Effect of Electroconvulsive Seizure on Acoustic Startle Reflex and Psychomotor Functions in rats.

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ABSTRACT - In spite of the abundance of literature on relationship between ECT and cognitive side effect, there seem to be little clarity and agreement in literatures concerning ECT. Related impairment of psychomotor functions. The aim of this study was to investigate the effect of electroconvulsive seizure on acoustic startle reflex and psychomotor functions. The research understudied electric shock on movement behavior in rat, interruption on learning and memory traces as well as studying the patterned behavior involved in psychomotor axis using the passive avoidance test, spatial learning and memory using the navigation test, assessment of the strength and will of the animals to hold on to object using the inverted screen test and adaptive locomotion (running, walking) mechanisms. A total of twenty-five male wistar albino rats weighing 180-200g were obtained and used for the test. The animals were subjected to graded voltages using electroconvulsive therapy machine which was calibrated to a current of 1.0mA 1.5mA and 2.0mA in order to induce a mild seizure. Consequently, upon passage of voltage, rat showed spike in visuo-spatial activities. Also, the rats demonstrated decline in cognitive function such as impaired avoidance ability and in significant deficit in locomotive performance including grasping and gripping, grooving objects and recognition ability, in the overall, significant adaptive behavior was observed. Nevertheless, the impairment seemed to increase with voltage amplification. Since this study showed no serious negative side effect on cognitive performance, the study recommended among others, that further study might be administered on particular memory centers to possess in-depth knowledge of effect. In case of clinical application.

Keywords: electroconvulsive seizure, psychomotor axis, locomotion, passive avoidance, memory

Background to the Study

Electroconvulsive therapy (ECT) has proven to be the foremost reliable and effective treatment of depression disorder, notwithstanding its overtime shown some potential cognitive side effect (Semkovska and Mcloughline, 2010). Research on the consequences of ECT on electro physical measurements are often traced back to the 1930. Nevertheless, there seem to be yet only a few studies on examination of the electrophysiological effects of ECT in comparison to other modalities (Abbolt *el al,* 2014). More so, the connection between the change in clinical response and cognitive function remains indefinable (Segi-Nishida *et al*, 2019).

Significantly, establishing useful relationship between ECT and possible clinical outcome response dosing should be guided by ECT finding of seizure sufficiency. Nevertheless, there has been no prospective study showing that this relationship has enough sensitivity and specificity to aide clinical deciding. Conversely, literatures have however shown that ECS end in impaired learning memory, which has relevancy to the cognitive side effects that are usually indicators during ECT. Evidence even have it that ECT or ECS elicited cognitive side effects are related to structural and functional change within the cholinergic system. It's also evident that activation of the immune cells, including increased microglia activity is necessitated after ECS which can successively induce short-term cognitive deficit.

In spite of the abundance of literature on relationship between ECT and cognitive side effect, there seem to be little 00m clarity and agreement in literatures concerning ECT. Related impairment of cognitive function. Some research have posited the absence of any effect on learning memory (Andrade, Arumugham and Thirthalli, 2016), many other studies has revealed various negative effect on different areas of cognitive function including greatest worldwide decrease concerning Bitemporal ECT, severe deterioration of verbal memory associated to bifrontal ECT, and great decrease in visual memory attributed to right unilateral ECT as measured by test like mini mental stet exam, trial making, Rey Auditory verbal autobiographical memory, visual memory and verbal memory (Dunne and mc Loughlin, 2012; Kellner, Knapp and Hussain 2010; Sienaeart, Vansterclandt and Demyhenacre, 2010; and Eschweiler, Vonthein and Bode 2007).

Materials and Method

The research was approved by the Ethic Committee of the University of Port Harcourt and was conducted according to the rules of humane use of laboratory animals in experimental work. The animals were maintained 5-6 to cage and had unlimited access to food and water. First, rats were habituated to the experimental conditions for six days by exposing them to randomly applied acoustic pulses. The habituation was performed in a testing chamber, where they were later exposed to ASR sessions.

Experimental Animal

A total of twenty-five male wistar albino 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 30ºC 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 four groups of five rats per group

Table 1 Experimental Design and Grouping of the Rats

Groups	Number of animals	Treatment:
Group I (Normal control)	5	Feed + Water ad libitum
Group II	5	Feed + Water ad libitum + ECT (1mA)
Group III	5	Feed + Water ad libitum + ECT (1.5mA)
Group IV	5	Feed + Water ad libitum + ECT (2mA)
Group V	5	Drug Epinephrine-treated (0.5ml/100g)

Experimental protocols 1 for electro-convulsion therapy shock

The animals here were subjected to a high shock using electroconvulsive therapy machine which was calibrated to a current of 1. 1.5 And 2.0mA in order to induce a mild seizure. When inducing the shock, the animals were restrained by holding their neck skin between forefinger and thumb and pressed gently against the table, the electrodes were then clipped to the animals' ears before inducing shock. After shock, the animals were then exposed to different maze tasks for about three weeks in order to study the effect of shock on their memory and defensive behavior.

Chemicals: Epinephrine was purchased from Sigma Chemicals (St Louis, MO, USA).

Experimental protocol 2 – (Investigating the Effects of ECT on the cognitive activities (learning, memory & perception) using Barnes-maze, passive –avoidance test and Navigational task in wistar rats.)

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. It was originally developed by Carol Barnes to study spatial memory in rats (Barnes, 1979) and later adapted for use in mice (Bach *et al.*, 1995). 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.

Passive –avoidance test – 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. Using modified method of Lipton et al, (1994), 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,

Navigational task – it is widely used in behavioral 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. 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 trial, the apparatus was cleaned to remove residual smell and fecal boil from first rat.

Object Recognition Test

The object recognition test (ORT), also known as the novel object recognition test (NOR), is a relatively fast and efficient means for testing different phases of learning and memory in mice. It was originally described by Ennaceur and Delacour in 1988 and used primarily in rats, however, since then, it has been successfully adapted for use in rats. Briefly, the rats were placed into the center of a 100 × 100 × 50 cm black polyvinyl chloride box for 5 min, and the trajectories were recorded After the open field, we analyzed NOR. To this end, the rats were placed into the arena as above which was equipped with two identical cylindrical objects (uniform gray color, diameter 4.5 cm, height 7 cm). This sample phase was performed on post-operative day 5 or 6 the first choice test was performed by replacing one of the cylindrical objects by a cuboid black-and-white colored object this choice test was repeated on the following day. For the analysis of the exploring behavior, we defined a circular area around the object and defined exploration as the time spent within this circle. In addition, we calculated the NOR index as the percentage of time spent at the novel object divided by the total time spent at both objects (i.e., within the diameter of 18 cm). In case the total time spent at both objects during the choice test was zero, these animals were not included for the NOR index calculation

Inverted Screen Test

Using the modified method of Kim et al, 2010, this test assesses the strength and will of the animals to hold on, even after being subjected to shock. In this test, the animals were held inversely, and put into the inverted screen box, ensuring that the animal grips the wire gauze properly. Once it gripped the wire gauze, the time started. The amount of time the animal held onto the wire gauze was recorded, once it drops into the box.

Acoustic Reflex Response Test

Using a modified method of Allen *et al*, 2008, at the start of each session, the rats was placed in the housing and allowed to acclimate to a constant background tone of 20 kHz and 80 decibel for 300s. The acclimation period was followed by “prepulse” and “startle only” trials. In prepulse trials, the prepulse stimulus comprised a frequency change consisting of a 1 ms linear ramp from the background tone to the prepulse tone, maintained at 80 dB SPL. Following the startle stimulus, tone was presented again until the prepulse of the next trial. In startle only trials, the prepulse consisted of a 1 ms ramp from f_1 to f_1, and thus maintained the ramp command while not actually introducing a frequency change. All trials were separated randomly by an inter-trial interval (ITI) ranging from 10-30s.. Trials were divided into three phases. The first phase comprised a series of startle only trials to allow for short-term habituation to the startle stimulus. The second block contained prepulse trials randomly interleaved with an equal number of startle only trials.

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 22.

RESULTS

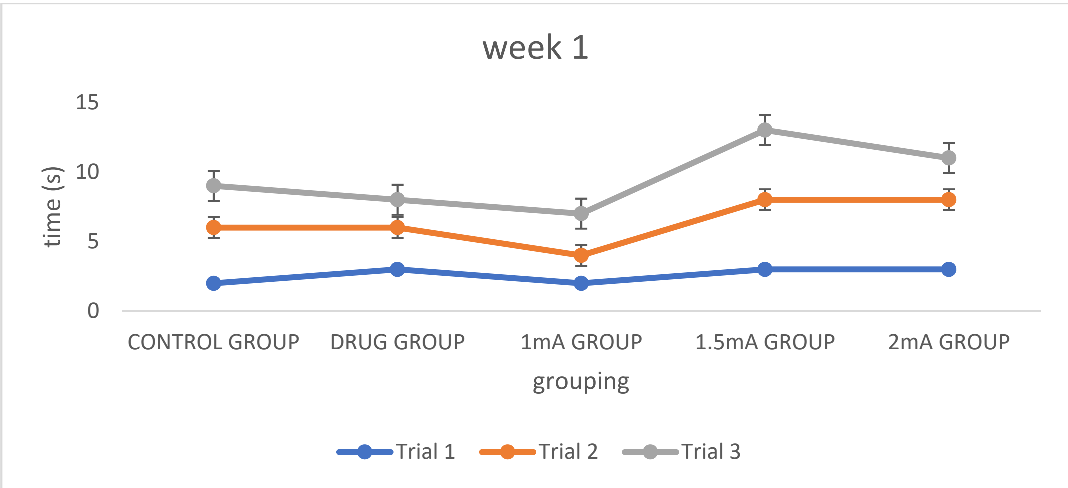

Figure 1. Acoustic Reflex Response Time (ARRT) recorded in various test and control groups exposed to ECT shock during trials 1, 2, and 3 at 30 minute interval at week 1 assessment

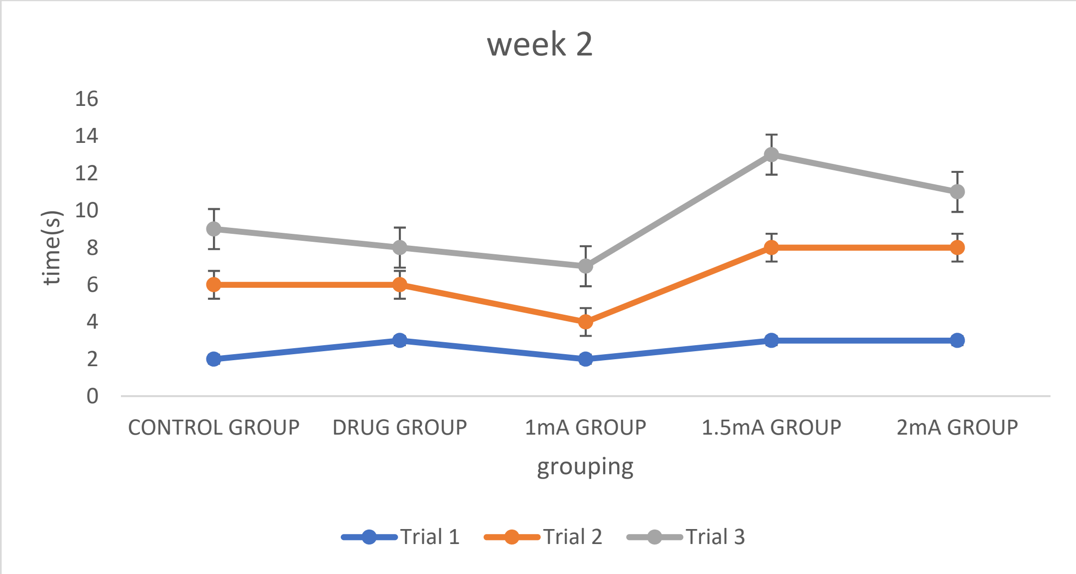

Figure 2. Acoustic Reflex Response Time (ARRT) recorded in various test and control groups exposed to ECT shock during trials 1, 2, and 3 at 30 minute interval at week 2 assessment

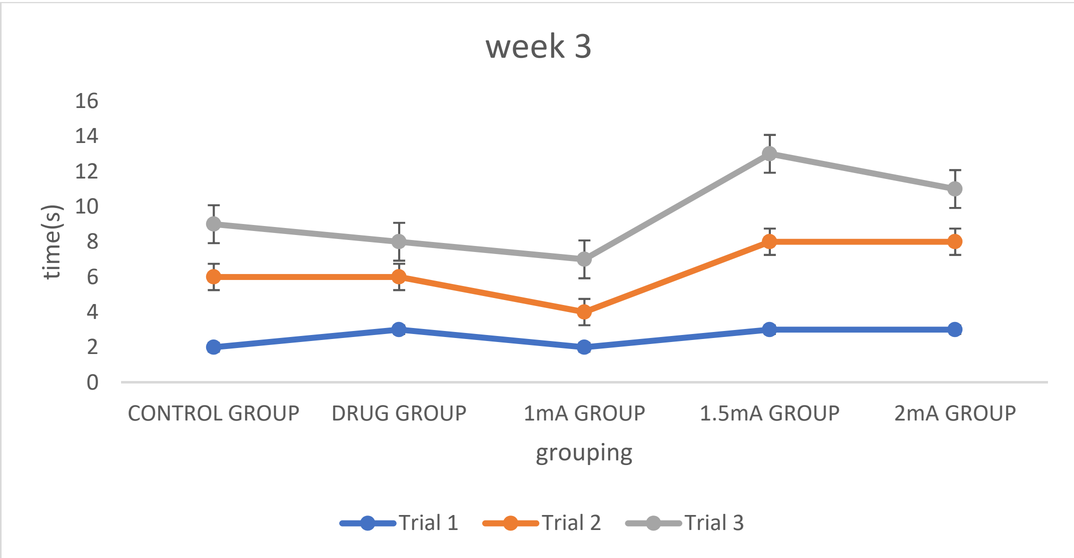

Figure 3. Acoustic Reflex Response Time (ARRT) recorded in various test and control groups exposed to ECT shock during trials 1, 2, and 3 at 30 minute interval at week 3 assessment

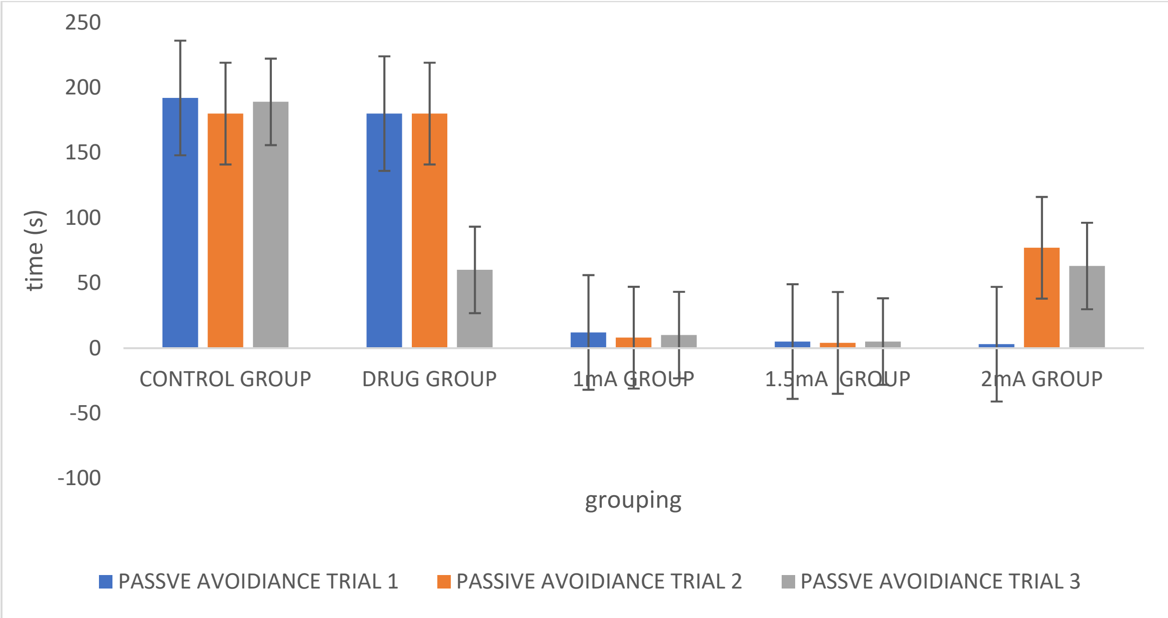

Figure 4. Patterns of response from various test and control groups using Passive Avoidance Test at week 1 study. N = 5, $P < 0.05$

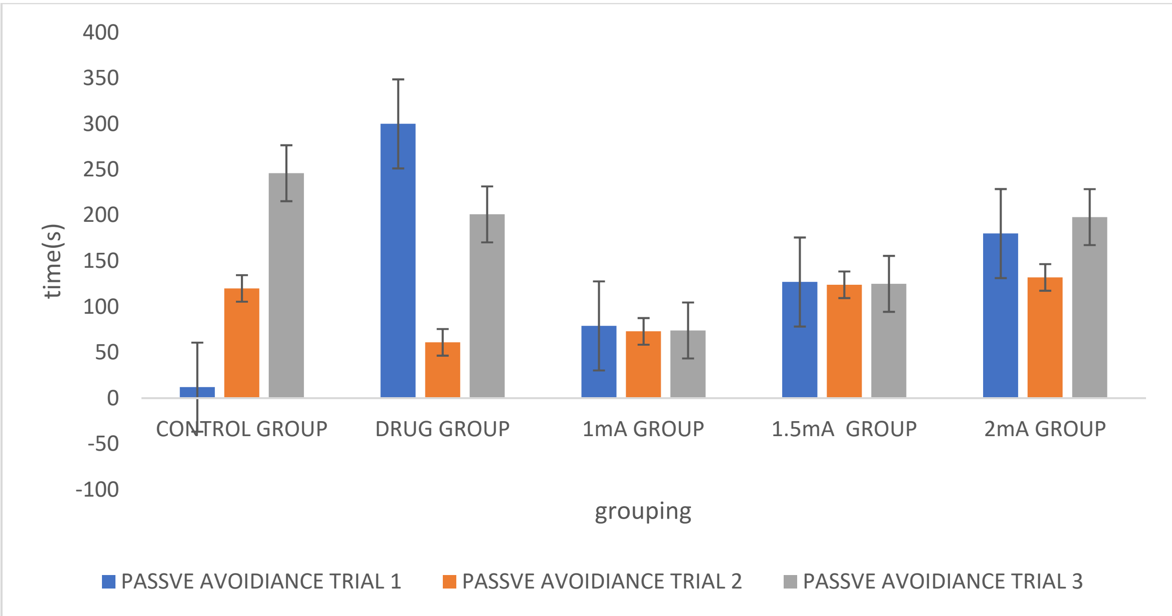

Figure 5. Patterns of response from various test and control groups using Passive Avoidance Test at week 2 study. N = 5, $P < 0.05$

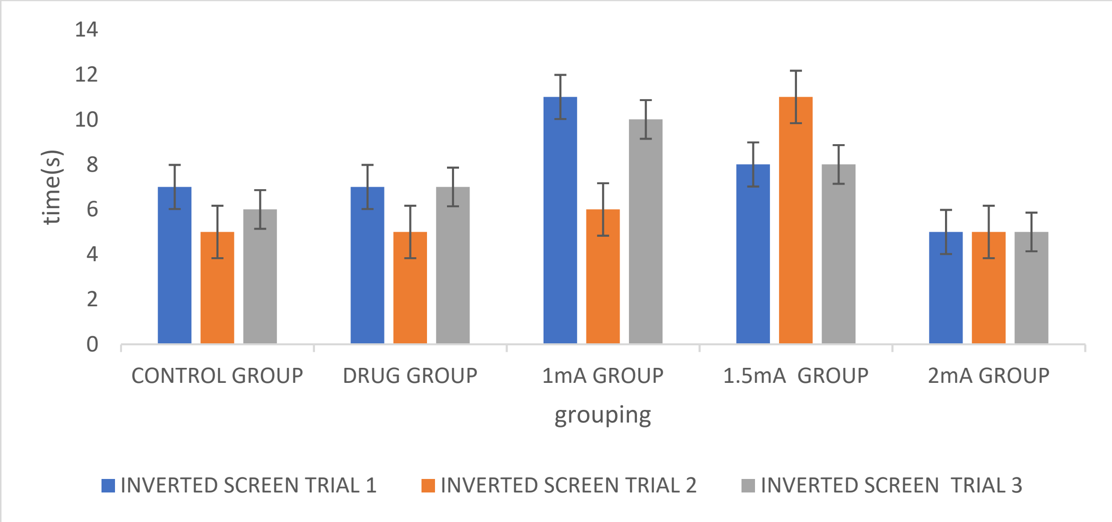

Figure 6. Patterns of response from various test and control groups using Inverted Screen Test at week 1 study. N = 5, P < 0.05

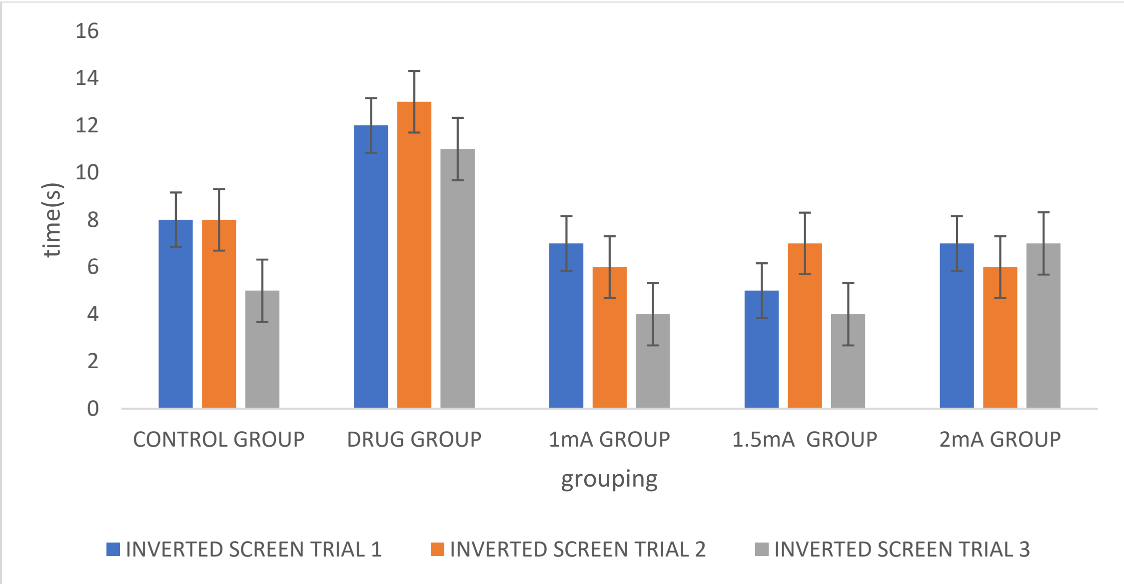

Figure 7. Patterns of response from various test and control groups using Inverted Screen Test at week 1 study. N = 5, P < 0.05

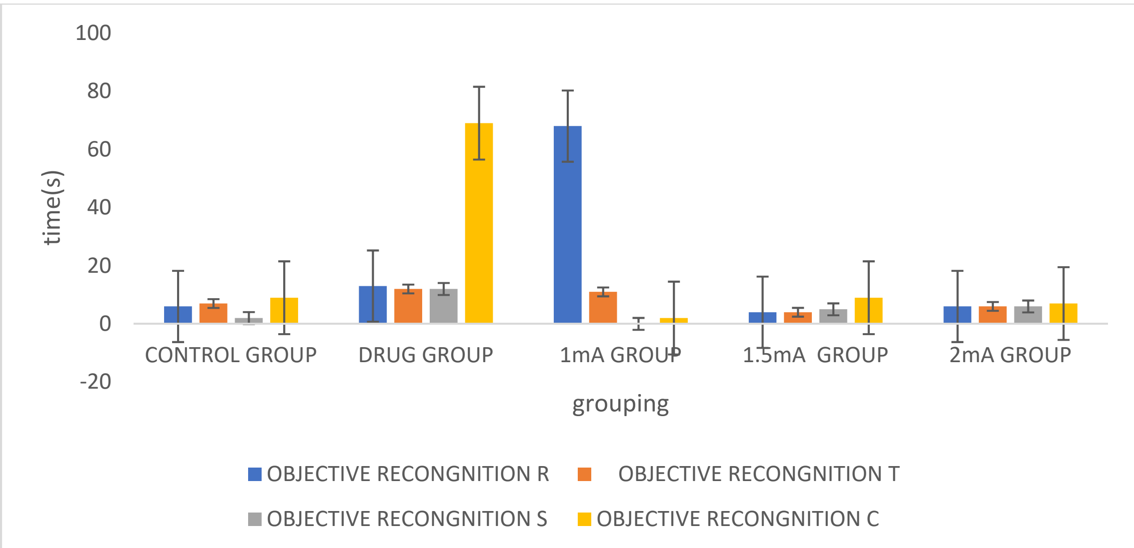

Figure 8. Patterns of response from various test and control groups using Inverted Screen Test at week 2 study. N = 5, P < 0.05

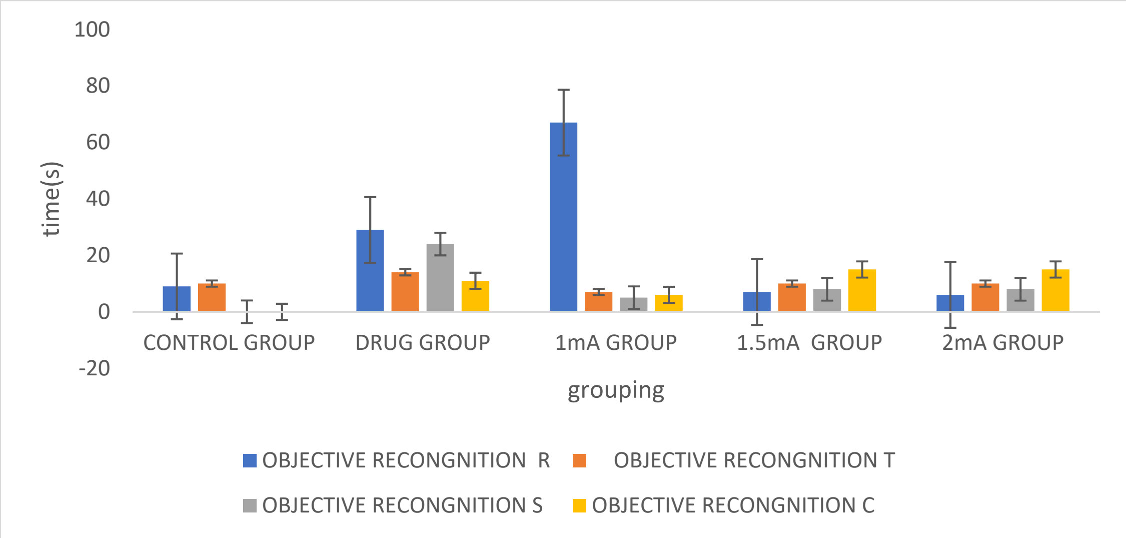

Figure 9. Patterns of response from various test and control groups using Object Recognition Test at week 2 study. N = 5, P < 0.05. (Round (R), triangular (T), square (S) and circular (C)

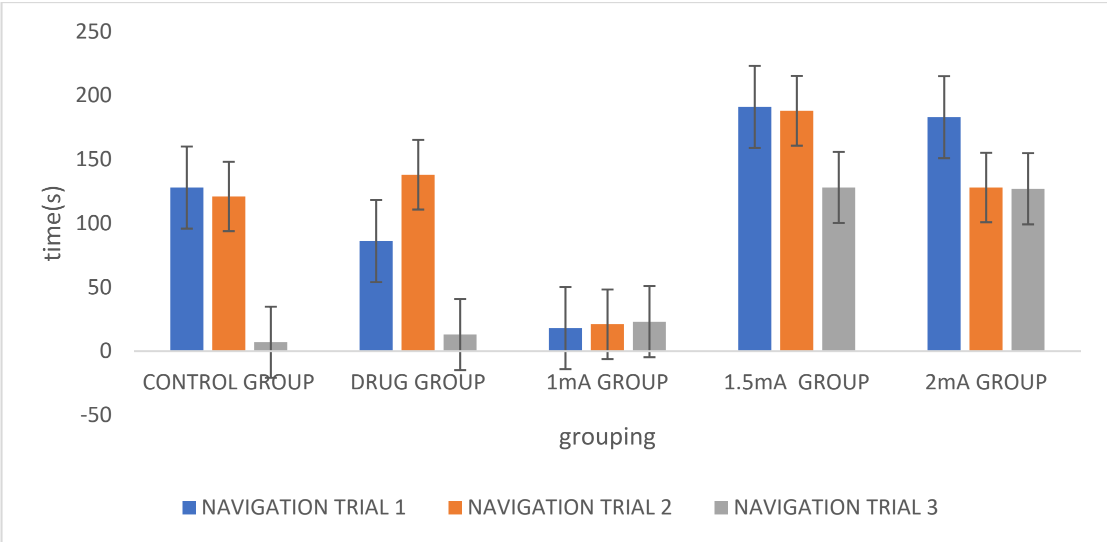

Figure 10. Patterns of response from various test and control groups using Navigation Test at week 1 study. N = 5, P < 0.05. (Round (R), triangular (T), square (S) and circular (C)

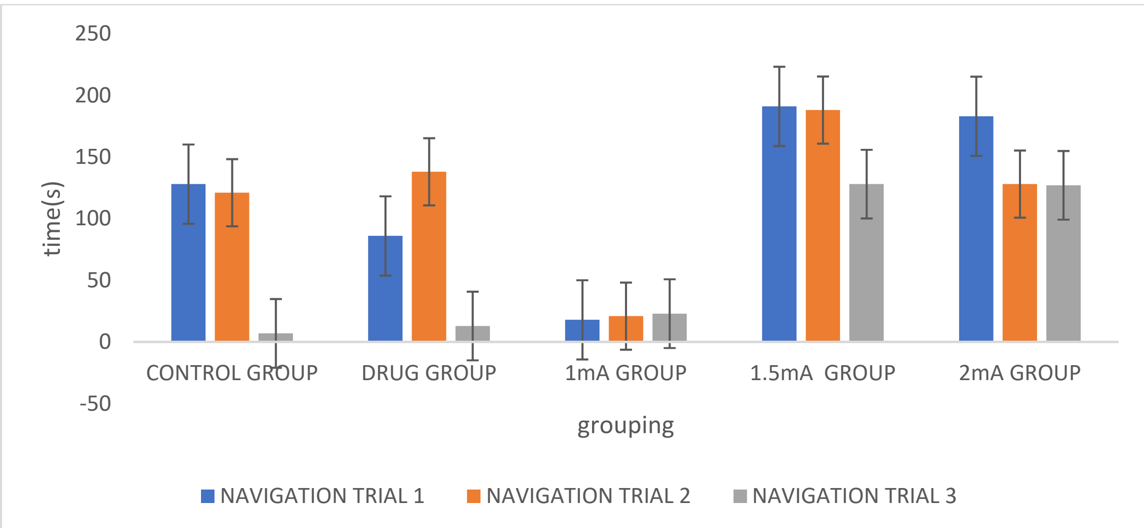

Figure 11. Patterns of response from various test and control groups using Navigation Test at week 2 study. N = 5, P < 0.05

Discussions

The current research demonstrated that interference of electroconvulsive seizure could disrupt acoustic startle reflex and psychomotor functions as revealed in this investigation. It equally demonstrated that electroconvulsive seizure has debilitating effect on cognitive performance especially in the presence of an electrical stressor on the rats.

Observations recorded from Acoustic Reflex Response Time (ARRT) showed that the threshold of response in group exposed to 1.5mA increased significantly when compared to those with lower voltages and the negative control. An indication that a strong voltage down-regulatory effects on reflex response and to an extent, leading to slowing in thought processing and decision making during stressful scenarios. Across the three trials at 30 minutes interval, patterns of activities were insignificantly different and adaptive responses were noted to be similar. The degree of reflex response from week 1 to week 3 did not allude any variations in reflexive adjustment especially in the test groups exposed to graded degree of electric shock. The group treated with epinephrine had their acoustic reflex response time significantly ($p< 0.05$) reduced and their reactions were much enhanced and this behavior was comparable to the group exposed to 1mA current. Summarily a significantly high voltage could impair adaptive responses and could worsen fight and flight response in rats.

The intrinsic ability to recall past noxious scenarios upon exposure to electric shock was observed to have interfered with memory and learning circuit in the hippocampus according to the study. Week 1 study revealed that groups exposed to 1.5 and 2 mA had significant ($p< 0.05$) amnestic tendency when compared to the 1 mA group and the control. Adaptive tendency seemed to improve this lopsided response in week 2. There was significant loss of memory upon exposure to high voltage shock. There seemed to be adaptive response from trial 1 to trial 3 and from week 1 to week 2 though slow and insignificant. The sum effect of memory loss recorded during the Passive Avoidance Test was an indication that electric shock could apparently lead to a huge decline in both anterograde and retrograde amnestic tendencies as demonstrated in the study.

Results from the inverted Screen test alluded to the fact that serial electric shock in rats interfere with muscular stability upon hanging on their four limbs. There was transient increase in tremor and shakiness. Ordinarily, muscles never work individually. They are activated by the brain, local or higher spinal reflexes, via the neuromuscular junctions and work within the constraints of other muscles and the skeletal system, therefore, electrical interference could significantly disrupted the motor units in the pathway and lead to a preponderance of misjudgment of motor commands from the motor cortices of the brain. Groups with higher shock (1.5 and 2mA) showed a huge decline in stability from week 1 to week 2 across the trials. Epinephrine group showed similar pattern of decline as their anxiety level was evoked. Unlike the control group and the group with 1mA that were enhanced and demonstrated significant steadiness and stability for a considerable amount of time in the study. This implies that rat still exhibited good cognitive performance under inducement with electroconvulsive shock indicating that, there was no show of stress or negative side effect which agrees with Andrade, Arumu and Gham (2016). Van buel *et al*, (2017) reveal that repeated ECS procedure resulted in weakened fear learning-memory.

The use of visual cues in the rats and recognition memory was observed to be positively enhanced with increasing potency from trial 1 to 3 and from week to week. Mild to moderate electric shock seemed to be beneficial in these attributes. Various shapes of objects were identified with various degree of dexterity in the groups. The groups with larger amount of shock adapted more rapidly with enhanced familiarity when compared with the control and low shock group. Object exploration time by the groups was measured and it was discovered that the novel object recognition ability could attest to the fact that moderate electric shock could ameliorate poorly enhanced visual cues as the case may be. Spatially dependent memory is basically characterized by spatial cues which could be subjected to increased concentrated attention in the awake state. Nevertheless, the recognition time increased with increase with amount of electric current discharged. This finding is in contrast with the research of (Dunne and Mc Loughlin, 2012 and Keelner, Knapp and Hussain 2010), which established that electroconvulsive shock result to impairment of visual memory.

Navigation through an interconnecting pathways within a given task time was taken as a measure of memory recall and cognito-motor performance. This was observed to be significantly poor in the 1.5 and 2mA groups while it was much improved in the 1mA group and the control groups. The speed of task performance was a measure of learning from pre-exposure and spatial memory. Failure in task completion as fast as possible could be tantamount to mental proficiency and retardation as amplified by electric shock from mild to moderate quantity and could leave a debilitating and irreversible pathway in the motor pathway. Locomotive deficit observed in these groups became worsen with retrograde amnesia with significant import. The study demonstrated that impedance in motor deficit could account for failure for successful motor task observed in these groups.

Conclusion

Effects of electroconvulsive seizure on acoustic startle reflex and psychomotor function were observed to have different implications on mental proficiency, visuo-spatial potentials, cognito-motor functions, and psychosomatic interactions as revealed in the study. It was specifically demonstrated that rats showed enhanced anxiolytic tendency in movement upon administration of electroconvulsive shock. There was impairment or negative effect of electroconvulsive seizure on certain cognitive functions like impaired avoidance ability upon administration of shock and in some test, investigation showed no impairment on cognitive performance including good ability and ability to steady on four limbs on inverted screen, good objects recognition ability, satisfactory adaptive locomotion ability. Nevertheless, the impairment seemed to aggravate with increase in current of electrical shock.

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