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Original Article

Orthopedic trauma or surgery does not effect on cognitive or behavioral capability of rats

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Abstract

Aim: The aim of this study is to investigate the probable effects of orthopedic trauma/surgery on anxiety, memory and learning capability in rats.

Material and Methods: Twenty-one Wistar albino rats grouped into three as control, sham operated, and tibia fracture. In control group, any surgery or fracture was done whereas in sham group the tibia of rats was fixed with intramedullar Kischner wire. In tibia fracture group, a closed fracture was occurred with Bonnarens and Einhorn's guillotine device then intramedullar Kischner wire sends to tibia. All rats were evaluated for memory, anxiety, and learning via behavioral tests respectively; water-maze test, elevated plus arm test and shuttle box test.

Results: One month after the fractures were done and the bone healing was seen on X-ray at all rats. According to the behavioral tests results, there was not any statistically difference between groups in terms of anxiety, learning capability, locomotor activity and memory ($p>0.05$).

Conclusion: Our results showed that, the orthopedic trauma or surgery itself does not affect the anxiety, learning capability, locomotor activity and memory in rats.

Keywords: Anxiety; elevated plus arm test; learning; memory; orthopedic surgery; orthopedic trauma; shuttle box test; water-maze test

INTRODUCTION

Post-operative cognitive dysfunction (POCD) is great concern for clinicians. In a multicenter trial, POCD was shown to be present in 25.8% of patients 1 week after surgery and in 9.9% of patients 3 months after surgery in patients older than 60 years (1). It is known to diminish the quality of the patient's life and increase the costs for hospitalization (2-4). Orthopedic surgeries and traumas are known to be a risk factor for reduction in memory, mental flexibility and learning capability (5). The exact mechanism for this circumstance remain unclear but aseptic trauma in surgery which activates bone marrow derived macrophages release proinflammatory cytokines which impairs long term potentiation in hippocampus, promoting inflammation and apoptosis of nerve cells and also causing disruption in blood brain barrier is thought to be related with cognitive decline (6).

In several studies the effect of POCD after orthopedic surgeries or traumas on memory, learning capability were investigated separately (7,8). Though Starr et. al. emphasizes that the trauma or orthopedic surgery itself is not influential on developing of anxiety, Moraes et al. emphasizes that trauma and prosthetic orthopedic surgery patients are prone to anxiety. This difference of these two studies may be related to the varies depending on the personality structure (7,8). In this perspective we aimed to investigate the probable effect of orthopedic trauma and surgery on anxiety, memory and learning capability in rats.

MATERIAL and METHODS

Preparation of animals

All experimental and animal care procedures were performed in accordance with the Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care (CCAC) (9) and the Animal Care and Local Ethics Committee of Gaziosmanpasa University (2016-HADYEK-25) approved the experimental protocols and procedures. 21 male Wistar albino rats 3 months old

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were used in experiments and were fed with standard rat chow and water ad libitum. The rats were kept in a room with temperature of 22 +/- 2 oC, and 55-60% humidity at 12 hours dark and 12 hours light cycles. Prior to the experimentation, all rats were handled for 20 minutes for 1 week by the same researchers to eliminate possible behavior biases during the experiments. At all experimental period, the same researchers were interested with rats. Elevated plus arm test and water maze test was recorded by a video tracking system (ANY-maze version 4.82). At the evaluation step of the recorded videos, investigator was blinded to the name of groups.

Experimental protocol

To investigate the effects of orthopedic surgery and tibia fracture on learning capability, memory, anxiety and locomotor activity rats were randomly divided into 3 groups namely; control (n: 7), sham operated (n: 7) and tibia fracture (n: 7).

All rats were given same medications during the experiment. They anesthetized with intraperitoneal administration of ketamine (50 mg/kg) and xylazine (5 mg/kg). Cefazolin 20 mg/kg was given for antibiotic prophylaxis. Analgesia was provided by buprenorphine (0.05 mg/kg; subcutaneously) and paracetamol (7.5 mg/kg; subcutaneously) for 2 weeks due to decrease in the probable pain sensation. Additionally 7.5 mg/kg paracetamol ad libitum was given in tap water for 2 weeks more. Right rear extremity was wiped with povidone iodine and an incision was done to the tuberosity tibia. In addition to those applied to the control group, after povidone iodine wipening, the incision was made on the tuberosity tibia in the sham operated group. Subsequent to incision, one 0.9 mm Kischner wire was sent intramedullary from tuberositas tibia and the incision was sutured.

In fracture/surgery group, a 0.9 mm Kischner wire was sent intramedullary from tuberositas tibia and the incision was sutured thereafter, a closed fracture was created with Bonnarens and Einhorn's guillotine device at the tibia. The fracture was assessed by the examination of the rotational instability.

Briefly, the only difference of sham operated group from control group is surgery. The only difference of fracture group from sham operated group is tibia fracture. Thus, we could able to compare the effect of orthopedic surgery and fracture separately.

Behaivoral Tests

Elevated plus arm test

The elevated plus arm test assesses the conflict between animals' natural tendencies to explore new environments and avoid vulnerable open spaces (10,11). Rats feel safe in the closed area due to their innate ingenuity. Being in an open area triggers anxiety. The purpose of this test is to assess anxiety. In this test, behavioral assessments indicating anxiety are; the extension of time on the closed arm, the increase of immobility, the decrease for line crossing, and the decrease of the time spent at open arm, the decrease of the number of access to open arm (12).

The device we used in this test consists of 4 arms those are 10 cm wide, 60 cm length, 60 cm high and perpendicular to each other. The two arms in the same direction are open and the other 2 arms are closed. The 3 walls of each of the 2 closed arms are 50 cm high (Figure 1a). On the fourth side, facing the center of these 2 closed arms, there is no wall due to allow rats to cross between the arms. In this system, the rats can freely go and return to the arm they want from the junction of the 4 arms. The open arms are called as ''zone'' in this test. In the Elevated plus arm test, the rats were left in the field with 4 arms intersected, with their faces facing open arms. Each rat was followed for a total of 5 minutes at elevated plus arm test. If the rat fell down in the course during the experiment, the video recording was automatically stopped and the rats

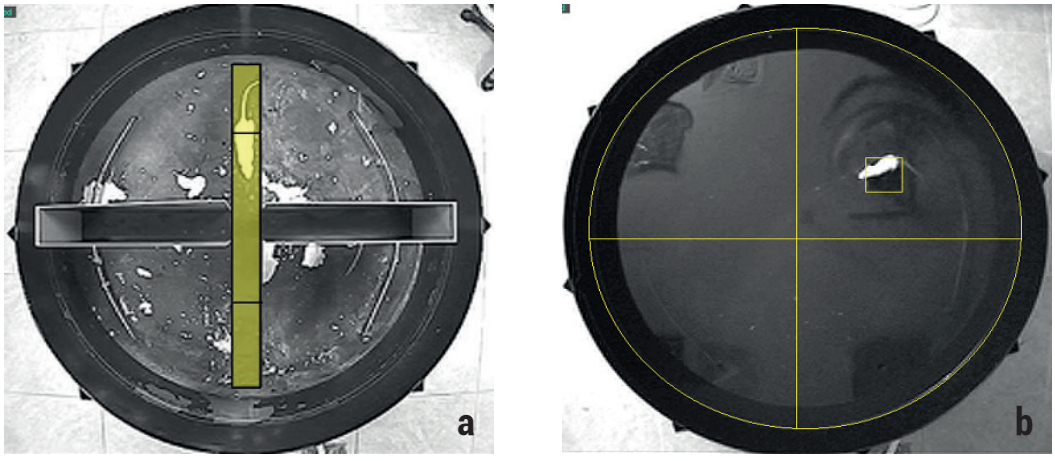

Figure 1. (a) The Elevated Plus Arm test. The colored area is called open arm. The transvers lines at the open arms are called ''line cross''. (b): The water maze test. The two imaginary lines cut each other perpendicularly at the center and makes 4 imaginary quarters. The small square specifies the escape platform that placed just below the water level. The imaginary quarter that escape platform is present in is called as zone

were put back into the same point where they fell and experiment continued from where they left down. In the computer program that the evaluation was made, a line was drawn perpendicularly to each arm imaginatively. This line is called line cross. Line cross divides the arms into two as the central and peripheral sides. Each transition of this line by the rats is called line crossing. The greater the number of line crossings, the less anxiety of the rat is accepted. Line crossing also specifies the locomotion of the rats.

Evaluation of locomotor activity

The parameters used to evaluate locomotor activity are; the total distance that rats have traveled (distance), the average speed of rats (mean speed), the total time that rats are mobile (mobile time), the total time that rats are immobile (immobile time), the number of zone entries, and the number of the line crossing.

Evaluation of anxiety

The parameters used to assess anxiety in rats are; the total time that rats are moving (mobile time), the total time that rats are immobile (immobile time), the number of zone entries, the number of the line crossing, the total time spent at zone (zone time total), the time that rats are mobile (Mobile zone time) and the time that rats are immobile (Immobile zone time).

Water maze test

Water maze test measures memory with spatial learning. In order to evaluate memory, rats firstly learned the location of the escape platform via initial 4 day by swimming trainings (13) (Figure 1b). Thus, 1 month after the applications were made to the groups, the escape platform was removed and probe trial test (fifth swimming test) was made to evaluate memory.

Water maze test protocol and rats' learning the place of escape platform

A black colored pool, 140 cm in diameter, 70 cm high was filled with 50 cm high fresh water.

The water temperature was set at 22-24 degrees. In order to provide the orientation of the rats in the space, white colored environmental hint marks were placed on each wall separately. Inside the maze, an escape platform with a height adjustable, 10 x 10 cm area was used in the same quarter. The quarter that the escape platform is inside named as ''zone''.

The maze is divided into 4 equal quarter circles in the east, west, north, south directions with 2 imaginary lines which perpendicularly cut each other at the center (Figure 1b). The escape platform was used to allow the rats to stand in the water. This escape platform always took place in the same quarter circle (zone) during the first 4 days of swimming training.

At the first 4 days, all rats had swimming training between 0900 and 1300 to learn the place of escape platform according to the protocol described previously (14). Each rat swim for five consecutive times for 60 seconds per each day. In each swimming training, rats were released from the other 3 quarters where the platform was not found. The rats were allowed to swim in the maze by rats are facing the center. They were kept on the platform for 30 seconds to recognize and learn environmental hints. After the swimming trainings at first 4 day, rats learned the location of the platform. During swimming training, each rat that came out of the pool after each swimming was dried with paper towels and placed in cages.

Probe trial test: Evaluation of locomotor activity - Evaluation of memory

On the fifth day of the Morris Water maze test, the swimming test is called as probe trial test. Memory and locomotor activity were evaluated in the probe trial test. The rats were left with their faces facing the center of the pool from the very opposite quarter. The swims were followed for a total of 30 seconds. Distance, mean speed and zone entries were used for assessment of locomotor activity. Zone distance, zone time were used for assessment of memory.

Shuttle box test- Evaluation of learning capability

Shuttle box, 40x40x80 cm in size, consists of 2 rooms

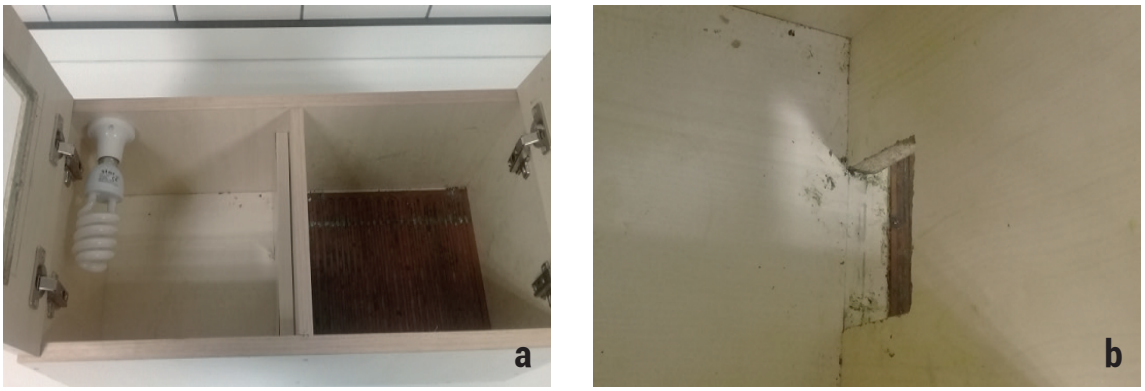

Figure 2. Shuttle box test. The two different room is present in this test (a). The room with a lamp is called bright room. The other room has electric shock at the floor is called dark room. The open room has a window at ceiling to observe the rats if they change the room. There is a door that allows rats to cross between the bright and dark rooms (b)

(Figure 2) (15). Between the two rooms, there is a wall. There is a gate on this wall that allows the passage of rats between rooms easily when they want. This gate is closed under the control of the investigator, thanks to a guillotine door that is on the wall. This gate is closed under the control of a guillotine door and a researcher. One of the rooms has a lamp of 100 watts. This room was called the bright room. The other room is completely dark and there is a 0.5 millimeter mechanism for electric shock at the floor. This room is called the dark room. Both the bright and dark rooms have lids on the ceilings for easy access to the rats. In the lid on the roof of the bright room there is a glass window for the researcher to observe the rat. Rats have higher anxiety in the bright environment and less in the dark environment. Therefore, rats instinctively tend to move into the dark room. The Shuttle box test utilizes the ability of rats to have anxiety in a bright environment. Bright room retention times are used to assess how strong the learning of the rats is.

The Shuttle box test was performed as previously described (15). In the shuttle box test, the day before the test procedure started, all the rats were placed in the shuttle box for twenty minutes each, for the purpose of recognizing the environment. On the first day of the procedure, the rats were left in the bright room section of the shuttle box. Rats were monitored by the glass windows on the ceiling of the bright room. Rats are instinctively passed to the dark room with the discomfort of the light. As soon as the rats passed through the dark room, the gate was closed with the guillotine door. The rats were exposed to 0.5 milliamps of electrical shock for 5 seconds in the dark room. The rats learned that they would be exposed to electrical shock if they enter the dark room at the end of the first day.

After 24 hours, on the second day of the test, learning was assessed. The rats were left in the bright room. Rats were followed for 5 minutes via a glass window on the ceiling of the bright room. The chronometer was run when the rats were left in the bright room. Because the rats knew about the risk of electric shock, they did not want to go to the dark room. The rats were expected to forget the risk of electric shock with the discomfort given by the high light and to pass the dark room. As soon as the rats passed from the bright room to the dark room, the chronometer stopped. If the rats were still in the dark room after 5 minutes, the test was terminated. The time spent by the rats in the bright room was used to assess the learning of the rats.

After all tests were completed, all rats were sacrificed by high-dose ketamine/xylazine (100/100 mg/kg) anesthesia and exsanguinations method. Tibias were dissected and x-rays taken.

Statistical Analyses

Statistical analyses were performed using R Statistical Software (www.r-project.org) a free software environment for statistical computing and graphics and SPSS 15.0. Baseline characteristics of the groups were presented as mean, standard deviation (SD) or median and interquartile range (IQR) with minimum and maximum values for quantitative variables where necessary. The normality of each variable within each group (control, sham, fracture) was tested using Kolmogorov Smirnov test. The variance heterogeneity was tested using Levene's test. The continuous variables (distance, mean speed, mobile time, immobile time for elevated plus arm experiment; distance, mean speed, zone distance, zone time for water maze experiment), which satisfied both the normality and variance homogeneity assumptions, were tested using one-way ANOVA, for other continuous variables (total zone time, mobile zone time, immobile zone time for elevated plus arm experiment; duration for box experiment) Kruskal Wallis H test was used. The negative binomial regression model was used to compare groups with respect to count variables (line crossing, zone entries) $p<0.05$ was considered statistically significant.

RESULTS

For the elevated plus arm experiment, the variance homogeneity assumption was satisfied for all continuous variables however the normality assumption was not satisfied for total zone time, mobile zone time, immobile zone time. The variables, which showed normality, were presented with mean and standard deviation and tested with parametric tests and others were presented with medians and IQR and tested with nonparametric tests in Table 1. The one-way ANOVA model was fit for distance, mean speed, mobile time/immobile time. The models were not significant with $F(2,18)=0.474$, $p=0.630$; $F(2,18)=0.508$, $p=0.610$; $F(2,18)=0.829$, $p=0.453$ respectively. Thus, we can conclude that the groups did not show any significant difference in terms of distance, mean speed, mobile time and immobile time (Figure 3). The negative binomial regression model was fit for the number of zone entries and line crossing. The goodness of fit test for zone entries revealed a chi square value of 9.7 with degrees of freedom of 18 and for line crossing, the test revealed a chi square value of 12 with degrees of freedom of 18. The tests were not significant with p values of 0.941 and 0.825 and respectively, which revealed that the fitted models were suitable. The negative binomial models for line crossing and zone entries were not significant with $\chi 2(2)=0.49$ ($p=0.782$) and $\chi 2(2)=0.03$ ($p=0.987$) respectively meaning the groups did not show significant difference with respect to neither zone entries nor line crossing. The Kruskal-Wallis H test revealed test statistics of $\chi 2(2)=1.46$ ($p=0.483$), $\chi 2(2)=1.81$ ($p=0.405$) and $\chi 2(2)=1.68$ ($p=0.432$) for zonetime total, mobile zonetime and immobile zone time respectively. The insignificant test results revealed that the groups did not differ significantly with respect to zonetime total, mobile zonetime and immobile zone (Table 1).

For the water maze experiment, Distance, mean speed, zone distance and zonetime satisfied the assumptions of both normality and variance homogeneity, thus one-way ANOVA was employed to compare groups with respect to these

variables (Figure 4). Time mobile and time immobile were constant in sham group, thus they could not be analyzed.

Zone entries being a count variable was analyzed using negative binomial regression. The goodness of fit test for zone entries revealed a chi square value of 4 with degrees of freedom of 18 (p=0.999). The negative binomial models for zone entries were not significant with $\chi 2(2)=0.073$(p=0.964) meaning the groups did not show significant difference with respect to zone entries (Table 2). The Shuttle Box experiment yielded the result that the groups were not significantly different in terms of duration spent in the dark room ($\chi 2(2)=0.07$; p=0.964) (Table 3).

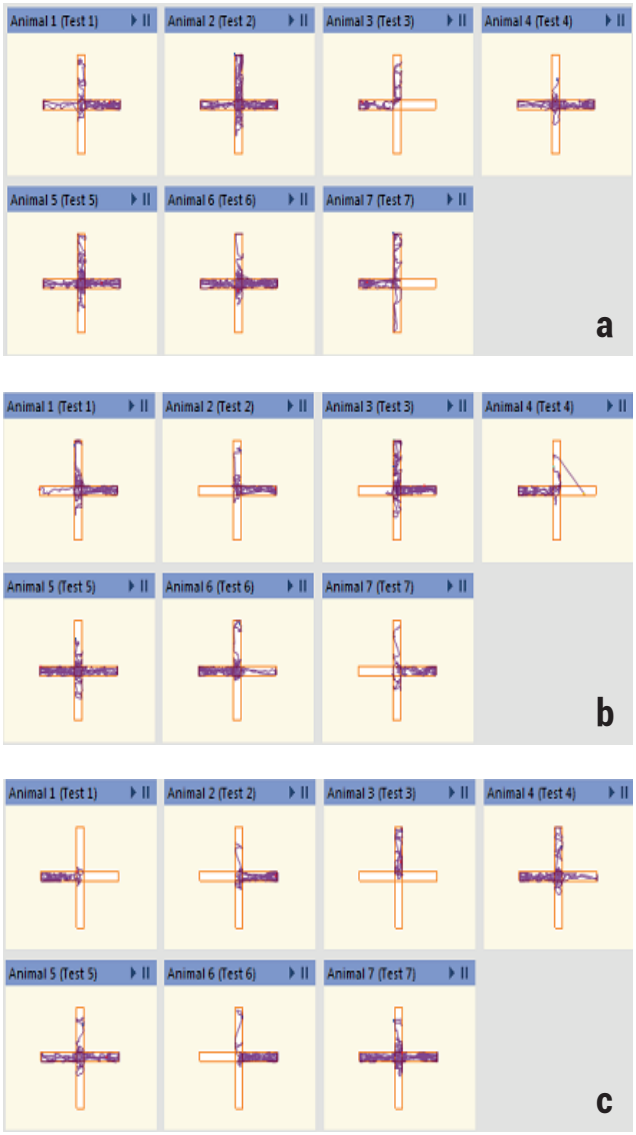

Figure 3. The results of elevated plus arm test in terms of the distance covered by using the video tracking system. The perpendicular arm is open arm, and the horizontal arm is the closed arm. The control group (a), the sham operated group (b), the fracture group (c)

Ray findings

At X-Rays we saw all tibias were healed completely. We did not see malunion, non union or infection at X-Rays. The most important finding on xrays between the sham operated and tibia fracture groups was the alignment difference of bending of Kischner wires those sent intramedullary. The Kischner wires were straight at fracture group and the Kischner wires were slightly bended at the sham operated group according to lateral tibia Xrays. But according to tibia ap xray views all Kischner wires were straight (Figure 5).

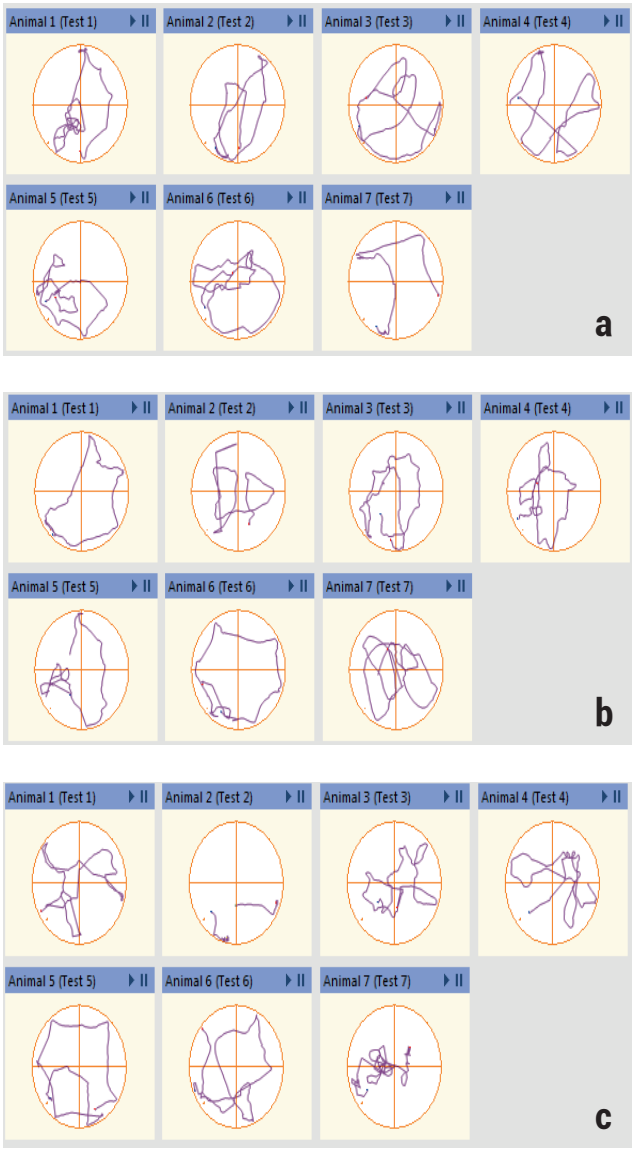

Figure 4: The results of Water maze test in terms of the distance covered by using the video tracking system. The control group (a), the sham operated group (b), the fracture group (c)

Interpretation of locomotor activity

Locomotor activity was evaluated via both the elevated plus arm and water maze tests. The distance, mean speed, mobile time, immobile time, the number of zone entries, and the number of the line crossing were statistically insignificant in terms of locomotor activity at elevated plus arm test between 3 groups (p>0.05) (Table 1). Distance, mean speed and zone entries were statistically insignificant in terms of locomotor activity at water maze

Table 1. Elevated Plus Arm experiment results

Variables	Control		Sham operated		Fracture		Test stat.	p
	Mean(SD)	Min;max	Mean(SD)	Min;max	Mean(SD)	Min;max		
Distance	7.00(3.42)	2.51;13.41	5.84(2.30)	2.73;9.87	5.40(3.70)	1.04;12.44	F(2,18)=0.474	0.630
Mean speed	0.02(0.01)	0.01;0.04	0.19(0.01)	0.01;0.03	0.02(0.01)	0.003;0.04	F(2,18)=0.508	0.610
Mobile time	78.43(42,63)	24;144.8	57.61(18.49)	32.3; 88.6	60.84(31.95)	15.8;118.4	F(2,18)=0.829	0.453
Immobile time	221.57(42.62)	155.2;276	242.39(18.49)	211.4; 267.7	239.16(31.97)	181.6;284.2	F(2,18)=0.828	0.453
	Median(IQR)	Min; max	Median(IQR)	Min;max	Median(IQR)	Min;max		
Line crossing	10(17)	4;30	12(16)	6;24	4(13)	1;36	$\chi 2(2)=0.49$	0.782
Zone entries	4(7)	1;9	3(5)	1;9	2(5)	1;13	$\chi 2(2)=0.03$	0.987
Zone time total	80.7(66.9)	22.1;145.7	52.6(76.7)	19.2;105.9	34.9(51.4)	12.9;283.8	$\chi 2(2)=1.46$	0.483
Mobile zone time	26.7(17.3)	10.2;64.8	11.5(12.9)	8.4;28.7	10.7(10.3)	4.4;30.7	$\chi 2(2)=1.81$	0.405
Immobile zone time	52.9(60.8)	11.4;87.4	41.7(57.3)	7.7;83.7	17.1(42.2)	8.4;273.1	$\chi 2(2)=1.68$	0.432

Table 2. Morris Water Maze experiment results

Variables	Control		Sham operated		Fracture		Test stat.	p
	Mean(SD)	Min;max	Mean(SD)	Min;max	Mean(SD)	Min;max		
Distance	5.48(1.11)	3.46;6.74	6.14(2.12)	3.94;10	5.29(2.01)	1.11;7.61	F(2,18)=0.430	0.657
Mean speed	0.183(0.04)	0.12;0.23	0.205(0.07)	0.13;0.33	0.177(0.07)	0.04;0.254	F(2,18)=0.428	0.658
Zone distance	1.09(0.48)	0.19;1.65	0.96(0.49)	0.53;1.65	1.02(0.65)	0; 1.78	F(2,18)=0.131	0.878
Zone time	5.80(3.60)	1;10.8	5.14(4)	2.9;10.3	5.67(4.8)	0; 10.4	F(2,18)=0.09	0.915
	Median (IQR)	Min;max	Median (IQR)	Min;max	Median (IQR)			
Zone entries	2(1)	1; 3	1(2)	1; 4	2(2)	0; 3	$\chi 2(2)=0.073$	0.964

Table 3. Shuttle Box experiment results

Variables	Control		Sham operated		Fracture		Test stat.	p
	Median (IQR)	Min;max	Median (IQR)	Min;max	Median (IQR)	Min;max		
Duration	14(288)	3; 300	112(290)	3; 300	103(295)	3; 300	$\chi 2(2)=0.07$	0.964

test between 3 groups (p>0.05) (Table 2). There was not any statistically difference between 3 groups in terms of locomotor activity.

Interpretation of anxiety

Mobile time, immobile time, the number of zone entries, the number of the line crossing, zone time total, mobile zone time and immobile zone time were statistically insignificant in terms of anxiety at elevated plus arm test between 3 groups (p>0.05) (Table 1). There was not any statistically difference between 3 groups in terms of anxiety.

Interpretation of memory

Zone distance, zone time were statistically insignificant in terms of memory at water maze test between 3 groups (p>0.05) (Table 2). There was not any statistically difference between 3 groups in terms of memory.

Interpretation of learning

The time spent by the rats in the bright room was statistically insignificant in terms of learning at shuttle box test between 3 groups (p>0.05) (Table 3). There was not any statistically difference between 3 groups in terms of learning.

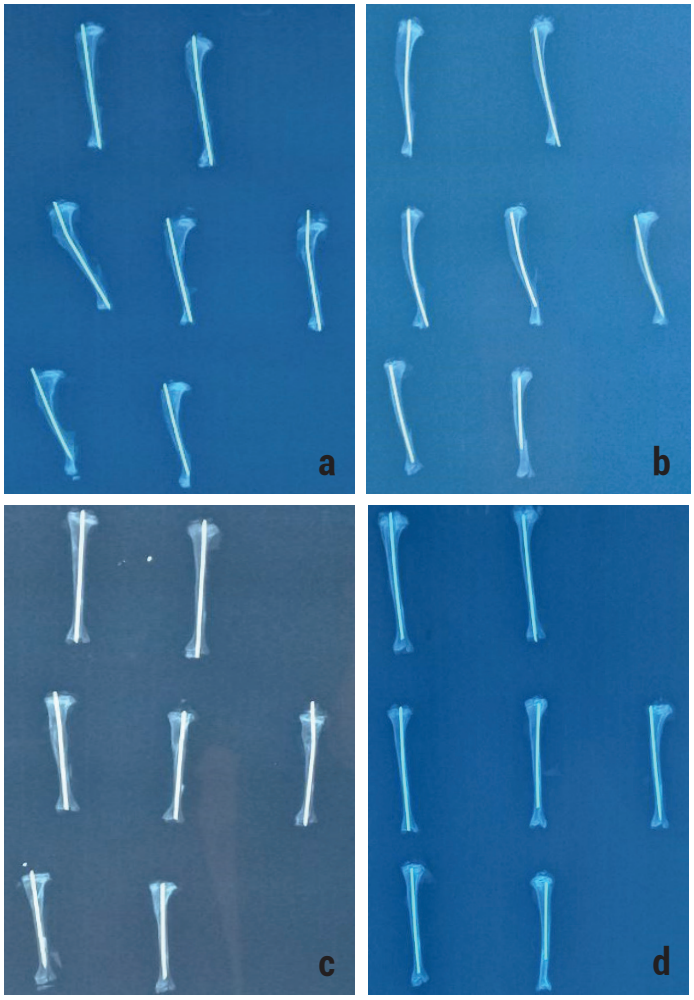

Figure 5. The x-ray views of tibias. The lateral tibia X-ray of; fracture group (a), sham operated group (b), the anteroposterior Xray of; fracture group (c), sham operated group (d). Please note the bending of the Kischner wires at sham operated group. But the other views show a straight Kischner wire alignment

DISCUSSION

In this study we aimed to investigate the effect of orthopedic trauma and surgery on anxiety, memory and learning capability in rats. According to our literature review until now, at orthopedic trauma patients, anxiety was studied only clinically previously, but memory and learning were not studied previously neither clinically nor experimentally. To the best of our knowledge, this is the first study in literature that investigates the effect of isolated orthopedic trauma or orthopedic surgery on behavioral manners as anxiety, memory and learning via animal experiment. Current experimental animal study revealed that isolated orthopedic trauma or orthopedic surgery does not affect the anxiety, memory and learning statistically (Table 1,2,3). These results lead us to give more importance to other predictors except orthopedic trauma and orthopedic surgery itself, in terms of anxiety and anxiety related diseases.

Anxiety is among the predictors of poor long-term quality of life (16). In clinic studies the relation between orthopedic trauma and mental disorders are investigated and trauma has often been shown as a potential cause of anxiety, and anxiety related disorders such as depression, acute stress syndrome, and posttraumatic stress syndrome. (4,17-21). The rate of anxiety related diseases has shown with a wide range 2% to 51% among adult orthopedic trauma patients. (2,7,22-25). Studies looking at the potential predictors to anxiety and anxiety related diseases after physical trauma have shown considerable variations owing to inconsistencies in methodological factors and inhomogeneous selection of patient groups, such as sex, age, culture, the severity of trauma, long-lasting trauma, additional traumas, education levels, locations, and psychosocial variables, socioeconomic variables, social support, pre-trauma mental health, geography, surgeon–patient communication, personality traits, usage of alcohol or drugs, absence of control group (2,4,17,20,22,26-32). The effect of traumatic brain injuries on mental disorders at orthopedic trauma patients is controversial, some authors found the increase and some decrease at mental disorders (3,17). Though the traumas and the applied treatments are same, patients' outcomes of life are variable (31). Older patients may have problems admitting and expressing their feelings (17). In addition, some patients may give limited or incomplete information because of the fear of becoming stigmatized with a psychological diagnosis in the social environment. (17). In the case of injuries covered by insurance, such as traffic accidents, patients' abuse of secondary earnings may affect the results of studies too. Such concerns are the controversial issues of clinic studies. Nevertheless, animals does not affected from these variables. When we interpret in terms of these concerns high lightened above of this paragraph, we claim the groups in current study are more standardized than clinical studies. In current study the age, sex, severity of traumas, surgeries, medicines, handling of rats, the tests were all standard and same. The only differences of the groups of current study are the orthopedic surgery and tibia fractures those we are investigating. Therefore, we claim current study is superior to clinic studies in terms of evaluating the subjective criteria as anxiety, memory, and learning.

Moraes et al found the rate of anxiety 45.7% at orthopedic trauma patients (8). Moraes et al associated this high anxiety rate with comorbidity of patients (8). However, the presence of comorbidity, which is different for each patient, is an important variable that affects anxiety in orthopedic trauma patients. They also stated that surgical stress was an important factor (8). In our study, there was no difference between the comorbidities of the experimental animals and the sham operated group was used to evaluate the surgical stress. Giannoudis et al found that the presence of anxiety was related to the severity of injury in the lower extremity (33). However, in our study the severity of trauma was standard thanks to the Bonnarens and Einhorn's guillotine device.

Other findings of current study are about memory and learning. In current experimental study, we put forth the

results of memory and learning due to behavioral tests, probably in the first time at literature. There was not any statistically difference between traumas, sham operated and control groups in terms of memory and learning. It is shown that the education about the rehabilitation and healing process prior to surgery reduces the anxiety levels (16,34). This shows the memory of patients' education prior to surgery about healing process improves the recovery. Wong et. al.'s study strengthens our findings in terms of the memory does not change with the isolated orthopedic trauma (34). In our review of clinic studies carried out with isolated orthopedic traumatology patients, we could not able to find any result about the memory and learning of orthopedic trauma patients. Probably this is because of not to having pre-trauma learning and memory knowledge of patients or because of not to able to carry out control groups. The absence of statistically difference between groups in terms of memory and learning may be related to 1) using anesthetic during performing the tibia fracture, 2) complete recovery of the fractures, 3) experimental animals' not having anxiety as shown above. According to our opinion, the amnesia effect of anesthetic drugs is one of the most important of these possibilities. It is known that, if the patient has head injury, patient forgets and does not remember the events at the time of the trauma (35,36). Moreover, in clinic studies head injury is highlighted why the orthopedic trauma patients with head injury have lower anxiety (17). Head injury with following amnesia can be protective against the development of mental disorders (35-37). This interpret at literature is very important in our study. Because the amnesia due to anesthetic application, that have protective effect against the mental disorders, may contribute to our groups for not having differences in terms of anxiety, memory and learning. We think this finding strengthens our findings orthopedic traumas and orthopedic surgeries itself doing not affect the results physically.

In addition, the lateral tibia xray finding of bending of Kischner wire at sham-operated group while the Kischner wire at fracture group is straight is firstly emphasized in current study. The probable cause of bending of the wire at sham-operated group is that the wire takes the shape of the intact tibia at sham group. However, at fracture group, fractured tibia takes the straight alignment shape of Kischner wire.

The limitations of current study are 1) not being a clinical study, 2) to use anesthetic drug, 3) to evaluate 1-month results, 4) not to evaluate the brain histopathological or the cytokines biochemically. There is a need for further studies with specific patient groups with large participants.

CONCLUSION

In conclusion ısolated orthopedic trauma or orthopedic surgery does not affect the anxiety, memory, and learning at rats. Our results support the clinical study by Starr et. al. (7). So as Star et. al. stated in the conclusion, the mental disorders are not related with the trauma itself, to know etiology would help physician to achieve an optimal recovery after orthopedic injury and clinicians must address both physical and psychological needs of their patients (38). Current experimental study shows us the orthopedic trauma and orthopedic surgery itself does not affect the mental health. Although reasons of mental disorders are not present at patients, mental illnesses can be observed due to the personality traits of the patients (17,31). So isolated orthopedic trauma patients should be examined to determine which patients are prone to mental disorders. By achieving this, individuals who are prone to mental illnesses can be consulted to psychiatry in orthopedic treatment process for a more successful treatment.

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