



## Can gestational exercise have a positive effect on cognitive functions resulting from brain injury? A rat study

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### ABSTRACT

**Aim:** The effects of gestational exercise on potential pathological conditions is not known yet. Therefore, in the present study, it was aimed to evaluate the effects of forced running exercise on lipopolysaccharide (LPS)-induced brain damage in pregnant rats.

**Method:** Pregnant females in the experimental group were forced to exercise 30 minutes daily for five days a week. Lipopolysaccharide (LPS)-induced brain injury model was created by administering 500 µg/kg body weight of LPS on gestational days 18 and 19. To evaluate injury histopathologically, brain tissues were fixed at the seventh postnatal day through transcardial perfusion (n=4 pups/group). When the remaining pups reached 30-day of age, Morris water maze test (MWM) was performed to assess memory and learning, open field (OP) and elevated plus maze (EPM) for testing anxiety, and Porsolt test (PT) for evaluating depression. The groups were defined as brain injury group (BI, n=13) and exercise+brain injury group (E+BI, n=7).

**Results:** The results obtained from MWM test indicated that animals found the platform in a shorter duration and distance at the day five compared to the day three. However, there was no significant difference between the groups. No significant difference was found in OP test regarding the distance traveled, time spent at the margins, movement at the center and the time spent as immobile. However, in the EPM test, the offspring at the BI group displayed higher mobility and increased number of entry to the open arms compared to the E+BI groups (p=0.01). There was no significant difference regarding mobility duration and total distance traveled in the PT test.

**Conclusion:** The results of the EPM test suggests that the gestational exercise can suppress the stress factors in the pregnant females with brain injury leading to the prevention of hyperactivity-induced negative learning behavior.

**Keywords:** Gestational exercise; cognitive functions; brain injury, neonatal, rat model; offspring.

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## **Introduction**

The benefits of gestational exercise on both mother and child health have been shown previously in both clinical and experimental studies. The aerobics and resistive exercises performed at the second trimester has been known to increase endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) levels and reduce free radicals in the placenta [1]. Recent studies have shown that swimming exercises during pregnancy improves future memory capacities, increases hippocampal neurogenesis and neutrophilic factors in offspring [2]. Likewise, it has also been shown that gestational exercise increase the antioxidant levels and mitochondrial mass in fetal brain [3,4]. Despite all these physiological effects, the impacts of gestational exercise on potential pathologies are not known. Therefore, in the present study, the effects of gestational forced running on lipopolysaccharide (LPS)-induced brain damage was evaluated.

## **Methods**

All the experimental procedures were approved by Abant Izzet Baysal University Institutional Animal Care and Use Committee. All the experimental procedures were carried out in Abant Izzet Baysal University Animal Research Laboratory as well as Alper Karakaş Research Laboratory of Faculty of Arts and Sciences. The food and water was provided ad libitum to all female Wistar rats. Animals were kept in standard cages with 12 h light: 12 h dark cycle, 50-60% of humidity and room temperature.

Three naive female rats, who were not pregnant before, were selected for running exercise. The females were acclimated to the exercise one week prior to the onset of the experiment. Moreover, three females were left in the standard cages. The vaginal smear was performed to detect the estrus day and experienced male rats were placed in the cages. The day of male cages introduced into the females were considered as Day 0 of pregnancy. The experimental group was forced to exercise either 3 meter/minutes for 5 minutes duration, 5 meter/minutes for 5 minutes duration or 8 meter/min for 20 min duration. Experimental groups were forced to exercise total of 30 minutes for 5 days in a week [5]. As it was shown previously in the literature, all female rats were administered 500 µg/kg body weight of LPS (*Escherichia coli*, serotype 055:B5, Sigma) intraperitoneally (i.p.) at the gestational day 18<sup>th</sup> and 19<sup>th</sup> [6]. In order to confirm the damage, the brain tissues of offspring at the postnatal day 7 (n=4 per group) were fixed through transcardiac perfusion with 4% paraformaldehyde under ether anesthesia. The brain-body proportions were determined and brain tissues were examined using hematoxylin-eosin staining. Following histological examination, experimental procedures were started. Morris water maze for memory and learning, open field test and elevated plus maze test for anxiety, porsolt test for depression were performed in offspring. The experimental procedures were recorded using Noldus Etho Vision software. The data was analyzed using SPSS version 18.0.

## **Learning and Memory – Morris Water Tank**

A 10 cm diameter of platform were placed in a pool, which has a diameter of 180 cm and

depth of 60 cm and the interior walls painted to black. The water temperature of the pool was  $28 \pm 1$  °C and some clues were placed in the pool helping the animals in order to find the platform. The colored paintings were hanged to the walls of the experimental room, where the animal can see them from water. Morris water maze were divided into 4 sections as north, south, east and west. For the first 3 days, the learning exercises were performed for 4 times a day and 10 min each time. At the day 4, no exercise were performed in order for animals to forget it. At the day 5, the time duration that the animals reach the platform, the speed, and the distance traveled were recorded [7].

### **Open Field Test**

The animals were placed into the center of the plexiglas cages, which has a dimension of 100x100x40 and divided into 16 equal squares. The time spent at the center and margins, number of times that they pass the line (both at the center and margins), total distance travelled and their mobility and speed were recorded for a total of 5 min experimental duration.

### **Elevated Plus Maze Test**

A maze having two open and two closed arms and 50 cm above the ground were set up in order to induce anxiety behavior. Closed arms were covered with 40 cm height walls. Open arms were only covered with 1 cm height plexiglas. Animals placed to the center and the number and time spent at the open and closed arms, mobility, and speed of animals were recorded for a duration of 5 min. The anxiety response were evaluated.

### **Prosolt**

An apparatus of 30 cm diameter and 50 cm height were filled with water at 28 °C. An

animal placed directly in water and the experimental parameters were recorded for 5 min duration including, the mobility and immobility time as well as the total amount of distance traveled. The depression response of animals were evaluated.

## **Results**

The histological evaluation of brain tissues in order to confirm the injury revealed an cytoplasmic shrinkage and presence of rodental bodies in brain injury (BI) and exercise + brain injury (E+BI) groups (Figure 1).

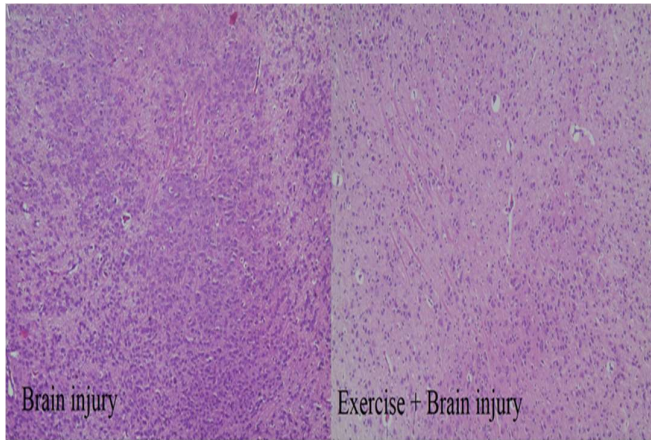
The brain/body proportion were not significantly different between the two groups (Figure 2). The offspring at one month of age were performed for behavioral tests (n= 13 and 7, respectively for BI and E+BI).

### ***Morris Water Maze Test***

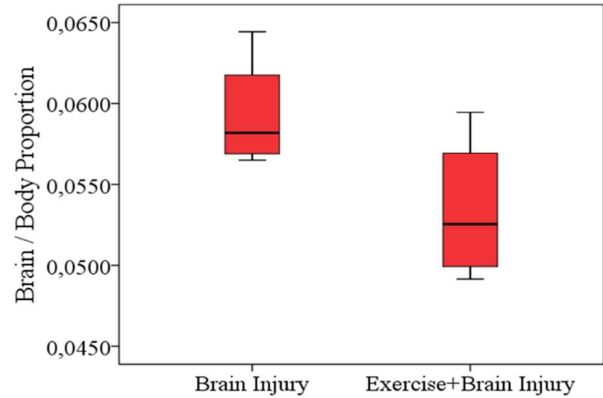
Morris water maze test were performed to evaluate the spatial memory. The animals were acclimated to the experiment for the first three days, then at the day four they were rested. The experiments were performed at day five. The platform was found by animals with less amount of distance traveled and in a shorter period of time at the day five compared to day three. However, the results were similar to each other (Figure 3).

### ***Open Field Test***

The total distance traveled, the time spent at the center and the margins, the mobility and immobility parameters at the center were similar between the two groups. The results of the open field tests demonstrated that in the LPS induced brain injury model, gestational exercise did not have any impact for the anxiety disorder (Table 1).

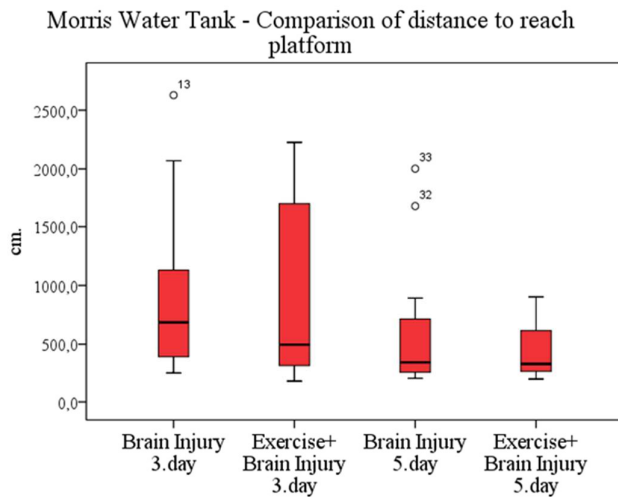


**Fig. 1.** The histological evaluation of brain tissues (H&E, x40). In brain injury (BI) and exercise + brain injury (E+BI) groups, an cytoplasmic shrinkage and presence of rodent bodies.

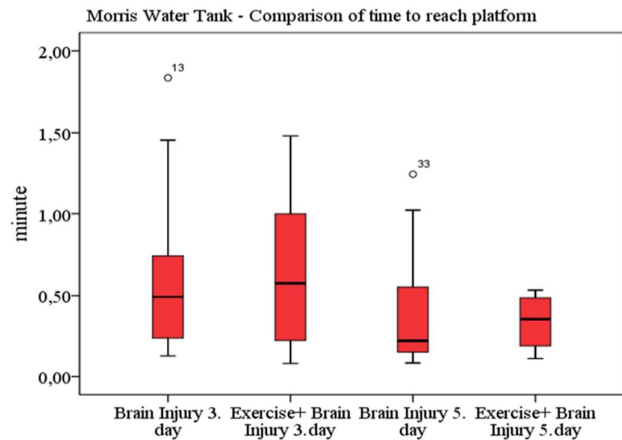


Levene's Test  $p=0.54$  / Student t test  $p=0.08$

**Fig 2.** The brain/body proportion evaluation. There was no significant difference between the two groups.



Mann-Whitney U test 3.day  $p=0.72$  / 5.day  $p=0.66$



Mann-Whitney U test 3.day  $p=0.96$  / 5.day  $p=0.96$

**Fig. 3.** Morris water maze test to evaluate the spatial memory. The results were similar to each other.

**Elevated Plus Maze Test**

The amount of time spent at the closed arms were reduced in the brain injury groups. However, the other parameters tested were not significantly different between the two groups. The offspring at the BI group demonstrated increased mobility and increased frequency of

open arm entries compared to the E+BI groups ( $P=0.01$ ) (Table 2).

**Prosolt Test**

Prosolt test was performed to evaluate depression. The results did not reveal any significant difference between the two groups



in terms of mobility time and the total amount of distance traveled (Figure 4).

### Discussion

It has been thought that the gestational exercise might have neuroprotective function in periventricular leukomalacia (PVL) through its antioxidant and anti-inflammatory activities that could positively impact neuronal plasticity during fetal development.

The studies investigating the impact of gestational exercise on behavior is now focused on the biological mechanisms underlying the observed behavioral changes. In addition to its impacts on neurogenesis, gestational exercise also increase the levels of BDNF, fibroblast growth factor-2 and c-FOS protein. However, whether the effects transmitted through neurochemical or

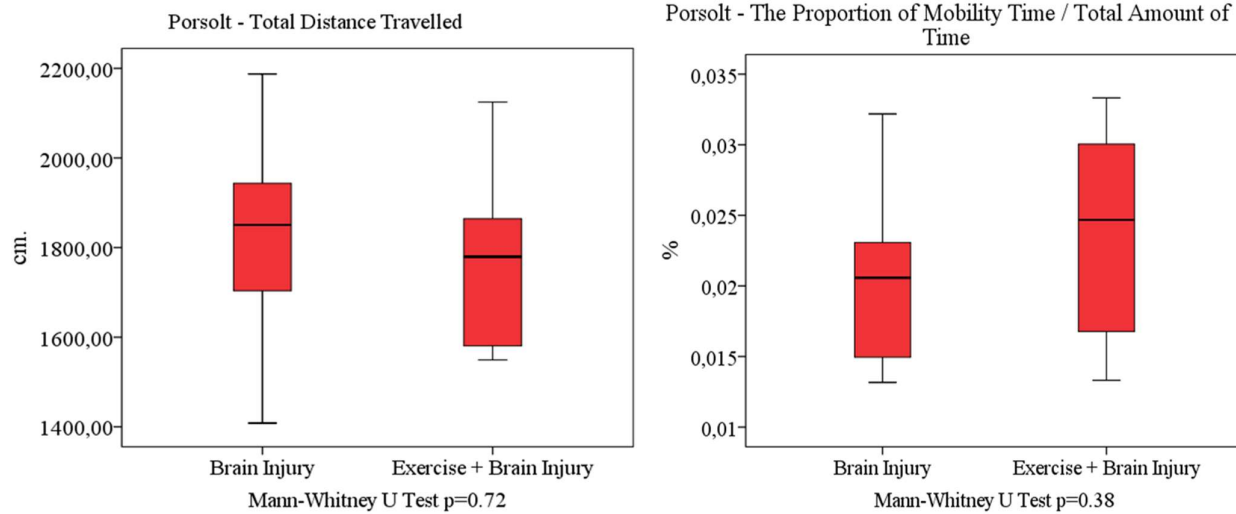
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**Table 1.** The results of the open field tests.

Open Field Test	Brain Injury			Exercise + Brain Injury			p
	Average ± SE	Min.	Max.	Average ± SE	Min.	Max.	
Distance Traveled (cm)	2516.8±429.5	1858.39	3364.53	2234.6±588.1	1607.1	3181.1	0.19
Time Spent at the Margins (min.)	4.9±0.17	4.36	5	4.9±0.09	4.74	4.99	0.36
Frequency of Staying at Margins	5.3±5.5	1	21	4.7±3.4	2	12	0.57
Time Spend as Immobile (min.)	4.9±0.01	4.95	5	4.9±0.00	4.98	5	0.14
Time Spent at the Center (min.)	3.9±0.27	3.384	4.258	3.7±0.52	3.038	4.467	0.36
Time Spend as Immobile at the Center (min.)	1.0±0.27	0.742	1.616	1.25±0.52	0.533	1.962	0.36
Speed (cm/min.)	504.5±87.0	371.68	676.69	447.8±117.2	323.06	636.23	0.19

**Table 2.** The results of elevated plus maze tests.

Elevated Plus Maze Test	Brain Injury			Exercise + Brain Injury			p
	Average ± SE	Min.	Max.	Average ± SE	Min.	Max.	
Distance Traveled (cm)	1519.2±312.8	1008.6	2205.3	1295.7±526.8	519.7	2138.2	0.25
Closed arms (min.)	3.6±0.5	2.8	4.3	4.1±0.5	3.08	4.65	0.01
Time in Open Arms (min.)	1.4±0.5	0.73	2.21	0.86±0.5	0.35	1.92	0.01
Frequency in Open Arms	8.0±2.7	5	14	6.6±4.5	2	15	0.23
Immobility Time (min.)	4.96±0.03	4.878	4.985	4.9±0.1	4.757	4.99	0.50
Immobility Frequency	39.0±11.6	20	61	24.4±9.07	13	40	0.02
Mobility Time (min.)	0.03±0.02	0.003	0.09	0.03±0.06	0.007	0.162	0.14
Mobility Number	23.5±12.1	3	52	12.4±4.3	8	20	0.01
Speed	304.6±62.1	203.4	441.05	262.7±105.8	104.5	429.94	0.25
Percentage in Closed Arms	0.71±0.1	0.56	0.85	0.82±0.10	0.62	0.93	0.01
Percentage in Open Arm	0.28±0.1	0.15	0.44	0.17±0.10	0.07	0.38	0.01



**Fig. 4.** Porsolt test to evaluate depression. The results were not revealed any significant difference between the two groups.

epigenetic mechanisms is not known. Likewise, its impact on maternal behavior is also not known. Therefore, how the effects are mediated are still not known [8]. Moreover, the effects of gestational exercise on additional stress factors, such as in the case of LPS induced brain injury models, are also not known.

Morris water maze test is used to evaluate the spatial memory and it is a useful tool to test the memory functions. In the present study, no significant difference was observed between the two groups. The current data in the literature showing the positive impacts of gestational exercise has been performed on healthy subjects. The results of the present study demonstrates a positive impact of gestational exercise for the fetal brain development. However, the results did not indicate any neuroprotective effects of the gestational exercise in the presence of additional stress factors, such as in the case of acute brain damage.

The data obtained from open field test demonstrated an increased locomotor activity,

mobility duration and distance travelled in offspring of exercise mothers compared to the offspring of sedentary mothers [9]. However, our results did not show such an effect. Therefore, this suggests that gestational exercise in a brain injury model does not have any protective effects. On the contrary, the offspring of mothers forced to exercise during gestation demonstrated an increased entry to the open arms in elevated plus maze test indication a reduced anxiety behavior [9]. LPS treatment has been previously shown to decrease the social behaviors, however, no effects has been reported on elevated plus maze test [10]. In the present study, offspring of LPS treated and exercised mothers demonstrated increased amount of time spent at the closed arms in elevated plus maze test compared to the offspring of PLS treated sedentary mothers. The increased time spent in the closed arms in LPS treated offspring, which is known to induce brain damage through inflammation, indicates an onset of depressive behavior in response to such a strong stressor.

In Porsolt forced swimming test, rat is placed in a deep water where there is no escape route. In such a test, the rat is first trying to swim. However, after certain time passed, rat give up swimming. Since the two groups traveled the same distance and exhibited similar motility, it can be concluded that gestational exercise has no effect.

In LPS induced brain injury models, impacts of exogenous neurotrophin reducing apoptosis and damage in myelination has been shown previously [6]. LPS treatment during critical stages of embryonic development has been shown to cause reduced adult hippocampal neurogenesis and BDNF levels, increased tendency of depressive behavior and long-term behavioral changes in adults [11]. Previously published studies in the literature demonstrates an increased BDNF and VEGF levels in the brain and improved open field and locomotor activity test results from offspring of exercised mothers during pregnancy [5, 9, 12-14]. Therefore, it is expected to observe an increased tolerance to the brain damage through endogenous trophic factors, such as BDNF. However, in the present study, all the tests performed on offspring of exercised and sedentary mothers revealed similar results.

The most striking results of the present study is that the offspring in the BI group demonstrated increased mobility and elevated frequency of entry to the open arms compared to the offspring in the E+BI group ( $P=0.01$ ). This situation implies that the offspring of BI mothers demonstrated a postnatal "hyperactivity" and the gestational exercise can potentially prevent this situation.

To our knowledge, this is the first study in the literature showing the protective effects of gestational exercise on acute brain damage model. However, in addition to the brain damage model performed in the current study,

effects of other medical and surgical damage models needs to be investigated. Moreover, in addition to the experimental parameters performed in the present study, advanced molecular and immunohistochemical analyses should be carried out for the further studies.

### **Compliance with ethical statements**

*Conflicts of Interest:* None.

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