Title: The Effect of Physical Activity on the Orexin and BDNF Expression on Kindling Model of Epileptic Rats

Running Title: Effect of Exercise on Orexin and BDNF in Epileptic Rats

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Abstract

Introduction: Epilepsy has been characterized by recurrent seizures associated with cognitive, mental, and social issues. Exercise has been well known as a non-pharmacological or complementary remedy to reduce the effective dose and side effects of pharmacological therapies. Orexin signaling pathway and brain-derived neurotrophic factor (BDNF) have an essential role in the pathogenesis of epilepsy. In this study, we investigated the effect of exercise on the modulation of the orexin-A (OXA) and BDNF signaling pathways in epileptic rats.

Methods: Male Wistar rats were divided into five groups: Normal saline (NS), seizure, physical activity (PA), PA+PTZ, and PA-PTZ. Assessment of seizure behaviors was done 30 min after any PTZ injection in the seizure, PA+PTZ, and PA-PTZ groups. Seizure behavior score (SBS) was monitored in seizure, PA+PTZ, and PA-PTZ. The expression of the OXA and BDNF in the CA1, CA3, and cortex was assayed by immunohistochemistry staining. The correlations were taken between the OXA and BDNF in different groups.

Results: SBC was reduced in the epileptic rats that had exercised. Seizure and physical activity increased the OXA expression in the seizure and PA groups. The OXA expression decreased in the CA1 and CA3 of the PA+PTZ and PA-PTZ and cortex of PA+PTZ compared to the seizure group. OXA was up-expressed in the PA-PTZ group compared to the PA+PTZ group. Seizure decreased the BDNF expression in the seizure group compared to the NS group. Physical activity elevated the BDNF expression in the CA1, CA3, and cortex of the PA group. BDNF was up-expressed in the cortex of the PA+PTZ and the CA1, CA3, and cortex of PA-PTZ. BDNF expression increased in the CA1 and CA3 of the PA-PTZ compared to the PA+PTZ. There was a significant correlation between the OXA and BDNF expression in the CA1, CA3, and cortex of the NS and seizure groups, as well as in the CA1 and cortex of the PA group.

Conclusion: Our results declared that physical activity had an amelioration effect on the seizure severity. Our finding suggested that the effect of physical activity on seizure might not rise from the interaction of the OXA and BDNF expression in epileptic rats.

Keywords: Epilepsy, Seizure, Exercise, Orexin, BDNF, Pentylenetetrazol

Abbreviations

BDNF: Brain-derived neurotrophic factor

PTZ: Pentylenetetrazol

SBS: Seizure behavior score

NS: Normal saline

PA: Physical activity

PA+PTZ: Group with Simultaneously physical activity and pentylenetetrazol (PTZ) injection

PA-PTZ: Group with physical activity for four weeks firstly, then Simultaneously physical activity and PTZ injection.

OXA: Orexin-A

GABA: Gamma-aminobutyric acid

i.p.: intraperitoneally

REM: Rapid eye movement

cAMP: cyclic adenosine 3′,5′-monophosphate

1. Introduction

Epilepsy as a neurodegenerative disorder has affected the lives of 50 million people worldwide. Three main goals of epilepsy treatment have included controlling seizure attacks, maintaining life quality, and decreasing the side effects of anti-epileptic drugs (Perucca 2021, Foutz & Wong 2022). Therefore, it is essential to use non-pharmacological treatment approaches for epilepsy (Alqahtani et al. 2020).

Physical activity has been introduced as a low-cost treatment for improving neurological function available to adults and does not have the intolerable side effects often caused by drug therapy (Matsuda, Sakakima, Ikutomo & Yoshida 2009, Perrochon, Borel, Istrate, Compagnat & Daviet 2019, Bareiss, Johnston, Lu & Tran 2022, Zhang et al. 2022). Association between epilepsy and physical exercise as a non-pharmacological approach has been noticed, and many researchers reported it as a non-pharmacological or complementary therapy for epilepsy (Popp et al. 2021, Zhang et al. 2022).

The orexin/hypocretin pathway has been involved in many neurological activities. Prepro-orexin mRNA has been located in the neurons of the lateral hypothalamus as a neuromodulatory system and led to the production of orexin-A and B peptides (Azeez, Igado & Olopade 2021, Abounoori, Maddah & Ardeshiri 2022). Orexin-A was usually presented in humans and consisted of 33 amino acids and bound to two orexin receptors-OXR1 and OXR2. By coupling to the Gq/11-alpha subunit and activating phospholipase C, OXR1 and OXR2 played as members of family G-protein coupled receptors, which stimulated the influx of cations and caused neuron depolarization and increased its excitability (Scammell & Winrow 2011).

It was supposed that orexigenic signaling was involved only in feeding, appetite, and energy homeostasis (Bonnavion & de Lecea 2010), but more recent research indicated that orexin had an essential role in the arousal and sleep and orexin dysregulation caused some sleep disorders such as narcolepsy and cataplexy as well as some neurodegenerative disorders (Liblau, Vassalli, Seifinejad & Tafti 2015, Pizza, Barateau, Dauvilliers & Plazzi 2022). Orexin was an essential factor in treating cognitive deficits in schizophrenia (Borgland & Labouèbe 2010). Orexin-A and neuronal cell numbers decreased in Alzheimer's disease (Um & Lim 2020) and Huntington patients (Petersén et al. 2005).

There was accumulating evidence that showed the signaling of orexin on the occurrence of seizures and epilepsy (Ng 2017, Sheibani, Shayan, Khalilzadeh, Ghasemi & Dehpour 2023). It has been reported that orexin receptor inactivation in the hippocampus by orexin antagonist reduced pentylenetetrazol (PTZ) induced seizures in male rats (Goudarzi, Elahdadi Salmani, Lashkarbolouki & Goudarzi 2015). The serum level of orexin-A was higher in epileptic individuals compared to healthy and individuals with pseudoseizures (Çikriklar et al. 2020). Intra-cortical injection of the orexins increased penicillin-induced epileptic activity in the male rats (Kortunay et al. 2012). The relation between epilepsy and orexin might be mediated through interaction with gamma-aminobutyric acid (GABA) and glutamate receptors (Razavi, Farivar, Etemad & Hosseinzadeh 2020, Manavi et al. 2022, Kinboshi et al. 2023).

Brain-derived neurotrophic factor (BDNF) was known as a member of the neurotrophic family of factors initially recognized as proteins responsible for neuron survival, differentiation, and neurogenesis (Kowiański et al. 2018). The decrease of BDNF protein in the brain of Alzheimer's patients showed its essential function in the pathological mechanism of this disease (Yulug et al. 2018, Ng, Ho, Tam, Kua & Ho 2019). The BDNF has been alleviated and accompanied by an enhancement in the deterioration of dopaminergic neurons in Parkinson's disease (Wang, Liu, Zhang, Soares & Zhang 2016, Lin, Chen, Yang, Chen & Hung 2017). In contrast, the serum level of BDNF was higher in amyotrophic lateral sclerosis compared to the control group (Riolo et al. 2022).

Several researches have accentuated the link between epilepsy and BDNF. BDNF has an amelioration effect against hippocampal epilepsy and apoptosis induced by seizure (Yu et al. 2019). The serum level of BDNF was lower in the epileptic adults compared to health (LaFrance, Leaver, Stopa, Papandonatos & Blum 2010).

Studies have shown some correlation between BDNF and orexin. Orexin-A elevated the protein level of BDNF in the dopaminergic neurons of the substantia nigra in the Parkinson's model of mice (Liu et al. 2018). Physical activity had an elevation effect on the levels of BDNF and orexin with antidepressive-like influence and memory-facilitatory function (Ghahfarrokhi et al. 2020). Also, one study reported a relationship between orexin-A and BDNF in schizophrenia patients after long-term clozapine treatment (Ren et al. 2022).

This study aimed to elucidate the effect of physical activity on orexin signaling and BDNF in PTZ-induced epileptic Wister rats.

2. Methods

2.1. Animals and Seizure Induction

Thirty male Wistar rats (weighing 250–300 g) were purchased one week before the beginning of the experiment and housed in the Central Animal Facility of Iran University of Medical Sciences under a specific condition with a 12-hour light/dark cycle (at 21 ± 2 °C) and free access to food and water.

All the experiments were carried out according to the protocol approved by the animal ethics of Iran University of Medical Sciences, Tehran, Iran. (IR.IUMS.FMD.REC.1401.198).

Randomly, the animals were divided into five groups (Figure 1):

Normal Saline (NS) group: normal saline (0.9% saline) was injected intraperitoneally (i.p.) three days a week for four weeks without any other intervention.

Physical activity (PA) group: rats forcedly ran for 30 minutes daily five days/week for four weeks without any injections.

Seizure group: seizures were induced by PTZ injection under the same protocol as the NS group injection.

PA+PTZ group: animals forcedly ran with the same protocol as the PA group. After five hours of running, seizure induction was carried out with the same protocol as the seizure group.

PA-PTZ group: first, rats received PTZ with the same protocol as the seizure group without physical activity for one month. After four weeks, rats forcedly ran and received PTZ with the same protocol as the PA+PTZ group for four weeks.

Seizure induction was performed by i.p. injection of 35 mg/kg of the PTZ (Sigma Aldrich, Germany), five hours after running in the PA+PTZ and PA-PTZ groups and at the same time and dose in the seizure group.

2.2. Exercise Protocol

The rats of the PA, PA+PTZ, and PA-PTZ groups were adapted to the motorized treadmill (pre-trained on the treadmill for three days (15 min/day) at a speed of 5 m/min. The forced running included 30 minutes of forced running on the treadmill for five days per week. The rate of the treadmill was started at 5 m/min and gradually increased every 5 min up to 25 m/min at 0 degrees of slope (Barzroodi Pour, Bayat, Navazesh, Soleimani & Karimzadeh 2021). Three

minutes were considered to warm up and cool down, respectively, in the beginning and at the end.

2.3. Assessment of Seizure behavior score

Seizure behavior score (SBS) was assessed by monitoring the rats from the moment of seizure induction to 45 min later. Convulsive behaviors were scored as follows: 0 = normal behavior; 1 = immobility; 2 = rigid posture; 3 = repetitive scratching, circling, or head bobbing; 4 = forelimb clonus, rearing, and falling; 5 = repeated occurrence of level four behavior, and 6 = severe tonic-clonic behavior (Barzroodi Pour et al. 2021).

2.4. Tissue Preparation

After the last intervention, rats were anesthetized with an i.p. injection of ketamine-xylazine (100–2 mg/kg of K-X, Sigma–Aldrich). The corpses perfuse through cardiac circulation using 250 ml of saline and 400 ml of 4% paraformaldehyde solution (PFA, pH 7.4). Animals were decapitated, and the brains were removed. Brain samples were embedded in paraffin blocks, and serial sections with eight μm thickness were taken by a microtome.

2.5. Immunohistofluorescence

Immunohistofluorescence was used to detect the OXA and BDNF expression in the CA1, CA3, and cortex. Three slides of each block with a 30 μm distance between the sections were selected and after deparaffined, they were rehydrated by a series of xylene and graded alcohol. They were washed with Tris buffer (pH 7.4) three times. The sections were boiled in the citrate buffer (pH 6.0) for 10 min and left to cool. After removing non-specific binding by washing with 1% bovine serum albumin for 10 min, three serial sections were incubated overnight at 4 ^{0}C with rabbit polyclonal anti-rat antibody of orexin-A (Biorbyt), and three sections incubated with rabbit polyclonal anti-rat antibody of BDNF (Biorbyt) at the same condition. Antibodies were diluted 1:250 in Tris buffer at pH 7.4. The sections were washed three times in Tris buffer and incubated with FITC-conjugated Goat Anti-Rabbit IgG antibody (Biorbyt) for two hours at room temperature (Salami, Bandegi, Sameni, Vafaei & Pakdel 2019). To the specificity of immune staining, negative control was carried out in the same protocol except for primary antibody incubation. Images for assessment were taken with a digital camera (Optika, objective lens × 40) connected to the microscope (Optika Ts100 fluorescence). The FITC-positive neural cells were counted by INFINITY ANALYZE 7 software and calculated in the unit of area (1 mm^2).

2.7. Statistical Analysis

Data were represented as mean ± S.E.M. The data were analyzed by GraphPad prim 9 software, multiple t-tests, and one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Normality and Lognormality Tests were used to prove the normality of the data. Correlation test used to assay correlation between data of different groups. The group size was six, and the statistical significance was $p < 0.05$.

3. Results

3.1. Seizure Behavior score

The mean of scores ± S.E.M of the seizure, PA+PTZ, and PA-PTZ groups were mentioned in Table 1.

As shown in Figure 2, SBS was reduced in the PA+PTZ and PA-PTZ groups compared to the seizure group in some days of seizure induction.

SBS decreased in the PA+PTZ group compared to the seizure group in the 3rd, 5^{th}, 8^{th}, 12^{th}, 15^{th}, 17^{th}, and 26^{th} days.

Also, SBS decreased in the PA-PTZ group compared to the seizure group on all PTZ injection days.

In the comparing of the PA-PTZ with the PA+PTZ groups, the reduction was indicated in the 10^{th}, 15^{th}, 17^{th}, 19^{th}, and 26^{th} days.

3.2. Expression of the OXA in the CA1 area

The mean number of immunofluorescence spots was counted in the CA1 area (Figure 3B). The expression of the OXA increased in the CA1 of the seizure, PA, PA+PTZ, and PA-PTZ groups compared to the NS group (0.11 ± 0.004, 0.084 ± 0.002, 0.081 ± 0.004, and 0.097 ± 0.003 vs. 0.058 ± 0.003 respectively ($p < 0.001$)).

Also, the OXA expression decreased in the CA1 of the PA, PA+PTZ, and PA-PTZ groups compared to the seizure group ($p < 0.001$, $p < 0.001$, and $p < 0.05$ respectively).

Additionally, OXA was highly expressed in the CA1 of the PA-PTZ group compared to the PA ($p < 0.05$) and PA+PTZ ($p < 0.05$). There wasn't a significant difference between the PA+PTZ compared to the PA group.

3.3. Expression of the OXA in the CA3 area

Data from the OXA-reacted cells in the CA3 area was analyzed (Figure 3C). The OXA expression increased in the CA3 of seizure, PA, PA+PTZ, and PA-PTZ groups compared to the NS group (0.12 ± 0.002, 0.085 ± 0.002, 0.095 ± 0.002, and 0.11 ± 0.003 vs. 0.056 ± 0.003, respectively ($p < 0.001$)).

Also, OXA was low expressed in the CA3 of the PA, PA+PTZ, and PA-PTZ groups compared to the seizure group ($p < 0.001$, and $p < 0.001$, $p < 0.01$, respectively).

OXA was highly expressed in the CA3 of PA-PTZ compared to the PA ($p < 0.01$) and PA+PTZ ($p < 0.05$). There was no significant difference between the PA+PTZ and PA.

3.4. Expression of the OXA in the cortex area

Analyzed data from the OXA-reacted cells in the cortex area (Figure 3D) showed significant differences in different groups.

The cortical expression of the OXA increased in the seizure, PA, PA+PTZ, and PA-PTZ groups compared to the NS group (0.66 ± 0.02, 0.43 ± 0.01, and 0.53 ± 0.02, 0.63 ± 0.02 vs. 0.29 ± 0.02 respectively ($p < 0.001$)).

The OXA expression decreased in the cortex of the PA and PA+PTZ groups compared to the seizure group ($p < 0.001$ and $p < 0.01$, respectively), but there wasn't a significant difference between the PA-PTZ and seizure groups.

The PA+PTZ and PA-PTZ groups showed high OXA expression in the comparison with the PA group ($p < 0.01$ and $p < 0.001$, respectively).

In addition, the physical activity increased the OXA expression in the PA-PTZ group compared to the PA+PTZ ($p < 0.05$).

3.5. Expression of BDNF in the CA1 area

The BDNF expression in the CA1 area was analyzed, and the results are indicated in Figure 4B.

The expression of the BDNF decreased in the CA1 of the seizure and PA+PTZ groups (0.057 ± 0.002 and 0.069 ± 0.002, $p < 0.001$ and $p < 0.01$, respectively) and increased in the PA group (0.10 ± 0.003, $p < 0.05$) compared to the NS group (0.087 ± 0.003).

There wasn't a significant difference in the BDNF expression between the PA-PTZ and NS groups.

The BDNF expression increased in the CA1 of the PA and PA-PTZ groups compared to the seizure group (0.10 ± 0.003 and 0.085 ± 0.003 vs. 0.057 ± 0.0029, $p < 0.001$), but the PA+PTZ group didn't have any significant difference in comparison with the seizure group (0.069 ± 0.002 vs. 0.057 ± 0.002, $p > 0.05$).

In the PA+PTZ and PA-PTZ groups, the expression of the BDNF decreased compared to the PA group (0.069 ± 0.002 and 0.085 ± 0.003 vs. 0.10 ± 0.003, $p < 0.001$ and $p < 0.01$ respectively).

The BDNF expression significantly increased in the CA1 of the PA-PTZ compared to the PA+PTZ ($p < 0.05$).

3.6. Expression of the BDNF in the CA3 area

The bar graph indicating the results of the BDNF expression in the CA3 area has been illustrated in Figure 4C.

The seizure reduced the BDNF expression in the seizure group compared to the NS group (0.065 ± 0.004 vs. 0.081 ± 0.003, $p < 0.05$).

The BDNF expression in the PA and PA-PTZ groups increased compared to the NS group (0.099 ± 0.003 and 0.098 ± 0.003 vs. 0.081 ± 0.003, respectively ($p < 0.05$)), but there wasn't a significant difference in the PA+PTZ group compared to the NS group (0.089 ± 0.003 vs. 0.081 ± 0.003, $p > 0.05$).

The BDNF highly expressed in the PA, PA+PTZ, and PA-PTZ groups compared to the seizure group (0.099 ± 0.003, 0.089 ± 0.003, and 0.098 ± 0.003 vs. 0.065 ± 0.004, $p < 0.001$, $p < 0.01$, and $p < 0.001$ respectively).

The BDNF expression decreased in the PA+PTZ group compared to the PA group (0.089 ± 0.003 vs. 0.099 ± 0.003, $p < 0.05$), but there wasn't a significant difference between the PA-PTZ and PA (0.098 ± 0.003 vs. 0.099 ± 0.003, $p > 0.05$).

The BDNF expression increased in the PA-PTZ compared to the PA+PTZ group (0.098 ± 0.003 vs. 0.089 ± 0.003, $p < 0.05$).

3.7. Expression of the BDNF in the cortex area

The comparison of different groups has been indicated in the Figure 4D.

The BDNF expression decreased in the seizure group compared to the NS group (0.40 ± 0.02 vs. 0.48 ± 0.01, $p < 0.05$) but increased in the PA group compared to the NS group (0.57 ± 0.013 vs. 0.48 ± 0.01, $p < 0.01$). There wasn't a significant difference in the PA+PTZ and PA-PTZ groups compared to the NS (0.49 ± 0.01 and 0.51 ± 0.02 vs. 0.48 ± 0.01, $p > 0.05$).

The BDNF expression increased in the PA, PA+PTZ, and PA-PTZ groups compared to the seizure group (0.57 ± 0.013, 0.49 ± 0.019, and 0.51 ± 0.02 vs. 0.4 ± 0.02, $p < 0.001$, $p < 0.05$, and $p < 0.01$ respectively).

The BDNF expression decreased in the PA+PTZ compared to the PA (0.49 ± 0.019 vs. 0.57 ± 0.013, $p < 0.05$), but there wasn't a significant difference between the PA-PTZ and PA (0.51 ± 0.02 vs. 0.57 ± 0.013, $p > 0.05$).

There wasn't a significant difference between the PA-PTZ and PA+PTZ (0.51 ± 0.02 vs. 0.49 ± 0.019, $p > 0.05$).

3.8. Correlation between expression of the OXA and BDNF in the CA1, CA3, and cortex of the different groups

The relationship between the OXA and BDNF expression in the CA1 regions has been presented in Figure 5. The correlation between the OXA and BDNF expression in the NS group showed that high OXA expression was associated with high BDNF expression (r = 0.74, $p < 0.05$). High OXA expression was associated with down BDNF expression in the seizure (r = -0.87, $p < 0.05$) and high BDNF expression in the PA groups (r = 0.87, $p < 0.001$). There was no group correlation between OXA and BDNF expression in the PA+PTZ and PA-PTZ groups.

The relationship between OXA and BDNF expression in the CA3 area has been presented in Figure 6. The correlation between OXA and BDNF expression in the NS group showed that high OXA expression was associated with high BDNF expression in CA3 of the NS group (r = 0.89, $p < 0.02$). High OXA expression was associated with down BDNF expression in the seizure group (r = -0.96, $p < 0.001$). There wasn't a significant correlation between OXA and BDNF expression in the PA, PA+PTZ, and PA-PTZ groups.

The relationship between the OXA and BDNF expression in the cortex has been presented in Figure 7. The correlation between the OXA and BDNF expression in the cortex of the NS

group showed that high OXA expression was associated with high BDNF expression in the NS group (r = 0.95, $p < 0.003$). High OXA expression was associated with down BDNF expression in the seizure group (r = -0.86, $p < 0.03$) and high BDNF expression in the PA group (r = 0.83, $p < 0.04$). There was no correlation between the OXA and BDNF expression in the PA+PTZ and PA-PTZ.

4. Discussion

Present study investigated the effect of aerobic exercise by treadmill as an interventional treatment without medicine therapy on score of seizure, expression of OXA and BDNF and correlation between the OXA and BDNF expression. As resulted from behavioral data, physical activity could have beneficial effect on prevention and treatment of seizure in epileptic rate. Also, physical activity could have different effect on OXA in healthy and epileptic male rat that increased OXA in healthy rats and decreased it in PTZ kindling-induced epileptic rat models. Also, as a same effect on OXA in health rats, exercise elevates the BDNF in health and compensates its reduction in epileptic rats. Also, there was a relationship between the OXA and BDNF expression in the CA1, CA3, and cortex of some groups.

Our findings indicated that exercise decreased the seizure intensity in the epileptic rats. The effects of physical activity on convulsive behaviors and the quality of life have been studied in individuals with epilepsy (Johnson, Helen Cross & Reilly 2020, Häfele et al. 2021). Treadmill exercise decreased the severity of seizure in PTZ-induced seizures in male rats (Barzroodi Pour et al. 2021). Aerobic training, resistance training, and stretching ameliorated the quality of life, stress levels, and physical fitness of epileptic patients and reduced their seizure frequency (Häfele et al. 2021). Physical activity decreases the seizure frequency in individuals with epilepsy and increases their quality of life (Lee, Ahn & Cucullo 2022). Swimming reduced the severity of seizures in rats (Souza et al. 2009, Tutkun, Ayyildiz & Agar 2010). Running on the treadmill (10 days) and swimming (6 weeks) diminished oxidative injury induced by PTZ injection in rats (Souza et al. 2009, Arabaci-Tamer, KAYA, YÜKSEL, YILDIRIM & YEGEN 2022).

We illustrated that four-week treadmill physical activity simultaneously with induction of seizure attenuated the severity of seizure in the male rats. In addition, physical activity pre-induction of seizure notably decreased seizure severity.

Our results indicated that exercise as a monotherapy for epilepsy has a potential effect on the severity of seizures. Additionally, it might be a prevention intervention to reduce the seizure severity.

There are accumulating studies that have investigated the role of orexin in epileptogenesis. Intracortical injections of orexin-A increased epileptic activity (Kortunay et al. 2012). Results raised from the Li et al. study indicated that hypothalamic inhibition of pre-seizure orexin activity reduced the severity of seizure in epileptic mice (Li, Viskaitis, Bracey, Peleg-Raibstein & Burdakov 2023). It has been shown that an OX1 receptor antagonist reduced seizure behaviors in the male Albino Swiss mice (Socała, Szuster-Ciesielska & Wlaź 2016). It suggested that the expression of both orexin types in the hippocampus elevated the excitability of hippocampal neurons during epileptogenesis (Morales et al. 2006). Our results illustrated that seizure increased the OXA in the CA1, CA3, and cortex of the sedentary male rats. The orexin pathway function in the wake-sleep cycle and regulation of the GABA and glutamate system highlighted the footprint in the pathogenesis of epilepsy. In this regard, the reduction of orexin activity was related to rapid eye movement (REM) sleep outset, and REM sleep was usually protective against epilepsy (Ng 2017).

Previous studies showed the effect of exercise on the orexin pathway. One theory that described the relationship between the orexinergic system and training was hypothalamic thermoregulation. Enhancing serum level of orexin-A and rectal temperature after ergometer exercise (Messina et al. 2016) and diminishing the simultaneously physical activity and core body temperature by blockage orexinergic system (Martin et al. 2019) directed according to this theory.

Another theory about the relation between exercise and the orexin system was the neurogenesis effect of exercise on the hippocampus. Studies showed that activating of orexin-A promoted neuronal proliferation, differentiation, and firing of neurons in the hippocampus and decreased animal immobility (Ito et al. 2008, Zhao et al. 2014, Chen, Chen & Du 2017). It has been indicated that the hypothalamic administration of orexin-A enhanced ambulation and reduced sedentary in the light and dark cycles of Sprague–Dawley rats (Kotz, Teske, Levine & Wang 2002). The injection of orexin-A in the hypothalamus and substantia nigra has enhanced the ambulation time, and it had a modulatory function on physical activity (Kotz et al. 2006).

Results illustrated that physical activity up-regulated the OXA expression in the CA1, CA3, and cortex, but it had a down-expression effect on the expression of OXA in the epileptic rats.

These findings suggested that physical activity changed its influence depending on conditions that increased the OXA expression in the healthy non-epileptic rat and decreased it in the epileptic rats. In fact, physical activity acted as a moderator in its effect on OXA expression. Additionally, four weeks of physical activity before seizure induction and then simultaneous physical activity with seizure induction increased the OXA expression compared to only physical activity during seizure induction simultaneously. This finding illustrated that physical activity increased the OXA expression in the first four weeks, then decreased the OXA expression when it was elevated by seizure. To explain how physical activity reduced the severity of seizures through OXA, we should review the effect of orexin-A and its antagonists on GABA and glutamate processes. It has been mentioned that blockage of OXR1 and OXR2 reduced the seizure by decreasing glutamate as excitatory and increasing GABA as inhibitory contents (Goudarzi, Salmani, Lashkarbolouki & Goudarzi 2015). Also, a dual orexin antagonist reduced seizure by elevation of GABAergic inhibition (Konduru et al. 2022). Similar to orexin antagonists, it has been reported that physical activity reduced GABAergic loss in the hippocampus (Lim, Shin, Lee & Seo 2015).

Our findings suggested that physical activity might improve the imbalance of the inhibitory and excitatory systems through the orexin signaling pathway in epileptic rats.

Previous studies showed the involvement of BDNF in epileptogenesis (Egbenya, Hussain, Lai, Anderson & Davanger 2023). Serum level of BDNF was lower in individuals with epilepsy (LaFrance et al. 2010). The hippocampus of the epileptic animals indicated less BDNF expression than in non-epileptics (Flores-Soto et al. 2021). Similarly, our results illustrated the reduction effect of seizure on BDNF in different brain areas.

Some studies showed the up-regulation effect of physical activity on the BDNF expression (Alomari, Khabour, Alzoubi & Alzubi 2013, Fang et al. 2013, Wrann et al. 2013, Venezia, Quinlan & Roth 2017). Immobilization reduced BDNF expression in the hippocampus (Nooshinfar, Akbarzadeh-Baghban & Meisami 2011), and treadmill exercise improved BDNF deficits (Fang et al. 2013). Forced running exercise restored the down-regulation of the hippocampal BDNF in male Sprague–Dawley rats (Ji et al. 2014). Similarly, our result showed the up-regulation effect of physical activity on BDNF expression. This suggestion arises from the up-regulation effect of exercise on the 5-HT and noradrenaline (Idorn & thor Straten 2017), which trigger the cyclic adenosine 3′,5′-monophosphate (cAMP) and cAMP active response element binding protein. Response element binding protein up-regulates the BDNF expression

through the tropomyosin receptor kinase B (Rumajogee, Madeira, Vergé, Hamon & Miquel 2002). Our results demonstrated that physical activity restored the decrease of the BDNF expression that occurred following seizures. Similarly, resistance exercise restored the BDNF levels reduction in the homogenized hippocampus in the pilocarpine hydrochloride-induced epileptic rat (de Almeida et al. 2017). The reduction influence of physical activity on the BDNF in epilepsy might be explained by the inhibition effect of the BDNF on the GABAergic in epileptic brain (Marty, Wehrlé & Sotelo 2000). Then, exercise might decrease the severity of seizure by increasing the BDNF, which elevates the density of the inhibitory synapses (Lu, Cheng, Lim, Khoshnevisrad & Poo 2010). Additionally, our results indicated that running on the treadmill as a pre-induced seizure had a potential effect on restoration of the BDNF reduced by seizure. These findings suggested that the possible reduction effect of physical activity as pre-induced seizure on the severity of seizure might rise from the possible effect of it on the BDNF expression.

Studies have shown some correlation between the expression of orexin and BDNF. The OXA and BDNF had anti-depressive effects and improved memory (Chieffi et al. 2017). The individuals with Parkinson's indicated low levels of OXA and BDNF (Drouot et al. 2003, Scalzo, Kümmer, Bretas, Cardoso & Teixeira 2010). Exercise increased both of the OXA and BDNF (Messina et al. 2016). Also, the positive correlation between OXA and BDNF was reported in schizophrenia patients (Ren et al. 2022). The OXA increased BDNF expression in Parkinson's, and this function of OXA might be mediated through OXR1 in Parkinson's disease (Liu et al. 2018). It has been demonstrated that microinjection of orexin-A in the hippocampus elevated expression of the BDNF that decreased by capsaicin administration in the capsaicin-induced orofacial pain Wistar rats model (Kooshki, Abbasnejad, Esmaeili-Mahani & Raoof 2018).

Our results showed a relationship between the OXA and BDNF expression in the CA1, CA3, and cortex of some groups. In the CA1, CA3, and cortex, high-expressed OXA correlated with high-expressed BDNF in the normal saline group and a negative correlation in the seizure group. In the CA1 and cortex, high-expressed OXA correlated with high-expressed BDNF in the PA group. These findings showed that the expression of the OXA and BDNF correlated in healthy rats and in rats, which intervened with seizure or physical activity separately. The correlation between the OXA and BDNF might indicate the signaling interaction between these two factors. Our results showed no correlation between OXA and BDNF expression in epileptic

groups with physical activity. The interruption of cross-talking of OXA and BDNF pathways by exercise in the epileptic animals emphasized the modulatory effect of physical activity in the pathological condition.

Conclusion

It might be concluded that physical activity had different roles depending on the pathological and non-pathological conditions. The anticonvulsant effect of physical activity seemed to be dependent on the disruption of interactions between the orexin and BDNF pathways.

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Legends:

Table 1. *The Mean ± S.E.M of seizure behavior scores.* *,** , and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$ in comparison of the PA+PTZ and PA-PTZ groups to the seizure group. # and ## indicate $p < 0.05$ and $p < 0.01$, respectively in comparison of PA-PTZ group to PA+PTZ group.

Figure 1. Schematic plan of normal saline injection, physical activity, and seizure induction.

Figure 2. The effect of exercise on the SBS. The line graph indicated that the SBS decreased in the PA+PTZ and PA-PTZ compared to the seizure in some days of seizure induction. Also, the SBS notably decreased in the PA-PTZ compared to the PA+PTZ on some days of seizure induction. *,** , and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$ in comparison of the PA+PTZ and PA-PTZ groups to the seizure group. # and ## indicate $p < 0.05$ and $p < 0.01$, respectively in comparison of PA-PTZ group to PA+PTZ group.

Figure 3: Distribution of the OXA in the CA1, CA3, and cortex areas. **A)** Immunofluorescent microscopic images of the OXA expression in the CA1, CA3, and cortex in different groups. **B, C,** and **D)** Bar graphs summarizing the OXA expression. * symbol indicates a significant difference compared to the NS group. # symbol indicates a significant difference compared to the seizure group. × symbol indicates a significant difference compared to the PA group. + symbol indicates a significant difference compared to the PA+PTZ group. One, two, or three repetitions of symbols indicated $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Figure 4: Distribution of the BDNF in the CA1, CA3, and cortex areas. **A)** Immunofluorescent microscopic images of the BDNF expression in the CA1, CA3, and cortex in different groups. **B, C,** and **D)** Bar graphs summarizing the BDNF expression. * symbol indicates a significant difference compared to the NS group. # symbol indicates a significant difference compared to the seizure group. × symbol indicates a significant difference compared to the PA group. + symbol indicates a significant difference compared to the PA+PTZ group. One, two, or three repetitions of symbols indicated $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Figure 5: The correlation between protein expression of the OXA and BDNF of the CA1 of different groups. A, B, C, D, and E are scatter plots of the NS, seizure, PA, PA+PTZ, and PA-PTZ, respectively. There is a strong correlation between OXA and BDNF expression in the CA1 of the NS, seizure, and PA groups.

Figure 6: The correlation between protein expression of the OXA and BDNF in the CA1 of different groups. A, B, C, D, and E, are scatter plots of the NS, seizure, PA, PA+PTZ, and PA-PTZ, respectively. There is a strong correlation between OXA and BDNF expression in the CA1 of the NS and seizure groups.

Figure 7: The correlation between protein expression of the OXA and BDNF of the cortex of different groups. A, B, C, D, and E, are scatter plots of the NS, seizure, PA, PA+PTZ, and PA-PTZ, respectively. There is a strong correlation between OXA and BDNF expression in the CA1 of the NS, seizure, and PA groups.

Table 1

Groups / Day of injection	Seizure	PA+PTZ	PA-PTZ
1	0.27 ± 0.11	0	0
3	1.5 ± 0.71	0.17 ± 0.11*	0
5	1.3 ± 0.11	0.42 ± 0.08***	0.17 ± 0.11***
8	1.8 ± 0.11	1.3 ± 0.11**	0.92 ± 0.15***
10	1.8 ± 0.17	1.4 ± 0.15	0.83 ± 0.11***#
12	2.2 ± 0.17	1.5 ± 0.18*	1.2 ± 0.20**
15	2.7 ± 0.17	1.9 ± 0.15**	1.9 ± 0.21***#
17	3.3 ± 0.25	2.5 ± 0.22*	1.7 ± 0.17***#
19	3.7 ± 0.25	3.0 ± 0.29	2.2 ± 0.21***#
22	3.8 ± 0.28	3.0 ± 0.29	2.3 ± 0.15***
24	4.4 ± 0.33	3.8 ± 0.31	3.3 ± 0.21*
26	5.3 ± 0.31	4.4 ± 0.2*	3.4 ± 0.2***##

Figure 1.

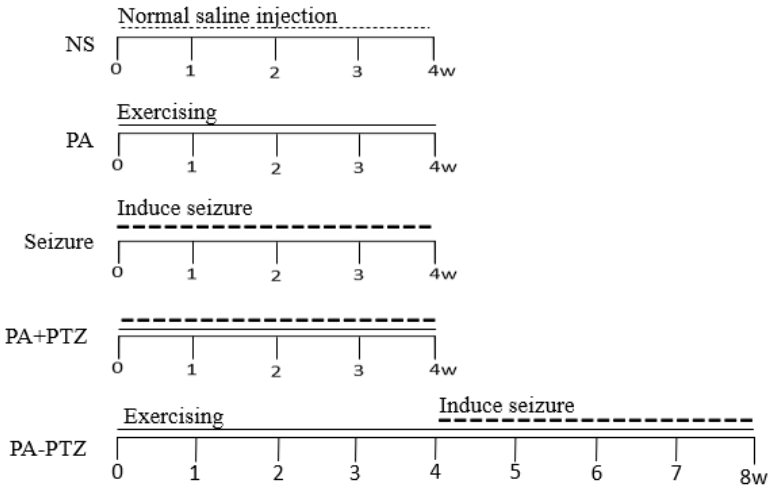

Figure 2.

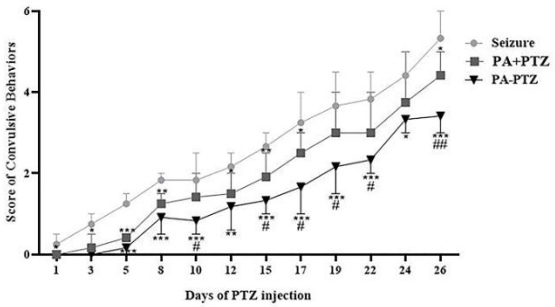

Figure 3:

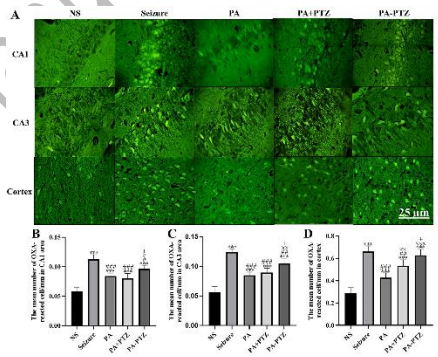

Figure 4:

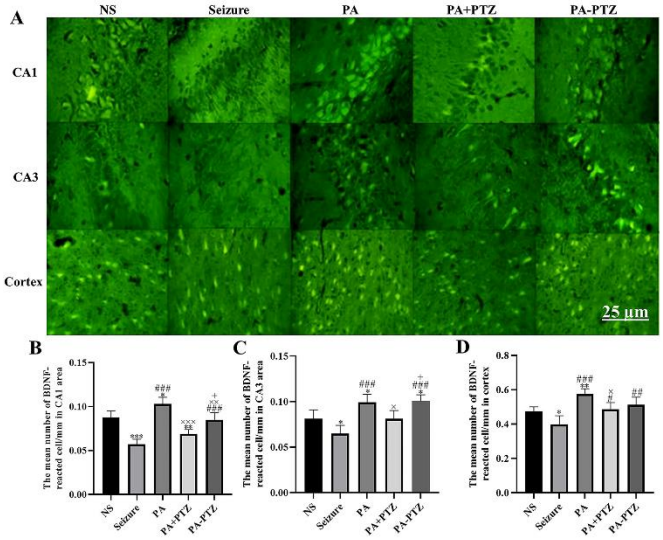

Figure 5:

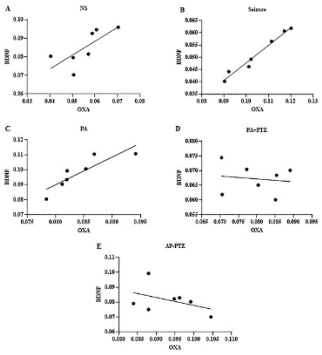

Figure 6:

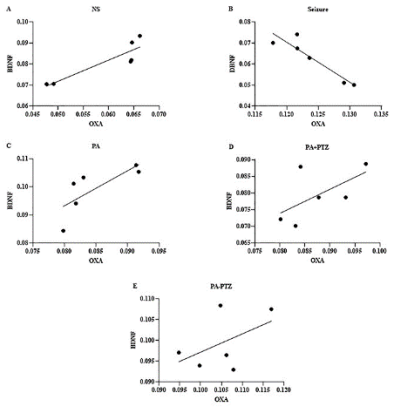

Figure 7: