

## Original Research Article

### **Neurobehavioral effect of anterior, superior and lateral head injury on adult male Wistar rats**

#### **ABSTRACT**

Background: Head injury is a common occurrence among sports men and women, the military and paramilitary as well as in road accidents. Victims of head trauma in Nigeria usually do not receive proper medical attention. This is because once the patient recovers and is able to move, healing is assumed. Therefore, this study was carried out to investigate the neurobehavioral effects of traumatic head injuries on adult male Wistar rats.

Results: The result of the hanging wire test for motor function showed that animals in the control group could hang on their limbs for longer duration throughout the three tests. Animals in group B had a slight decrease in duration as the tests progressed. However, animals in groups C and D had a significant decrease in duration as the tests progressed. The result of the Morris water maze test for spatial learning showed that it took the rats in the control group less time to locate the escape platform compared to rats in the experimental groups. The Open field test for model of anxiety-like behaviour evaluated the animals' response level to centre freezing, line crossing, rearing, grooming, urination, faeces and freezing. The result of the open field tests showed that animals in the control group responded better to the tests than animals in the experimental groups.

Conclusions: Head injury resulted in weakness, poor memory performance, high level of fear and anxiety and higher tendency to be static (freezing). These tendencies became worse days after the injury. The study also noted that lateral head injury produced worse effects compared to superior and anterior head injuries.

**KEYWORDS**

Blunt injury, Brain injury, Hanging wire, Head trauma, Morris water maze, Open field, Traumatic brain injury.

UNDER PEER REVIEW

## **BACKGROUND**

Head injury is a broad term that describes a vast array of injuries that occur to the scalp, skull, brain, and underlying tissue and blood vessels in the head. Head injuries are also commonly referred to as acquired brain injury, brain injury, or traumatic brain injury (TBI), depending on the extent of the head trauma [1]. Head injuries are rising dramatically – about 1.7 million people have a TBI each year and it is more common in children, adults up to 24 years, and those older than 75 years [2]. Head injuries are one of the most common causes of disability and death in adults. The injury can be as mild as a bump, bruise (contusion), or cut on the head, or can be moderate to severe in nature due to a concussion, deep cut or open wound, fractured skull bone (s), or from internal bleeding and damage to the brain [1]. A TBI is caused by an excessive force, blow, or penetrating injury to the head. The CDC reported the 2013 rates for principal mechanisms of TBI-related injuries, as associated with Falls (47.2%), being struck by or against objects (15.4%), and motor vehicle crashes (13.7%) [3].

Head injury is common today among men and women in sports, armed forces, victims of domestic and criminal violence, and road traffic accidents [4-6]. This is even worse in underdeveloped and developing countries where infrastructure is poor and medical care is deficient. Varying degrees of head injury at one time or the other in an individual's life may affect the person's behavior and may lead to trauma and brain injuries of a permanent nature that predispose the individual to long term adverse effects. Head or brain injury causes substantial disability and mortality. It occurs when a sudden injury damages the brain and disrupts normal

brain function. TBI may have profound physical, psychological, cognitive, emotional, and social effects [7].

According to the US Centers for Disease Control and Prevention (CDC), there were approximately 2.8 million TBI-related emergency department visits, hospitalizations, and deaths in the United States in 2013. The vulnerability to TBI is far higher in developed countries although there is poor documentation [3]. Road traffic accidents from car crashes, motor bike accidents, fights, sports and environmental hazards are common. To make matters worse, as a result of poverty, many victims prefer self-medication or treatment from road side patent chemist shops except when there are fatalities [8]. Therefore, many cases of TBI go without proper diagnosis and medical treatment. The result is that many victims move around in the society with untreated TBI and the consequences of the injury. This predispose many to near madness attributes like easy and out of proportion anger, paranoia, wrong channeling of grievances, and poor motor coordination [9,10]. This in turn predisposes the society to further head injuries from accidents, gang fights, suicide falls, home accidents, etc.

Traumatic head injury can be primary or secondary. The primary injury is caused by mechanical force and occurs at the moment of injury. The two main mechanisms that cause primary injury are contact like an object striking the head or the brain striking the inside of the skull. Secondary injuries are not mechanically induced; it may be delayed from the moment of impact, and it may superimpose injury on a brain already affected by a mechanical injury [11].

This study was aimed at investigating the effects of lateral, anterior and superior traumatic head injuries on the neurobehavioral activities of adult male Wistar rats.

## **METHODS**

### **Study location**

This research was carried out at the Department of Anatomy, Nnamdi Azikiwe University, Nnewi campus, Anambra state, Nigeria.

### **Experimental animals**

A total of 48 adult male Wistar rats were used for this study. The animals were housed in a room with a 12-hour light / dark cycle with a temperature of  $25\pm 1^{\circ}\text{C}$ . They were given standard laboratory chow – growers mash from “Top feeds”, and water ad libitum.

### **Experimental procedures**

Animals with similar weights were assigned into four groups – A, B, C and D of 12 animals per group. Group A served as the control, whereas groups B, C and D served as the experimental groups. Animals in groups B, C and D were traumatized by hitting the lateral, superior and anterior aspects of the head with a blunt device respectively. The blunt device is a mechanical device, that has a round adjustable knob with a resting weight of 160g (measured on a digital weighing scale) falling from a height of 45cm inclined at an angle of 85 degrees. After trauma the rats were allowed to regain consciousness before they were transferred back to their cages. Twenty-four hours after the trauma (test 1) was impacted, all the rats (control and experimental groups) were weighed and then subjected to the three neurobehavioral tests – Morris water maze,

open field, and hanging wire tests. This was repeated 48 hours and 96 hours post trauma following a geometric sequence.

### **Hanging wire test**

The test procedure used for this study was adapted from Brandeis *et al.* [12] and was used to assess the muscle strength and balance. The procedure measured the limb hang time in seconds. Each rat was suspended with both forepaws on a horizontal steel wire 80 cm long, diameter 2 mm. When the rat grasped the wire, it was released, and the latency to fall was recorded with a stopwatch.

### **Morris water maze (MWM) test**

Morris water maze test was carried out according to the method reported by Gehring *et al.* [13] Rats were trained 3 times in the water bath of 120cm wide and 60cm deep. Water was used to fill the container up to the 50cm mark while an escape stage was placed at the center of the bowl, a little above the water level. The rats were first placed on the escape stage for 10 seconds after which they were pushed into the water to see how fast they will return to the safe platform. This was done three different times for each rat during the two weeks period of acclimatization. Each training phase lasted a maximum of 180 seconds (3 minutes). After the 3<sup>rd</sup> training phase the rats were exposed to a real experimental scenario where the escape stage was removed and the water made opaque using powdered milk. This was done to ascertain the time it took the rat to trace the escape stage back to its usual location as during the training.

### **Open field test**

This involves the use of square open field box measuring 128 x 128 x 60 cm high, with the base divided into 16 squares of 8cm each. The rats were initially placed at the center square and allowed uninterrupted locomotor activities for a maximum duration of 180 seconds (3 minutes). Each investigation was recorded using a digital camera suspended from the ceiling above according to the method described by D'Hooge and De Deyn [14]. At the end of the exposures, videos were analyzed and parameters extracted include center square duration, line crossing, rearing, grooming, urination, defecation and freezing.

### **Statistical analysis**

Data were curated and analyzed using Statistical Package for Social Sciences IBM series version 25 which were represented in tables. Independent sample t-test was used to compare the difference in the mean values of the results of the neurobehavioral tests between the control group and the experimental groups (lateral, anterior and superior head injuries). Values were considered significant at  $p \leq .05$ .

## **RESULTS**

### **Hanging wire neurobehavioral test**

The result of the hanging wire test for motor function showed that animals in the control group could hang on their limbs for longer duration throughout the three tests. Animals in group B had a slight decrease in duration as the tests progressed. However, animals in groups C and D had a significant decrease in duration as the tests progressed (Table 1).

The mean duration of completing the first hanging wire test by the control group was ( $\bar{x} = 1.02$ ,  $SD = 0.71$ ). Group B completed the first hanging wire test with a mean duration of ( $\bar{x} = 1.07$ ,  $SD = 0.83$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha > p = .451$ , indicating that there was a statistically insignificant difference in the duration of completion. Group C completed the first hanging wire test with a mean duration of ( $\bar{x} = 1.12$ ,  $SD = 1.08$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha > p = .427$ , indicating that there was a statistically insignificant difference in the duration of completion. Group D completed the first hanging wire test with a mean duration of ( $\bar{x} = 1.26$ ,  $SD = 0.89$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha > p = .312$ , indicating that there was a statistically insignificant difference in the duration of completion (Table 1).

The mean duration of completing the second hanging wire test by the control group was ( $\bar{x} = 1.67$ ,  $SD = 0.74$ ). Group B completed the second hanging wire test with a mean duration of ( $\bar{x} = 1.45$ ,  $SD = 1.25$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha > p = .360$ , indicating that there was a statistically insignificant difference in the duration of completion. Group C completed the second hanging wire test with a mean duration of ( $\bar{x} = 0.71$ ,  $SD = 0.66$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha < p = .019$ , indicating that there was a statistically significant difference in the duration of completion. Group D completed the second hanging wire test with a mean duration of ( $\bar{x} = 0.74$ ,  $SD = 0.60$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the

result showed that  $\alpha < p = .019$ , indicating that there was a statistically significant difference in the duration of completion (Table 1).

The mean duration of completing the third hanging wire test by the control group was ( $\bar{x} = 1.31$ ,  $SD = 0.72$ ). Group B completed the third hanging wire test with a mean duration of ( $\bar{x} = 0.47$ ,  $SD = 0.05$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha < p = .008$ , indicating that there was a statistically significant difference in the duration of completion. Group C completed the third hanging wire test with a mean duration of ( $\bar{x} = 0.32$ ,  $SD = 0.17$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha < p = .004$ , indicating that there was a statistically significant difference in the duration of completion. Group D completed the third hanging wire test with a mean duration of ( $\bar{x} = 0.28$ ,  $SD = 0.41$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha < p = .006$ , indicating that there was a statistically significant difference in the duration of completion (Table 1).

### **Morris water maze neurobehavioral test**

The result of the Morris water maze test for spatial learning showed that it took the rats in the control group less time to locate the escape platform compared to rats in the experimental groups. However, results of the subsequent tests showed that it took animals in the experimental groups a longer time to locate the escape platform when compared with the animals in the control group (Table 2).

The mean duration it took the control group to locate the escape platform was ( $\bar{x} = 0.63$ ,  $SD = 0.49$ ) in the first test. Group B located the escape platform during the first test with a mean duration of ( $\bar{x} = 0.59$ ,  $SD = 0.49$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha > p = .445$ , indicating that there was a statistically insignificant difference in the duration of locating the escape platform. Group C located the escape platform during the first test with a mean duration of ( $\bar{x} = 0.14$ ,  $SD = 0.08$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha < p = .018$ , indicating that there was a statistically significant difference in the duration of locating the escape platform. Group D located the escape platform during the first test with a mean duration of ( $\bar{x} = 0.18$ ,  $SD = 0.15$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha < p = .029$ , indicating that there was a statistically significant difference in the duration of locating the escape platform.

The mean duration it took the control group to locate the escape platform was ( $\bar{x} = 0.12$ ,  $SD = 0.10$ ) in the second test. Group B located the escape platform during the second test with a mean duration of ( $\bar{x} = 0.13$ ,  $SD = 0.08$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha > p = .488$ , indicating that there was a statistically insignificant difference in the duration of locating the escape platform. Group C located the escape platform during the second test with a mean duration of ( $\bar{x} = 1.12$ ,  $SD = 1.45$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha > p = .062$ , indicating that there was a statistically insignificant difference in the duration of locating the escape platform. Group D located the escape platform during the second test with a mean duration of ( $\bar{x} = 0.24$ ,  $SD = 0.07$ ); which was compared to the

control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha < p = .017$ , indicating that there was a statistically significant difference in the duration of locating the escape platform.

The mean duration it took the control group to locate the escape platform was ( $\bar{x} = 0.14$ ,  $SD = 0.15$ ) in the third test. Group B located the escape platform during the second test with a mean duration of ( $\bar{x} = 1.04$ ,  $SD = 0.04$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha > p = 3.873$ , indicating that there was a statistically insignificant difference in the duration of locating the escape platform. Group C located the escape platform during the second test with a mean duration of ( $\bar{x} = 2.06$ ,  $SD = 1.04$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha < p = .001$ , indicating that there was a statistically significant difference in the duration of locating the escape platform. Group D located the escape platform during the third test with a mean duration of ( $\bar{x} = 2.17$ ,  $SD = 0.91$ ); which was compared to the control group using an independent sample t-test ( $\alpha = .05$ ); and the result showed that  $\alpha < p = .001$ , indicating that there was a statistically significant difference in the duration of locating the escape platform.

### **Open field neurobehavioral test**

The Open field test for model of anxiety-like behavior evaluated the animals' response level to center freezing, line crossing, rearing, grooming, urination, feces and freezing. Animals in the control group had a significantly high center freezing time compared to the experimental groups during the three test periods. There was a statistically significant difference in duration of the

first center freezing test between the control group and experimental groups; a statistically significant difference in duration of the second center freezing test between the control group and groups B and D; and a statistically insignificant difference in duration of the third center freezing test between the control group and experimental groups (Table 3).

The first line crossing test showed that animals in groups C and D crossed more lines than animals in the control group and group B. The difference between the number of lines crossed between the control group and groups B and C was statistically significant; whereas the difference between the number of lines crossed between the control group and group D was statistically insignificant. The second line crossing test showed that animals in the control group and group B had similar number of lines crossed; whereas animals in groups C and D significantly crossed more lines than animals in groups B and A. The difference in the number of lines crossed in the second test was statistically insignificant between the control group and groups B and C; whereas it was statistically significant between the control group and group D. The third line crossing test showed that animals in the control group crossed more lines than the animals in all the experimental groups. The difference in the number of lines crossed in the third test was statistically significant between the control group and groups C and D; whereas it was statistically insignificant between the control group and group B (Table 4).

The first rearing test showed that animals in groups B and D showed less rearing after trauma compared to groups A and C. There was a statistically insignificant difference in the first test on rearing between the control group and experimental groups. Animals in the experimental groups showed less rearing compared to the control group during the second test. The difference in

rearing of the second test between the control group and groups C and D was statistically insignificant; whereas it was statistically significant between the control group and group B. The third test showed that animals in all the groups had similar rearing except group B which had lesser rearing. The difference in rearing of the third test between the control group and groups C and D was statistically insignificant; whereas it was statistically significant between the control group and group B (Table 5).

Animals in the experimental groups groomed more than animals in the control group during the first test; and there was a statistically significant difference in the grooming frequency between the control group and groups C and D. However, the difference in the first grooming test between the control group and group B was statistically insignificant. Animals in the experimental groups groomed more than animals in the control group during the second test; and the difference in grooming frequency was statistically insignificant. Animals in the control group groomed more than animals in group C but had similar grooming frequency with animals in group B and lesser grooming frequency than animals in group D in the third grooming test. The difference in the third grooming test between the control group and groups C and D was statistically significant; whereas it was statistically insignificant between the control group and group B (Table 6).

Animals in the control group produced lesser urine than animals in groups B and D, and also produced more urine than animals in group C during the first test; and the difference in the urine production between the control group and experimental groups was statistically insignificant. In the second test, animals in the control group produced more urine than animals in groups B and

D, and produced lesser urine than animals in group C; and the difference in urine production between the control group and experimental groups was statistically insignificant. In the third test, animals in the control group produced lesser urine than animals in the experimental groups. The difference in the third urination test between the control group and groups B and D was statistically insignificant; whereas it was statistically significant between the control group and group C (Table 7).

In the first feces test, animals in the control group produced lesser feces than animals in groups B and C, but had similar feces production rate with animals in group D; and the difference in the feces production between the control group and experimental groups was statistically insignificant. In the second test, animals in the control group produced lesser feces than animals in groups B and D, but produced more feces than animals in group C. The difference in the second feces test between the control group and groups C and D was statistically insignificant; whereas it was statistically significant between the control group and group B. In the third test, animals in the control group produced more feces than animals in the experimental groups. The difference in the third feces test between the control group and groups C and D was statistically insignificant; whereas it was statistically significant between the control group and group B (Table 8).

Animals in the control group had a lower freezing time compared to the experimental groups during the three test periods. The difference in the first freezing test between the control group and groups B and D was statistically significant; whereas it was statistically insignificant between the control group and group C. The difference in the second freezing test between the

control group and groups B and D was statistically insignificant; whereas it was statistically significant between the control group and group C. The difference in the first freezing test between the control group and groups C and D was statistically insignificant; whereas it was statistically significant between the control group and group B (Table 9).

## **DISCUSSION**

The result of the hanging wire test shows that rats in the control group could hang on their forelimbs for increasingly longer duration throughout the three tests. The same was observed in group B rats with lateral head injury. However, groups C and D rats had a steady decline in the duration of time on the hanging wire even with increasing exposure. This is in line with the report of Feng *et al.* [15] that reported short and long-term motor deficits following traumatic brain injury. Same was reported by Yan *et al.* [16].

The result of the Morris water maze test for memory shows that it took rats in the control group lesser time to identify the escape stage with progression of the experiment. After training, the animals learnt to locate the stage at an increasingly shorter time as the period of exposure progresses from day 1 to days 3 and 7. The result was however contrary for rats in the test groups which showed increasing time spent to identify the escape stage across all the experimental groups B, C and D which had lateral, superior and anterior head trauma. But we see that the figures were higher for group B exposed to lateral head trauma than the other groups. All the rats exposed to head trauma across the three exposure groups B, C and D showed decline in time spent at the center square upon placement in the open field chamber after trauma compared to before trauma (initial).

The open field results reveal lots of neurobehavioral responses from the rats. Shorter freezing time at the center square is indicative of more locomotion and exploration tendencies and by implication less anxiety [17]. The decline in this measure indicates fear and agitation and animals resorted to thigmotaxis as acclimatization to fear [16]. The number of line crosses and the frequency of rearing are both measures of locomotor activity, exploration and anxiety. A high frequency of these behaviors indicates increased locomotion and exploration and/or a lower level of anxiety. Rats in the control group A had increased line crossing and rearing with decreased grooming. This implies increased locomotion, exploration and low level of anxiety. The same is not true of rats exposed to trauma. Rats in groups B and D had decreased line crossing and rearing but increased grooming. Kinder *et al.* [18] reported decrease in exploratory activity in open field test after traumatic brain injury. This clearly indicates high level of anxiety but low explorative and locomotor activity compared to the control animals. Some reports state that anxiety or emotional stress can impair spatial learning and memory [15,19,20]. Severe cognitive impairments in young children in the acute phase after TBI have been found to worsen rather than recover over time, suggesting that acute cognitive impairments may be a sensitive predictor of long-term outcomes [21]. Animals in group C are a little exception with reduced rearing but increased grooming and line crossing. Increased grooming and line crossing show increased anxiety and locomotion. Grooming behavior is a displacement response and is expected to be displayed in a novel environment [22]. Therefore, grooming behavior should decrease with repeated exposure to the testing apparatus. All the animals in the experimental groups showed increased grooming when compared to their control counterparts.

## CONCLUSIONS

Traumatic head injury resulted in weakness, poor memory performance, high level of fear and anxiety and higher tendency to be static (freezing) in rats with reference to their performance before the impact of the head trauma. These tendencies worsened as the days post trauma increased. We also observed that traumatic lateral head injury produced worse effects compared to superior and anterior traumatic head injuries in virtually all tested variables. We also observed that the impact of head trauma does not just go away by mere passing of time.

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## TABLES

Table 1: Results of the Hanging wire test

Group	Test 1 (Mean $\pm$ SD)	<i>P</i> -value	Test 2 (Mean $\pm$ SD)	<i>P</i> -value	Test 3 (Mean $\pm$ SD)	<i>P</i> -value
A	1.02 $\pm$ 0.71		1.67 $\pm$ 0.74		1.31 $\pm$ 0.72	
B	1.08 $\pm$ 0.83	0.451	1.45 $\pm$ 1.25	0.360	0.47 $\pm$ 0.05**	0.008
C	1.12 $\pm$ 1.08	0.427	0.70 $\pm$ 0.66*	0.019	0.32 $\pm$ 0.17**	0.004
D	1.26 $\pm$ 0.89	0.312	0.74 $\pm$ 0.60*	0.019	0.28 $\pm$ 0.01**	0.006

\*. t-test is significant at the .05 level.

\*\* . t-test is significant at the .01 level.

Table 2: Results of the Morris water maze test

Group	Test 1 (Mean $\pm$ SD)	<i>P</i> -value	Test 2 (Mean $\pm$ SD)	<i>P</i> -value	Test 3 (Mean $\pm$ SD)	<i>P</i> -value
A	0.63 $\pm$ 0.49		0.12 $\pm$ 0.10		0.14 $\pm$ 0.15	
B	0.59 $\pm$ 0.49	0.445	0.13 $\pm$ 0.08	0.488	1.04 $\pm$ 0.04	3.873
C	0.14 $\pm$ 0.08*	0.018	1.12 $\pm$ 1.45	0.062	2.06 $\pm$ 1.03**	0.001
D	0.18 $\pm$ 0.15*	0.029	0.24 $\pm$ 0.07*	0.017	2.17 $\pm$ 0.91**	0.001

\*. t-test is significant at the .05 level.

\*\*.. t-test is significant at the .01 level.

Table 3: Result of the Open field test – Centre Freezing

Group	Test 1 (Mean ± SD)	<i>P</i> -value	Test 2 (Mean ± SD)	<i>P</i> -value	Test 3 (Mean ± SD)	<i>P</i> -value
A	25.33±13.88		16.50±5.46		17.83±5.49	
B	4.17±2.48**	0.002	9.17±5.04*	0.018	2.50±0.55	2.357
C	2.00±0.89**	0.001	1.33±0.52	2.478	1.50±0.55	1.382
D	2.67±0.82**	0.001	3.33±3.61**	0.001	1.67±0.52	1.501

\*. t-test is significant at the .05 level.

\*\*.. t-test is significant at the .01 level.

Table 4: Result of the Open field test – Line Crossing

Group	Test 1 (Mean ± SD)	<i>P</i> -value	Test 2 (Mean ± SD)	<i>P</i> -value	Test 3 (Mean ± SD)	<i>P</i> -value
A	27.17±15.47		22.50±9.93		58.67±6.65	
B	14.50±7.01*	0.049	21.33±13.68	0.434	15.5±1.64	1.333
C	42.83±12.61*	0.042	33.83±15.30	2.478	43.00±9.51**	0.004
D	36.0±21.48	0.216	50.17±10.21**	0.001	33.67±12.40**	0.001

\*. t-test is significant at the .05 level.

\*\*.. t-test is significant at the .01 level.

Table 5: Result of the Open field test – Rearing

Group	Test 1 (Mean ± SD)	<i>P</i> -value	Test 2 (Mean ± SD)	<i>P</i> -value	Test 3 (Mean ± SD)	<i>P</i> -value
A	5.17±4.71		9.50±5.65		6.50±3.51	
B	2.83±3.66	0.180	1.83±2.56**	0.006	3.50±0.55*	0.033
C	5.17±4.45	0.500	5.83±2.32	0.086	6.17±0.75	0.412
D	4.33±3.44	0.367	5.83±1.17	0.075	6.50±0.55	0.500

\*. t-test is significant at the .05 level.

\*\*.. t-test is significant at the .01 level.

Table 6: Result of the Open field test – Grooming

Group	Test 1 (Mean ± SD)	<i>P</i> -value	Test 2 (Mean ± SD)	<i>P</i> -value	Test 3 (Mean ± SD)	<i>P</i> -value
A	2.00±0		2.67±3.08		3.00±0.89	
B	2.67±1.21	0.104	3.17±0.75	0.353	3.00±1.10	0.500
C	2.67±0.52**	0.005	3.50±1.38	0.279	2.17±0.41*	0.032
D	2.83±0.98*	0.032	4.00±1.55	0.182	4.50±1.64*	0.039

\*. t-test is significant at the .05 level.

\*\*.. t-test is significant at the .01 level.

Table 7: Result of the Open field test – Urination

Group	Test 1 (Mean ± SD)	<i>P</i> -value	Test 2 (Mean ± SD)	<i>P</i> -value	Test 3 (Mean ± SD)	<i>P</i> -value
A	0.83±1.17		1.50±1.05		0.50±0.55	
B	1.17±0.98	0.302	1.33±1.63	0.419	0.67±0.52	0.500
C	0.50±0.55	0.271	2.00±1.90	0.292	3.50±1.64**	0.001
D	1.33±1.51	0.267	0.83±1.17	0.161	2.17±0.41	6.819

\*. t-test is significant at the .05 level.

\*\*.. t-test is significant at the .01 level.

Table 8: Result of the Open field test – Feces

Group	Test 1 (Mean ± SD)	<i>P</i> -value	Test 2 (Mean ± SD)	<i>P</i> -value	Test 3 (Mean ± SD)	<i>P</i> -value
A	1.67±2.33		1.50±1.05		3.00±2.61	
B	2.67±2.07	0.225	3.83±1.94*	0.013	0.50±0.55*	0.022
C	3.17±2.56	0.157	1.00±0.63	0.170	1.67±0.52	0.124
D	1.67±1.86	0.500	3.67±2.88	0.057	1.83±0.98	0.165

\*. t-test is significant at the .05 level.

\*\*. t-test is significant at the .01 level.

Table 9: Result of the Open field test – Freezing

Group	Test 1 (Mean ± SD)	<i>P</i> -value	Test 2 (Mean ± SD)	<i>P</i> -value	Test 3 (Mean ± SD)	<i>P</i> -value
A	31.83±9.81		20.17±16.96		37.50±21.60	
B	127.33±54.54**	0.001	92.67±25.66	8.962	71.33±28.52*	0.022
C	52.17±43.53	0.145	68.17±53.11*	0.031	36.50±16.98	0.465
D	69.83±50.01*	0.049	30.17±10.25	0.122	36.50±4.93	0.457

\*. t-test is significant at the .05 level.

\*\* . t-test is significant at the .01 level.