Original Article

Regular Aerobic Voluntary Exercise Increased Oxytocin in Female Mice: Cause to Decrease Anxiety and Increase Empathy-Like Behaviors

Yüksel et al. Effects of Exercise on Empathy- and Anxiety-Like Behaviors

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Background: It’s known that regular physical activity reduces anxiety. Low anxiety levels affect mood, emotion, and empathy behavior. Oxytocin is a powerful hormone that regulates social interaction, sexual reproduction, maternal-infant bonding, milk release, empathy, and anxiety. Empathy is important in living community; and also important for sportsmen’s life during sportive competition as well as except competition period, because of sportsmen also seen as role model by other people.

Aims: The aim of this study is to investigate the effects of voluntary physical activity on oxytocin, anxiety and empathy levels and the relationship between them.

Study Design: Animal experiment.

Methods: Male and female mice were exercised in running wheel cages for 6 weeks. Their anxiety and empathy levels were evaluated, and their brain and blood oxytocin levels were measured.

Results: Empathy-like behavior was improved in both genders of exercised groups (door opening time decreased in both gender of exercised groups, both of p=0.0001). As a response to exercise, brain and serum oxytocin levels increased in female mice (both of p=0.0001); but oxytocin levels increased in only brain of males (p<0.05). Anxiety levels decreased in all exercised groups (increased time spent in the middle area of open field test, both gender of p=0.002; increased time spent in the open arms of elevated plus maze test, females p=0.004, males p=0.0001). There was a strong negative correlation was found between serum oxytocin levels and door opening time of helping behavior equipment and moderate negative correlation was found between brain oxytocin levels and door opening time of helping behavior equipment in females. Whereas; there wasn't any correlation between both brain and serum oxytocin levels and empathy behavior in males. And also there were very strong positive correlations between low anxiety indicators and both brain and serum oxytocin levels in both gender.

Conclusion: Our findings suggest that voluntary physical activity decreases anxiety and increases empathy-like behavior in mice; which is associated with increased oxytocin levels in female mice whereas it is not associated with oxytocin levels in male mice. Further research is needed to investigate the mechanisms of exercise effect on anxiety and empathic brain pathways in males.

Keywords: Exercise, anxiety, empathy, oxytocin, brain, gender
There is substantial literature demonstrating that regular exercise and physical activity leads to many health benefits, including prevention and improvement of metabolic diseases such as type 2 diabetes mellitus, metabolic syndrome, obesity, heart conditions, stroke arthritis. The benefits of physical activity are not limited to physical conditions but also compass our psychological well-being. Research has shown that regular physical activity has significant benefits for psychiatric patients, rendering it an effective therapeutic strategy (1). Our previous studies documenting correlations between exercise and changes in brain biochemistry also support the positive effect physical activity has on mental health (2, 3).

Many studies have shown that oxytocin is a neuropeptide associated with emotional behavior, including empathy. It plays an important role in the development of social signals during evolution from lower vertebrates to higher primates (4). Oxytocin has been shown to support attachment, trust, empathy, generosity, and positive social participation following intranasal administration in humans (5). Expression levels of oxytocin in the brain as well as the neural architecture of empathy have both been observed to be altered by life experiences through epigenetic modifications (6). Animal and human studies show that exercise increases oxytocin levels. A ten-minute running exercise increased the level of salivary oxytocin (7). Increased oxytocin levels following exercise were reported in the hypothalamus, brainstem, and nucleus solitaries of rats (4, 5).

Physical activity positively affects cognitive brain areas such as the hippocampus, prefrontal cortex, and amygdala, by increasing cognitive functions and lowering anxiety and depression levels. Exercise improves one’s mood and supports emotional progress (8, 9); which can also be observed as a reduction in anxiety-like behavior in rats, following voluntary exercise (10). Anxiety has been shown to be positively associated with help behavior and empathy (11).

Empathy is the recognition and internalizing of someone else’s feelings, condition or behavior. In 1934s, Alfred Adler described empathy as “to see with the eyes of another, to hear with the ears of another, and to feel with the hearth of another” (12). Empathy is important for the survival and maintenance of society by preventing aggression against others and establishing healthy communication among members (13). Empathic behavior has been shown to increase activity in temporo-parietal cortex, the ventromedial prefrontal cortex, hippocampus, and amygdala regions of the brain. Empathy observed in many animal species such as humans, primates, and rodents (14).

Empathy affected from many things such as stress situation, pain, depression, and autism (11, 15). We showed that empathy increased with low intensity acute stress in our last experiment (16). Empathy is a significant variable in the effectiveness of sporting activity. The empathy skills of individuals are important in the team performance, especially during high stress sports events. In team sports, the empathy skills displayed by a team member towards his/her teammates, coach and competitors can be an important factor in team spirit and success. It will allow a player to anticipate behavior and outcomes in advance (17). On the other hand, the reverse relationship between sports/physical activity and empathy -the effects of physical activity on empathic behavior- has not been reported. The aim of this study is to investigate the effects of voluntary physical activity on oxytocin, anxiety and empathy levels and the relationship between them.

**MATERIALS AND METHODS:**

Thirty-two adult male and female Balb-c mice were used in this study. With free access to laboratory chow and water mice were housed in individual cages. Mice were kept in in a 12-h light/12-h-dark cycle at constant humidity (60%) and room temperature (22±1°C). Animal Care Committee of Dokuz Eylul University School of Medicine’s approval for the study was obtained. Mice were divided into four groups: (1) control females (n= 8), (2) running wheel exercised females (n=8), (3) control males (n=8), (4) running wheel exercised males (n=8). After an adaptation period of one week, a running wheel with a diameter of 11.5 cm is placed in the cage that the voluntary exercise group had free access for 6 weeks (2). Then mice were harbored in the same environment 14 days prior to the study for habituation; two mice were placed in each running wheel cage. Study design is shown in Figure 1A.

**Voluntary wheel running**

Voluntary wheel running is a common model to investigate physiological effects of aerobic exercise. Mice run freely inside a plastic wheel. Daily running distance can be calculated by the number of rotations of the wheel, which is counted by a digital counter connected to the wheel (18, 19).

**Empathy-like behavior Test**

All mice were trained via Helping Behavior Test Equipment (Figure 1B). Following the exercise period, all mice (including controls) were subjected to the Helping Behavior Test during 11-days training period. Previously described experimental equipment is used to test empathy-like behavior (16, 20). Opening the door and saving cage-mate was defined as empathy-like behavior. Mice were trained to open the door of the empathy test equipment for a total of 11 days, and tested on the 12th day. Each mouse assumed the roles of both the rescue mouse and the savior. Mice in pairs were subsequently placed in the platform’s rescue section and water section as shown in Figure 1B. Testing sessions were 5 minutes per mouse. Learning criteria was opening the door within 60 seconds for 3 days successively. As mice could be affected by each other, which may interfere with their empathy-like behavior, we have reduced contact between mice during behavioral assessment. After
empathy test, anxiety levels were evaluated with and elevated plus maze and open field tests. All behavioral experiments were conducted in the morning between times 9:00 and 13:00.

**Open Field Test**

Open field is a square area with 1 m borders surrounded with walls in 50cm height. Open Field Chamber was placed in a soundproof room with controlled illumination (100lx). After placing each mouse in the center of the chamber locomotor activity of mouse was recorded with a video camera mounted 2.5m above the chamber for 5 minutes. Open field is divided to 16 equal squares (4 central, 12 peripheral). The distance travelled by mice and time spent in different parts of the open field was calculated. Time spent in the central part was considered as an index of anxiolyis (21).

**Elevated Plus Maze**

The elevated plus maze is a plus shaped apparatus commonly used to evaluate anxiety in rodents based on rodents’ aversion behavior to open spaces. Maze was 50cm above the floor level and consisted of 4 arms, two of them were open and two of them were closed with walls 40cm in height. Mice were placed in the center of the apparatus facing open arm. Locomotor activity of mouse was recorded for 5 minutes. Time spent in on the closed and open arms and total numbers of entries into the closed and open arms were calculated. Time spent in the open arms of equipment was considered as an index of anxiolyis.

**Biochemical Analysis**

Thoracotomy was performed under carbon dioxide anesthesia and blood sample was obtained from the ventricle by cardiac puncture, followed by extraction of brain tissues. Tissue samples (blood and brain) were kept in laboratory refrigerator (−85°C) until analysis. Mouse Oxytocin ELISA kit (catalog no. E-EL-0029, Elabscience, Wuhan, China) was used for oxytocin analysis. Assay sensitivity was determined to be 9.38pg/mL and detection range 15.63-1000pg/mL.

**Statistical evaluation**

SPSS software for Windows, Version 11.0 (SPSS, Chicago, IL) is used for statistical analysis. GLM-repeated measure post-hoc Bonferroni was used to analyze differences in empathy learning period. Mann-Whitney U test is used to analyze differences between groups. Pearson correlation analysis was used to calculate correlations between groups. Results are presented as mean ± SD. Because multiple comparisons were performed to prevent an alpha (type 1) error, (α/n = 0.05/5), p < 0.01 was considered statistically significant (22).

**RESULTS:**

Mean running distance for the exercise group in the running wheel cages is 2.4 ± 0.3 km/day and running time is 143 ± 5.18 min/day.

In Helping Behavior Test Equipment, the mean door opening duration progressively decreased with time in all groups (p<0.0001)(Figure 2A and B). Exercised groups opened the door quicker comparing the control groups in the empathy-learning period (females, 5th day, exercised females 186 ± 103,9; control females 217 ± 101,2 p=0.027; and 7th day, exercised females 141 ± 48,56; control females 153,89 ± 102,9 p=0.001; males, 5th day exercised males 76,7 ± 42,9; control males 132,9 ± 50,1 p=0.01; and 9th days exercised males 136,4 ± 123,6; control males 214,3 ± 65,6 p=0.001)(Figure 2A and B). On test day (day 12), opening door time was observed to decrease in the exercised mice; exercised females 125,8 ± 45,4; control females 150,6 ± 40,8; exercised males 60,7 ± 16,9; control males 69,6 ± 22,4; both genders p=0.0001)(Figure 2C).

Times spent in the center of the open field chamber were higher in exercise groups (exercised females 33,4 ± 5,7; control females 6,1 ± 2,8; exercised males 41,4 ± 2,5; control males 15,6 ± 1,9 both genders p=0.002)(Figure 2D). There wasn't any difference between males and females.

In the elevated plus maze test, the exercise groups spent more time in the open arms when compared to same gender of control groups (exercised females 80,4 ± 19,2; control females 50,1 ± 18,9 p=0,004; exercised males 130,5 ± 22,6; control males 40,1 ± 5,9 p =0.0001)(Figure 2E).

Brain and serum oxytocin levels were found to be higher in exercised female mice (Brain; exercised females 0,87 ± 0,13; control females 0,55 ± 0,09 p=0.0001; exercised males 2,44 ± 0,39; control males 1,87 ± 1,02 p=0.039; Serum; exercised females 431,49 ± 60,76; control females 123,38 ± 21,15 p=0.0001; exercised males 34,49 ± 4,79; control males 31,59 ± 5,88 p>0.05)(Figure 3A and B); whereas statistically no difference was observed in males (p>0.05).

In females; a strong negative correlation was found between serum oxytocin levels and door opening time of helping behavior equipment (r = - 0.702, p=0.0001) and moderate negative correlation was found between brain oxytocin levels and door opening time of helping behavior equipment (r=-0.430, p=0.036). Whereas, there wasn't any correlation between both brain and serum oxytocin levels and empathy behavior in males.

In females; there were very strong positive correlations between activity in the central area of open field and both brain and serum oxytocin levels (brain oxytocin, r= 0.815, p=0.0001; serum oxytocin, r= 0.914, p=0.0001)(Figure 3C). Serum oxytocin levels were strongly correlated with activity in the central area of elevated plus maze test in both genders.
levels of males. These results suggest that brain and blood oxytocin levels are related with anxiety and empathy-like behavior only in exercised females, and other mechanisms have a role on the positive effects of exercise on anxiety and empathy-like behaviors in males. There is a need for further investigation of the mechanisms of exercise effect on anxiety and empathic brain pathways in males.

DISCUSSION:
Voluntary physical activity decreased anxiety and increased empathy-like behavior in both male and female mice in this study. These results are related with increased oxytocin levels in females, but not in males. To our knowledge, this is the first study that examines the physical activity relation with oxytocin, anxiety and empathy-like behavior.

Voluntary exercise is recommended in stress-related disorders like anxiety disorders. Although conflicting results had been reported about anxiety and chronic voluntary exercise relationship (23,24) chronic voluntary exercise reduced anxiety in our previous studies (2). Also in this study exercised mice of both gender showed reduced anxiety in behavioral tests. Empathy is the psychological identification with the feelings, thoughts, or attitudes of another. It also forms the basis of the feeling of interest, warmth and closeness to those in difficult situations. Empathy related helping behavior (prosocial behaviors) involves helping others in a difficult situation (13). Empathy-related behaviors in sports consist of behaviors such as helping a falling opponent, encouraging teammates etc. (25). There is a great amount of research on the relationship between empathy and prosocial behaviors, stating that in the lack of empathy, prosocial/helping behavior is not observed. A significant positive relationship between sportsmanship orientation (prosocial/helping behavior) and empathy has been previously reported (25, 26). Differing results of empathy-like behavior during a sporting event has been observed. Empathy towards an opponent may conflict with the “achievement goal”; while empathizing with a teammate may hinder focusing on personal performance (27). In some cases coaches intentionally may not want their athlete to worry about the opponent (28). Although there is research on the effects of empathic behavior during a sporting event; the effect of regular physical activity on social empathic behaviors is not known. In our study we found that exercised mice displayed higher levels of empathy when compared to control.

We found that brain and serum oxytocin levels were higher in the exercised females, but unchanged males. Exercise increases oxytocin release from nucleus tractus solitaries. Oxytocin is important in autonomic changes resulting from voluntary exercise that stimulates the parasympathetic nervous system, reducing cardiac tone through nervus vagus, and limiting exercise-induced tachycardia (29). There are a few studies reporting the effects of exercise on oxytocin levels. Martins et al reported that chronic treadmill exercise increased oxytocin and oxytocin receptor levels in the paraventricular nucleus of hypothalamus and it’s projection to dorsal brain stem male rats (30). Kim et. al reported that the running wheel exercise, reversed depression and increased oxytocin and oxytocin receptor levels in the basolateral amygdala in male mice (31). Interestingly, chronic resistance exercise was reported to reduce oxytocin levels in the paraventricular neurons of the hypothalamus in male rats (32). In addition Bakos et al. showed that weeks of running wheel exercise decreased pituitary and serum oxytocin levels in male Sprague Dawley and Lewis rats (33). In one of human study, Hew-Butler et al. showed that blood oxytocin levels increased in both ultramarathon runners and high intensity exercising groups, while it did not change in the steady-state control group. Their subjects consisted of both males and females (34).

The majority of the studies report significantly higher levels oxytocin in female species than in males; but to our knowledge this is the first study to compare oxytocin levels in regular voluntary exercised subjects. Also in our study, high blood and brain oxytocin levels positively correlated with low anxiety levels and increased empathy-like behavior levels in females.

Our findings suggest that voluntary physical activity decreases anxiety and increases empathy-like behavior in both male and female mice. In females, blood and brain oxytocin levels were also found to be significantly higher than controls, and were correlated with behavioral results. Whereas there wasn’t any change in oxytocin levels of males. These results suggest that, brain and blood oxytocin levels are related with anxiety and empathy-like behaviors only in exercised females, and other mechanisms have a role on the positive effects of exercise on anxiety and empathy-like behaviors in males. There is a need for further investigation of the mechanisms of exercise effect on anxiety and empathic brain pathways in males.

Conflict of Interest: No conflict of interest was declared by the authors.
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REFERENCES


FIG. 1: (A) Timeline of the study. (B) Empathy test equipment.
Door opening time of males in empathy equipment (second)

- **CONTROL**
- **EXERCISE**

**DAYS**

Door opening time in empathy equipment at 12th day (second)

- **Exercised F**
- **F**
- **Exercised M**
- **M**
FIG. 2: Behavioral test results. (A) Door opening time of the empathy test equipment during the total of 12 days - female rats. (B) Door opening time of the empathy test equipment during the total of 12 days - male rats. (C) Door opening time on day 12. (D) Time spends in middle area of open field test. (E) Time spends in the open arms of elevated plus maze. F: females, M: males. *p<0.05 compared to same sex control group.
Brain oxytocin levels (pg/mg protein)

Exercised F  F  Exercised M  M

Serum oxytocin levels (pg/ml)

Exercised F  F  Exercised M  M
FIG. 3: Biochemical investigation and correlation results. (A) Brain tissue oxytocin levels. (B) Serum oxytocin levels. F: females, M: males. *p<0.05 compared to same sex control group. (C) Scatterplot that activity in the middle area of open field and both brain and serum oxytocin levels in female mice. (D) Scatterplot that activities in the open arms of elevated plus maze test and serum oxytocin levels in female mice.