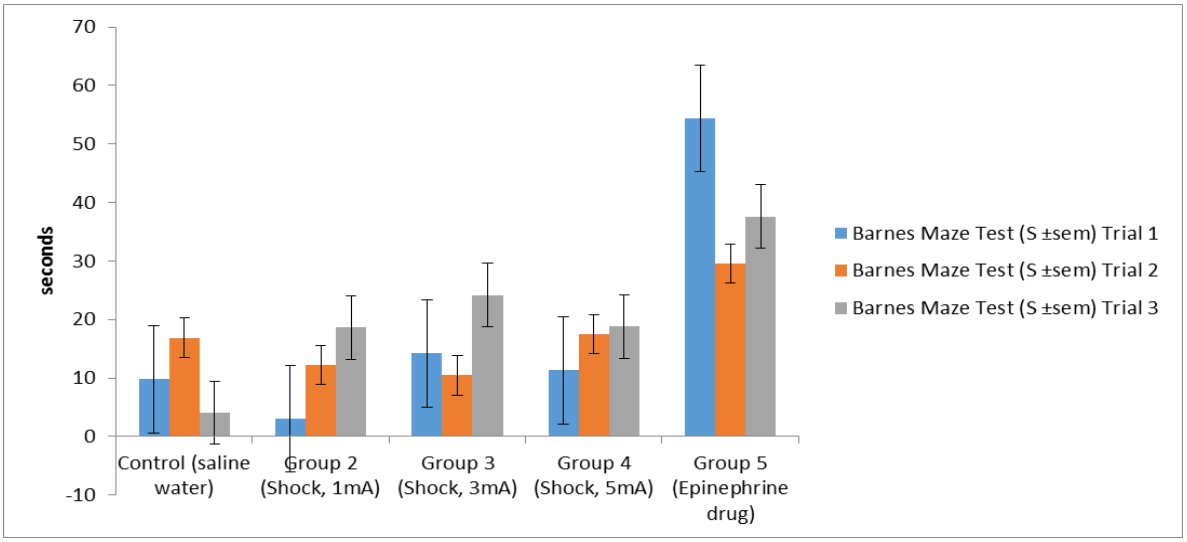

Figure 7: Pattern of spatial memory as displayed in the test and control groups using Barnes Maze in week 1.

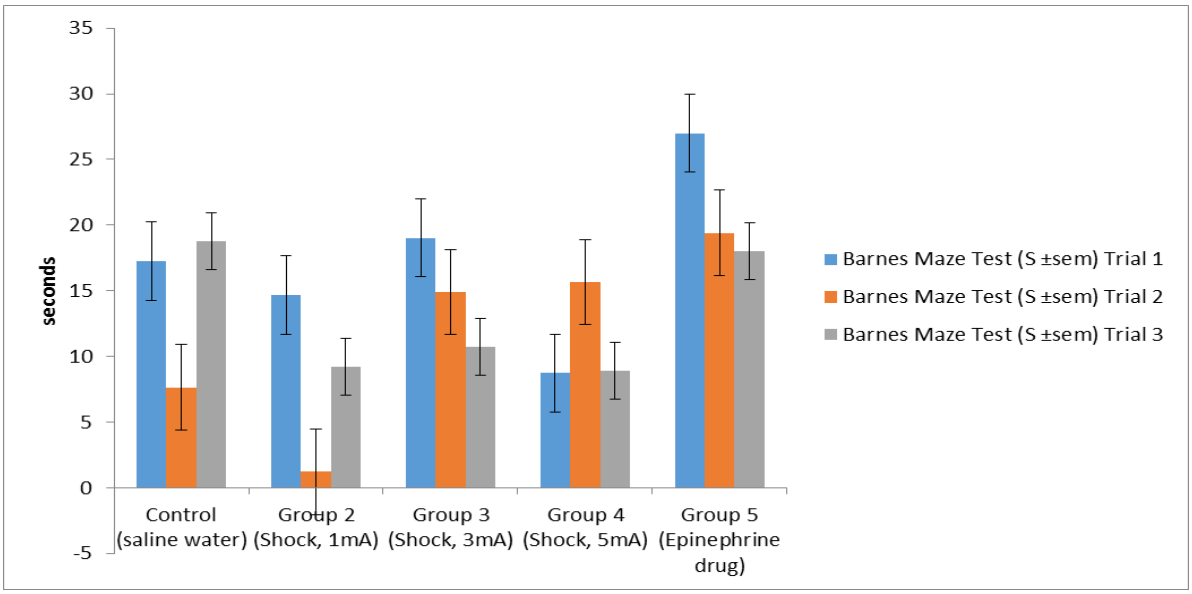

Figure 8: Pattern of spatial memory as displayed in the test and control groups using Barnes Maze in week 2.

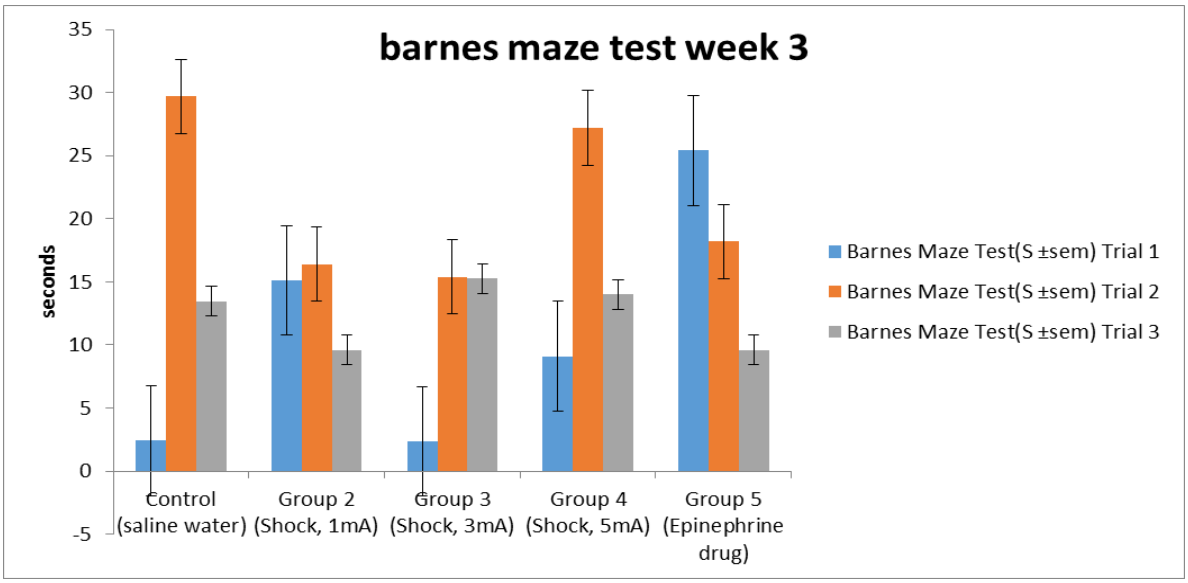

Figure 9: Pattern of spatial memory as displayed in the test and control groups using Barnes Maze in week 3.

Table 2: Pattern of Acetyl cholinesterase activities in the test and control groups.

Acetylcholinesterase (U/ml)	
Group1 (control)	37.433 ± 2.21
Group2 (2mA shock)	101.02 4± 1.10*
Group3 (3mA shock)	105.534 ± 1.10*
Group4 (5mA shock)	93.357 ± 3.31*
Group5 (Epinephrine)	113.652 ± 3.31*

Values are presented as mean ± sem, n= 5 * means values are statistically significant when compared to the control groups.

DISCUSSION

The present study was designed to examine the effect of non-lesion neuro- n stimulation using ECT on brain acetyl cholinesterase and memory activities in albino wistar rats. The experimental procedure was done using the following test; Navigational maze test, passive avoidance test, Barnes maze test.

Navigational maze test is a test for cognitive motor function for rats that rely on cues to navigate from start location to exit end through a labyrinth.[8] Navigational maze is employed in behavioral neuroscience to study spatial stated that the test could be a very precise study of learning memory and spatial working and is also capable of accessing damages to cortical regions of the brain. From the current study the navigational test involving three trials for the total period of three weeks; In week one (figure 1); there was a significant increase in the time spent by the test groups 2, 3, & 4 in comparison with the control group. The time taken to perform the navigational is a clear reflection of cognitive astuteness of the animal in challenging situations when life is hard. Hence there is a significant improvement were recorded for the animals exposed to epinephrine when compared to different shock levels and control. However; group 5 which took 0.1ml/100g of epinephrine showed improvement in the performance than groups 2, 3, & 4. This result may justify the report that epinephrine (also known as adrenaline is a neurotransmitter in the sense that, within the brain, it help neurons to communicate with one another and inaction concerns available physical and motivational resources.

For week two (figure 2); initially there was no increase in group 2 with 1mA with comparison with the control group. Group 3 showed an increase in improvement at trial and group 4 increased at trial 3 in comparison with the control group while group 5 which is epinephrine group has the rapid increase response. Observations in week three (figure 3) showed that there was a significant increase in the time spent by the test group 1 (control) and group 2, 3. At group 4 with 5mA shock there was no improvement. However group 5 (epinephrine drug) has a significant improvement.

The general observation of the navigational maze task showed that quality of adaptive locomotion improved significantly ($p<0.05$) the test groups exposed (especially at 5mA) to shock from week 1 to week 3 and thus up regulated cognition aspect of motor function when compared to the control groups.[9]

The Passive Avoidance test is useful for evaluating the effect of novel chemicals entities on learning and memory as well as studying the mechanism involved in cognition. From the current study the passive avoidance test involving three trials for the total period of three weeks.

Activities in week one (figure 4) revealed that learning and memory tend to be increased in all test groups including that of epinephrine drug group. Similarly, the pattern of activities were not different in week two (figure 5), thus, from the current study the degree of alertness and cognitive awareness significantly increased as revealed by the time spent by the test groups at different shock rate very much comparable to the epinephrine drug group. In week three (figure 6) current at 2 and 3mA prolonged relapse but at 5mA, it was interesting to discover a decline memory. In other words, the degree of awareness declined significantly at week 3 with 5mA portending danger on prolonged exposure to electroshock which of course, could be detrimental to mental health. As started earlier, this study showed that the task is useful for evaluating the effect of novel chemical entities on learning and memory.

Among the methods valuable for accessing spatial learning and memory impairments in rats, the Barnes maze test deserves special attention. It is based on the assumption that the rats placed into the aversive environment should learn and remember the location of an escape box located below the surface of the platform. Different trails of this test allow to measure spatial learning, memory retrieval, and cognitive flexibility.

As recorded in the first week (figure 7), in group 1(control) and group 2, 3, & 4 it was observed that the rats spent lesser time to locate the escape box while group 5(epinephrine drug) spent the highest time to locate the escape box. Therefore, group 2(1mA shock) had the least time spent (quickest response) in locating the escape box when compared to group 1,3,4,5. This exposure to electroshock led to preponderance of cognitive awareness and attention as displayed with respect to stress response. This allows rapid memory developing within a few seconds. In the second week trials as recoded (figure 8) in group 2(trial 2) had the least time to locate the escape box while group 4(5mA shock) had the lesser time with group 5(epinephrine drug) having recorded the highest time to locate the escape box in comparison to control group and group 3. This showed that shock at 5mA down regulated cognitive processing and thus has an impaired effect on memory processes. In week three (figure 9) it was observed that group 3 had the least time to locate the

escape box in comparison to control group, group 2,4 and group 5.

After these few trials in the period of three weeks shorter latencies to reach the escape box were observed in Barnes maze test because the rats changed their strategy from random to spatial. This is in accordance with[10] who observed that in Barnes maze, rats take advantage of the natural preference for dark and quiet environments. Weak aversive stimulation which is often applied to provide inhospitable conditions and increase the motivation to escape from the platform, doing so without undue physical or mental stress.

Acetylcholine (Ach) promotes memory formation. The primary role of AchE is to terminate neuronal transmission and signaling between synapses to prevent Ach dispersal and activation of nearby receptors.[11,12] The present study revealed that the effect of shock on AchE activity in brain memory function is dependent upon the level of shock. This study showed that the brain memory was affected by shock but not in a uniform pattern. At group 2 (2mA), AchE increased significantly compared to that of control (saline water) which means shock contributes to the depletion of AchE. At group 3 evidently showed in table (2) at a shock of 3mA, AchE increased more significantly in comparison to that of control group. More slow but intermittent shock could lead to memory decline, amnesia, dementia as observed in groups 2 and 3 when level of AchE increased which would destroy Ach as observed in the study. Interestingly, group 4 with more shock of 5mA, AchE activity was significantly decreased. This shows that lesser shock produces significant changes in brain AchE activity in rats whereas more shock could improve learning and memory function.[13] At group 5(epinephrine drug) evidently showed a significant increase in AchE activity as relative to group 2(2mA), group 3(3mA). There was significant decrease in the AchE activity in group 4 which experienced the highest shock level of 5mA but did not experience that effect in group 2 and 3. It is expected that lowered AchE activity may enhance cholinergic activity by raising Ach level (inhibition of metabolism) thereby maintaining the cognitive functions, which happened in case of group 5(5mA) but not in other group.[14, 15]

CONCLUSION

This study investigated the effects of ECT-induced electroshock on brain acetyl cholinesterase level and spatial and memory behavior of wistar rats using neuro-stimulation method to induce shocks after which they underwent some cognitive and spatial tasks such as navigational maze test, passive avoidance test, Barnes maze test. It was demonstrated that electroshock can cause elevation response in Brain Acetyl cholinesterase level alongside improved spatial learning activities with a delayed onset of cognitive decline in Rats.

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