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SUB-ANESTHETIC DOSE OF N-METHYL-D-ASPARTATE RECEPTOR ANTAGONIST (KETAMINE) DOWN REGULATES SPATIAL LEARNING AND IMPAIRS VISUAL SCENE-BASED MEMORY IN WISTER RATS

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ABSTRACT
The study was designed to investigate the effect of sub-chronic administration of N-methyl-D-aspartate receptor (ketamine) antagonist on spatial learning and visual scene-based memory in rats. Twenty-five albino wistar rats were arranged in five groups; group1 (Normal control), group 2(1mg/kg Ketamine), group 3(3mg/kg Ketamine), group 4(6mg/kg Ketamine), and group 5 (epinephrine drug). Drug treatments lasted for 4 weeks across the groups The rats in various groups were made to undergo a total of nine (9) trials of some spatial learning and memory tests which include: navigational maze (adaptive locomotion), passive avoidance test (episodic memory), Barnes maze test (visual scene-based memory). The neurobehavioral activities exhibited by the test and control groups were recorded and analyzed using ANOVA. Observations from the adaptive locomotion study revealed that there was a significant decline ($p< 0.05$) in motor function especially as the treatment progressed and in a dose-dependent pattern. The quality of locomotion task performance also decreased as the dose of ketamine increased. Results from visual scene-based memory test revealed that in the ketamine-administered groups, quality of visual perception and memory retrieval became significantly ($p< 0.05$) abysmal especially in group 4(6mg/kg ketamine) in the week 3 of the study when compared to both the negative and positive controls. Assessments for the episodic memory study interestingly showed ketamine treatment across the 3-dose level up regulated memory retrieval and the experience was dose-dependent. Pain episode seemed to consolidate spatial learning aspect of memory. From the results obtained in the study, ketamine at these doses could be favorable towards consolidation of episodic memory but could antagonistic to visual scene-based memory alongside decline of adaptive locomotion and motor coordination. In order words, it can be seen that the use of different doses of ketamine can cause a decrease in spatial behavior in albino wistar rats.

KEYWORDS: Ketamine, wister rats, Navigation maze, Adaptive locomotion, Visual scene-based memory.

INTRODUCTION
Amnesia is a deficit in memory caused by brain damage or disease, but it can also be caused temporarily by the use of various sedatives and hypnotic drugs. The memory can be either wholly or partially lost due to the extent of damage that was caused.[1] There are two main types of amnesia: retrograde amnesia and anterograde amnesia. Retrograde amnesia is the inability to retrieve information that was acquired before a particular date, usually the date of an accident or operation. In some cases, the memory loss can extend back decades, while in others the person may lose only a few months of memory. Anterograde amnesia is the inability to transfer new information from the short-term store into the long-term store. People with anterograde amnesia cannot remember things for long periods of time. These two types are not mutually exclusive; both can occur simultaneously.[2]

Case studies also show that amnesia is typically associated with damage to the medial temporal lobe. In addition, specific areas of the hippocampus (the CA1 region) are involved with memory. Research has also shown that when areas of the diencephalon are damaged, amnesia can occur. Recent studies have shown a correlation between deficiency of RbAp48 protein and memory loss. Scientists were able to find that mice with damaged memory have a lower level of RbAp48 protein compared to normal, healthy mice.[3] In people suffering with amnesia, the ability to recall immediate

information is still retained and they may still be able to form new memories. However, a severe reduction in the ability to learn new material and retrieve old information can be observed. Patients can learn new procedural knowledge.[4] In addition, priming (both perceptual and conceptual) can assist amnesiacs in the learning of fresh non-declarative knowledge. Amnesic patients also retain substantial intellectual, linguistic, and social skill despite profound impairments in the ability to recall specific information encountered in prior learning episodes*[5]*). The cause of amnesia can be either primarily *organic*, resulting from neurological conditions such as stroke, tumor, infection, anoxia, and degenerative diseases that affect brain structures implicated in memory; or it can be primarily *functional or psychogenic*, resulting from some traumatic psychological experience.[6,7] For almost a decade, basic scientists have known that the excitatory amino acid (EAA) receptor, N-methyl-D-aspartate (NMDA) plays a critical role in animal models of chronic pain. These studies have motivated clinical investigators to systematically evaluate the potential analgesic effects of several NMDA receptor antagonists in chronic pain patients.[8]

Ketamine is an anesthetic and a popular abusive drug. Ketamine has been used as a general anesthetic agent for over 30 years. In the 1980s, this dissociative anesthetic was discovered to have NMDA receptor antagonist properties. Ketamine got its start as an anesthesia medicine in the 1960s.[9] It was used on the battlefields of the Vietnam War. At lower doses, it can help ease pain. Ketamine helps sedatives work and may help people need fewer addictive painkillers, like morphine after surgery or while caring for burns. When misused, ketamine can change your sense of sight and sound. You can have hallucinations and feel out of touch with your surroundings -- and even from yourself. It can make it hard to speak or move, and it's been abused as a date-rape drug. Despite the relatively small sample sizes, lack of longer-term data on efficacy, and limited data on safety provided by these studies, they have led to increased use of ketamine as an off-label treatment for mood and other psychiatric disorders. Hence this study is aimed at investigating the effects of chronic administration of N-methyl-d-aspartate receptor antagonist (ketamine) on spatial learning and visual scene-based memory in rats.

MATERIALS AND METHODS

Experimental Animals

A total of twenty-five male wistar albino rats weighing 180-200g were obtained from animal house of the University of Port Harcourt. 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 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 (Normal control)	5	Feed + Water ad libitum
Group II	5	Feed + Water ad libitum + Ketamine (1mg/kg)
Group III	5	Feed + Water ad libitum + Ketamine (3mg/kg)
Group IV	5	Feed + Water ad libitum + Ketamine (6mg/kg)
Group V (Positive control)	5	Drug Epinephrine-treated (0.3ml/100g)

Experimental protocol 1 – Investigating the Effects of N-methyl-D-aspartate receptor antagonist (ketamine) spatial learning and visual scene-based memory in rats using Barnes-maze, passive –avoidance test and Navigational task in Wister rats.

Barnes maze – (Using the modified method of Barnes) it is a visual- spatial learning and memory task designed for rats.it consists of an elevated circular surface with holes around the edge.

Principles – it is a dry-land based rodent's 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 takes to locate the escape hole into the dark box beneath the maze should be 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 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 spined 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 fecaloid from the first rat.

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.

Principle – The testing apparatus is a trough-shaped alloy divided into two distinct compartments 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 0.1ml – 0.3ml ketamine was administered to the rat.
- 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 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 fecal solid from first rat.
- No aversive stimulus applied to animals upon re-entry into the dark compartment during testing.

Navigational Test – 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.

Principle – it is basically used to test mnemonic function in rats. These tasks are designed in such a way that the rats have 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 3 trials carried out on each of the days.
- After each trial, the apparatus was cleaned to remove residual smell and fecaloid from first rat.

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 23.

RESULT

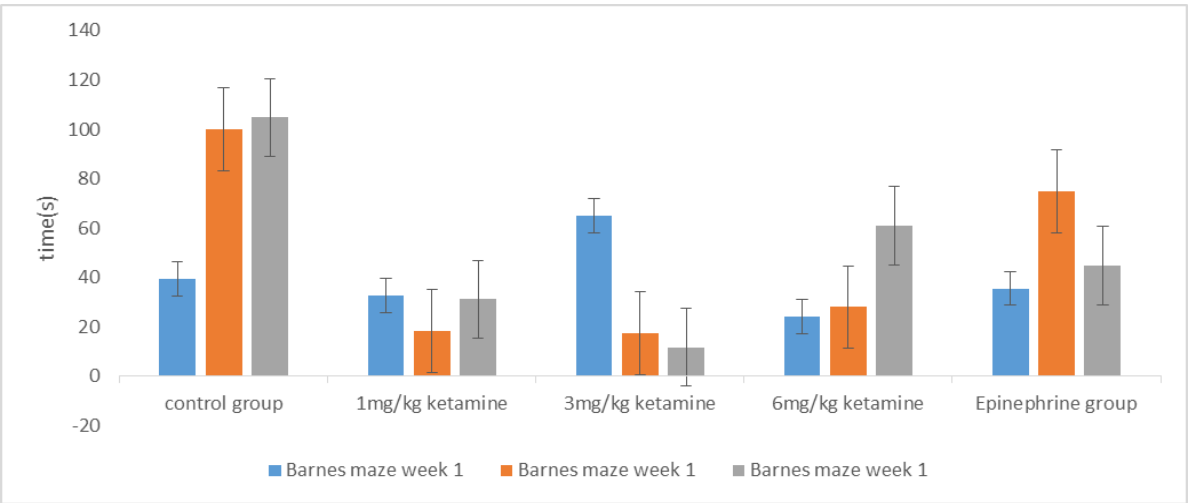

Figure 1: Pattern of visual scene-based memory test performance in the test and control groups in week 1.

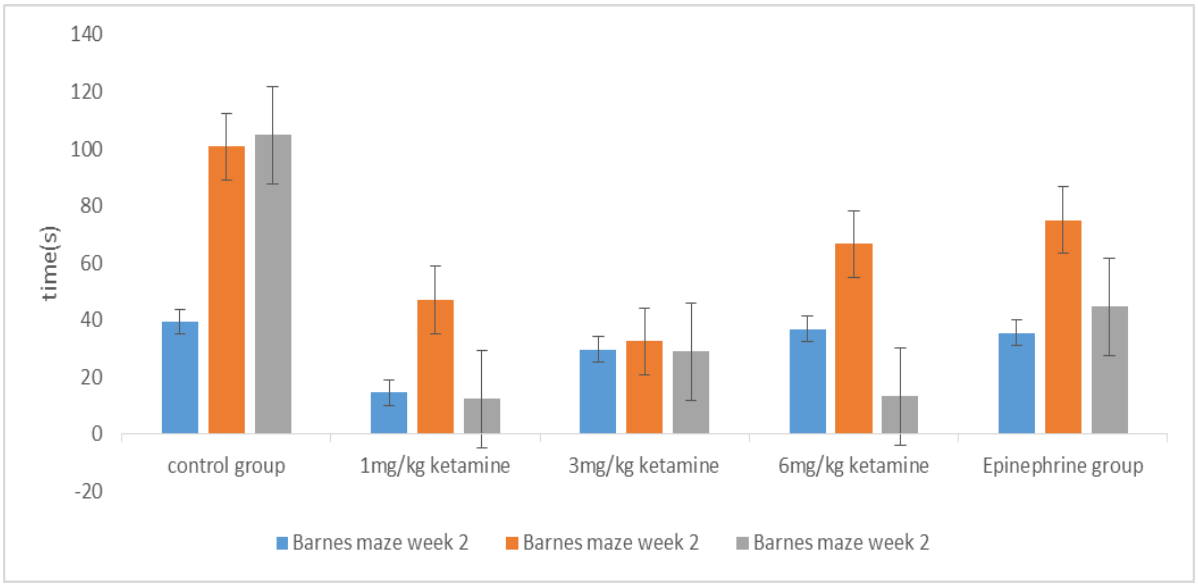

Figure 2: Pattern of visual scene-based memory test performance in the test and control groups in week 2.

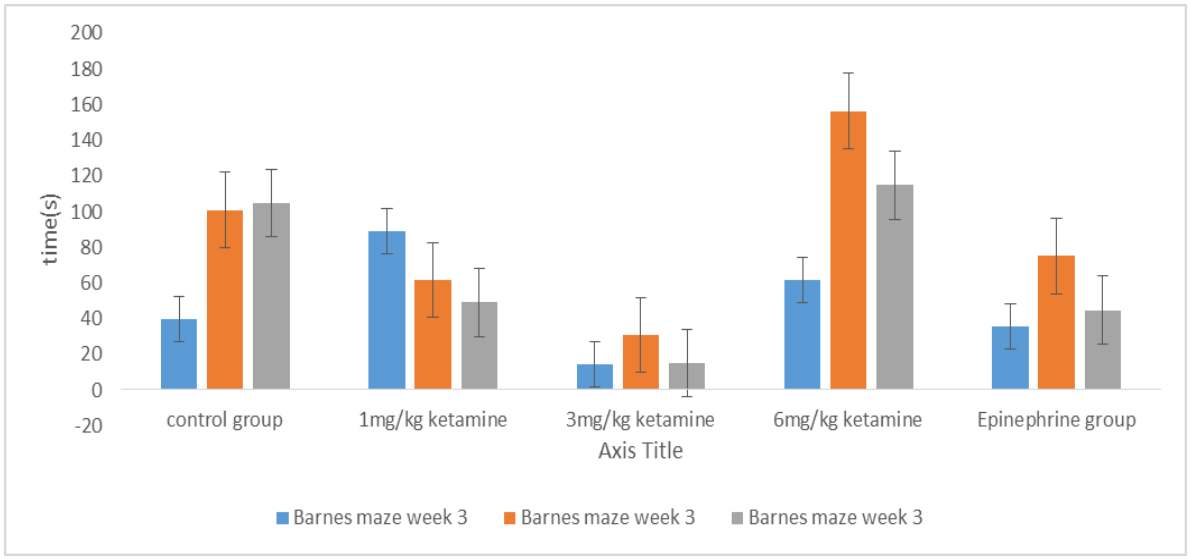

Figure 3: Pattern of visual scene-based memory test performance in the test and control groups in week 3.

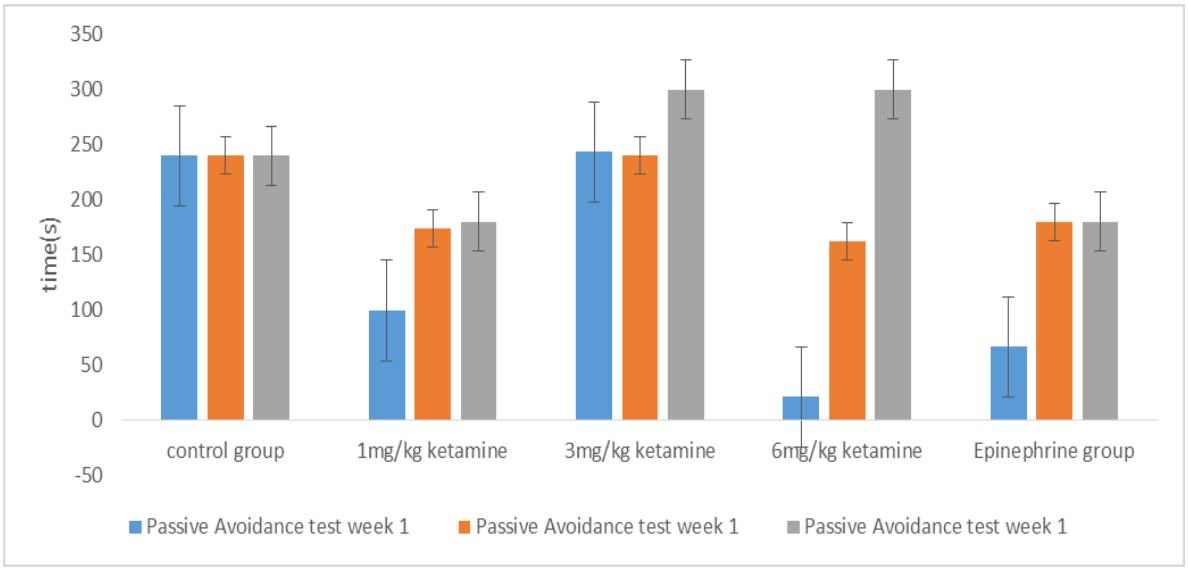

Figure 4: Pattern of Amnestic expression in the test and control groups using Passive Avoidance Test in Week 3.

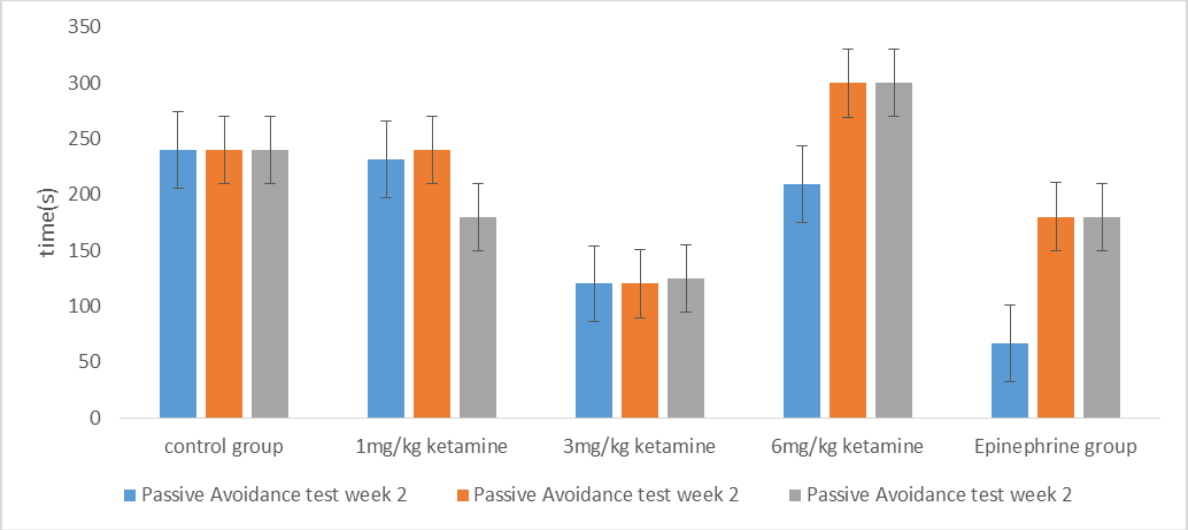

Figure 5: Pattern of Amnestic expression in the test and control groups using Passive Avoidance Test in Week 2.

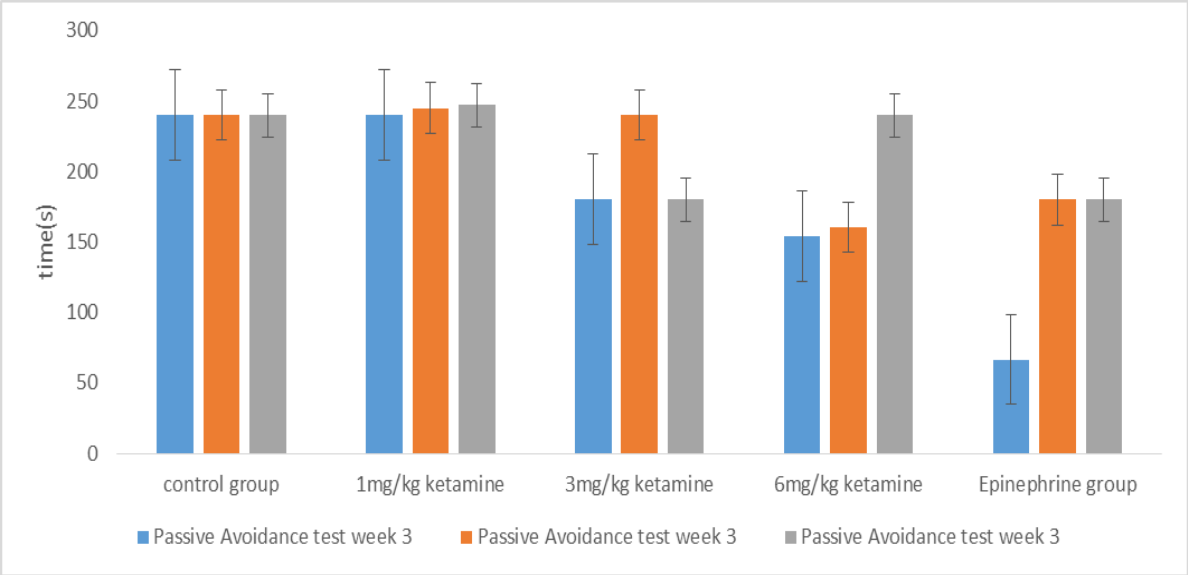

Figure 6: Pattern of Amnestic expression in the test and control groups using Passive Avoidance Test in Week 3

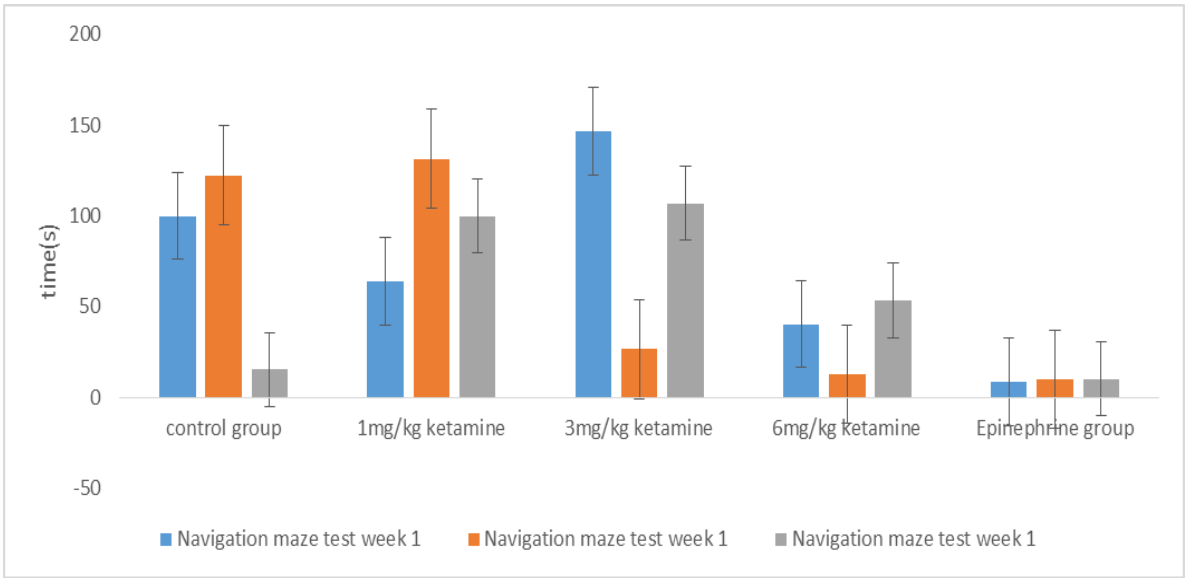

Figure 7: Pattern of adaptive locomotion exhibited in the test and control groups in Week 1

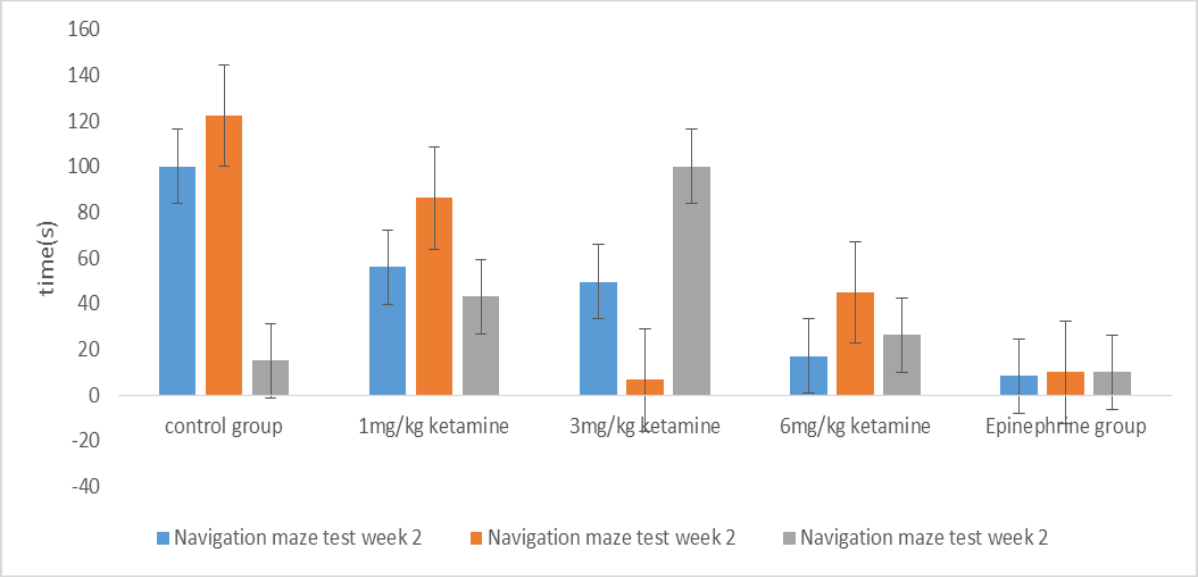

Figure 8: Pattern of adaptive locomotion exhibited in the test and control groups in Week 2.

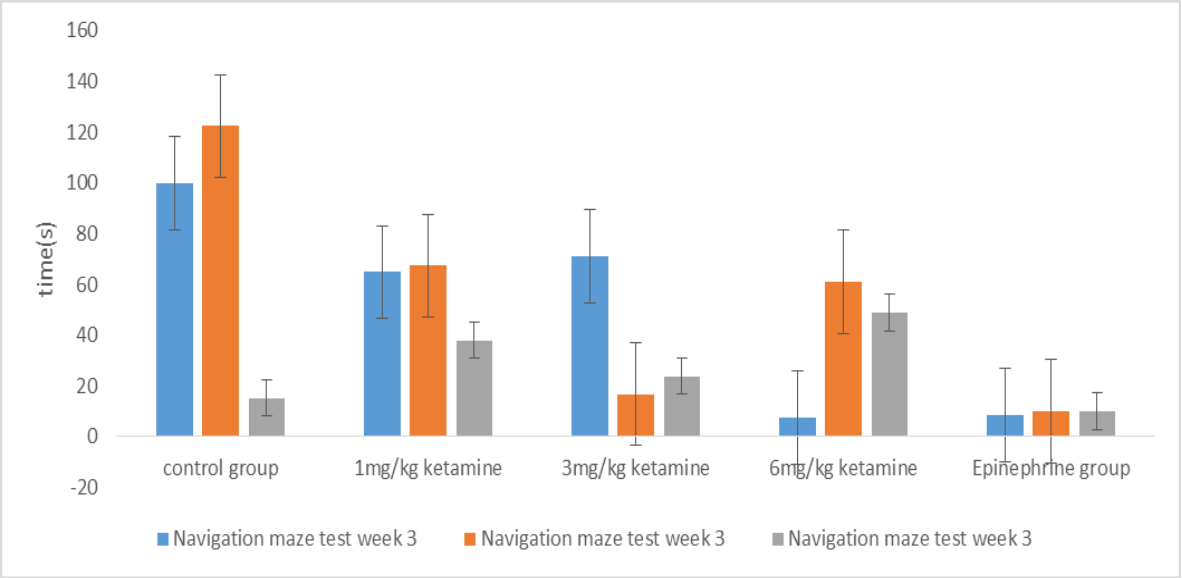

Figure 8: Pattern of adaptive locomotion exhibited in the test and control groups in Week 3.

DISCUSSION

Ketamine is an intravenous general anesthetic. It is clinically used as an anesthetic for surgery or anesthesia inducer. It has a certain mental dependence and impact on learning and memory.[10] Ketamine has an anesthetic effect at high doses (>80 mg/kg), but the drug can cause DNA damage in rodent models within 12h.[11] At low doses, it can simulate an analgesic effect. Therefore, ketamine can be used as an analgesic after surgery.[12] Considering that ketamine has hallucinogenic and addictive properties, it often appears in various entertainment venues and has become a substance that is increasingly.[12] This study was therefore, set up to investigate the effect of sub-chronic administration of N-methyl-D-aspartate receptor (ketamine) antagonist on spatial learning and visual scene-based memory in rats.

The Barnes maze (spatial memory) is a dry-land based behavioral test that has been shown to be sensitive to spatial learning related to neurodegeneration.[13] The result obtained from Barnes maze test in the study showed that in week 1, the time spent in in the performance of the visual scene-based memory task was distorted across the test groups when compared to the group treated with epinephrine and similar result was obtained in week 2. At week 3, a sharp decline and mental retardation and dullness was observed in group 4 (6mg/kg) indicating a dose- and time-dependent pattern of effect as presented in figures 1, 2, and 3. It appeared from the study that ketarnine induces inhibitory effects on spatial learning and memory via blocking the NMDA receptors, previous studies have reported that Ketamine abuse primarily affects brain tissue, causing mental problems such as anxiety, confusion, and memory loss.[26] Arc/Arg3.1 fosters the maturation of

hippocampal network activity necessary for learning and memory storage.[27] The destruction of Arc mRNA can affect the learning and memory function of hippocampus.[12] Other recent reports in similar manner equally reported that some anesthetics have been reported to inhibit the transcription of a large number of genes, including the Arc, thereby affecting memory consolidation.[28] In addition, Penrod RD's study confirmed that Arc might play a regulatory role in drug addiction.[29]

The passive avoidance test (memory recall) 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. Figures 4, 5, and 6 showed the result obtained in passive avoidance test and it showed that in week 1 ketamine administration was reported to cause retention in episodic memory from 1 to 6mg/kg in an increasing manner leading to memory consolidation. Step through latency was significantly declined. At week 2, the episodic memory enhancement was further expressed with pronounced pre-pulse inhibition and this observation was significant when compared to both control groups. Week 3 study results revealed that ketamine could inherently up regulate alertness with concentrated attention in the animals as it increased awareness of the aversive stimulus (electric grid in the dark compartment) thereby allowing the animal to spent more time in the awareness unlike in the negative control group that declined in their episodic awareness and retention potential.[14] However, these observations modify slightly that obtained by Shi *et al*. (2021) where it was recorded that injection of ketamine caused a decline in learning and memory. In other words, Assessments of the episodic memory study interestingly showed ketamine treatment across the 3-dose level up regulated memory retrieval and the experience was dose-dependent. Pain episode seemed to consolidate spatial learning aspect of memory.

Navigational maze test (cognitive activity) is a test for cognitive function for rats that rely on cues to navigate from start location to exit end through a labyrinth *[15*. Navigational maze is employed in behavioral neuroscience to study spatial behavior and could be a very precise study in 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 involved three trials for the total period of three weeks**.** Observations from Figures 7, 8, and 9 showed that in week 1, there were no significant effects on the tested rats the trial for the three weeks at 1mg/kg ketamine. However, slight effects were seen in trial 2 week 1. At 3mg/kg ketamine, tested animals in week 1 for all the trials showed no significant effect across the trials. The result obtained in week 2 also showed no significant effect from trial 1 to trial 3 coupled with week 3 where no significant changes was seen. However, slight performances in some of the treated rats were seen.

As observed in the study, ketamine administration seemed to occlude motor nerve terminals and down regulate neuronal activities at the final common paths. These pattern of activities were similar across the three weeks of study and became more significant with increasing dose administered. The general outcome of the result of Navigation test showed that ketamine did not produce a significant increase in motor learning activity compared to the control group as revealed. The result showed that ketamine, at all the doses used did not induce increase in the performance of motor behavior and no change was significantly obtained. This result does not align with that of Shi.[16] where it was observed that treated rat with ketamine increased significantly because the high body temperature caused changes in the learning and memory-related indicators of rats but observations from the adaptive locomotion study revealed that there was a significant decline ($p< 0.05$) in motor function especially as the treatment progressed and in a dose-dependent pattern. The quality of locomotion task performance also decreased as the dose of ketamine increased.

1. CONCLUSION

This study investigated the effect of sub-chronic administration of N-methyl-D-aspartate receptor (ketamine) antagonist on spatial learning and visual scene-based memory in rats. This present study concluded that the use of different doses of ketamine can cause significant alteration and certain modification in spatial behavior in animal. From the results obtained in the study, ketamine at these doses (1, 3, and 6mg/kg) could be favorable towards consolidation of episodic memory but could antagonistic to visual scene-based memory alongside decline in adaptive locomotion and motor coordination. In other words, it can be seen that the use of sub anesthetic dose of ketamine could lead to a substantial decline in learning and memory.

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