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Chronic exercise buffers the cognitive dysfunction and decreases the susceptibility to seizures in PTZ-treated rats

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ABSTRACT

Epilepsy is a serious neurological disorder posing a severe burden to our society. Cognitive deficits are very common comorbidities of epilepsy. It is known that enhanced cognition has been demonstrated as an indicator for successful treatment of epilepsy. Physical exercise shows a positive consequence on cognition in healthy individuals and improves health and life conditions in people with epilepsy. However, there is no direct evidence to determine the role and the potential mechanism of physical exercise on the cognitive impairment and the relationship of susceptibility to seizures. The goal of the current investigation was to explore whether sustained physical exercise improves the cognitive dysfunction and simultaneously decreases the susceptibility to seizures in rats with epilepsy. Rats were treated with pentylenetetrazole (PTZ) (35 mg/kg, i.p. [intraperitoneally]) for 36 days to induce chronic epilepsy. During the induction period, rats were exposed to voluntary wheel running or forced swimming 30 min prior to each PTZ injection from the 16th day. The cognition of rats was evaluated by object recognition test and passive avoidance test. The susceptibility to seizures was evaluated by seizure frequency and duration. The levels of synaptic-related proteins including PSD95 (postsynaptic density 95), Synapsin, GluA1, and BDNF (brain-derived neurotrophic factor) were measured to evaluate the hippocampal synaptic plasticity. Furthermore, the GAD67 (glutamic acid decarboxylase) levels and GABA (γ-aminobutyric acid)ergic function in PTZ-treated rats were also determined. Finally, antagonist of $GABA_AR$ ($GABA_A$ receptors) bicuculline was used to explore the reversal effects of physical activity on seizures and cognition. The results showed that rats subjected to voluntary wheel running or forced swimming showed a significant reduction of seizure frequency and duration in PTZ-treated group relative to rats without running or swimming. In addition, both running and swimming improved cognitive function as measured by enhanced performance in object recognition test and passive avoidance test. Furthermore, the reduced levels of synaptic-related proteins and GABAergic function were reversed by exercise compared with rats without exercise. Moreover, antagonism of hippocampal CA3 (cornu ammonis 3) GABAergic neurons blocks the reversal effects of physical activity on seizures and cognition in PTZ-treated rats. These data showed that chronic physical exercise reduced the frequency of seizures and improved the cognitive function in a rat model of chronic epilepsy through normalization of CA3 synaptic plasticity and GABAergic function. Our findings suggest that chronic physical exercise has beneficial effects on controlling seizure through enhancement of cognition and highlights the possibility to translate into reduced seizure recurrence in people with epilepsy.

1. Introduction

Epilepsy is a serious neurological disorder caused by neuronal hyperexcitability in the brain. There are approximately 3.4 million people suffered from epilepsy in the United States in 2015, posing a severe burden to individuals, families, and society [1]. Cognitive deficits are very frequent conditions in patients with epilepsy as reflected by impaired learning and memory in both preclinical and clinical studies. It is seen that cognitive deficits are as high as 70–80% in patients with chronic epilepsies [2]. In addition, several animal models of epilepsy provide the evidence that epilepsy and seizures damage the brain and consequently result in functional decline and behavioral problems. In particular, better cognition has repeatedly been demonstrated as a marker of greater treatment outcome of epilepsy [3,4]. Consequently, some interventions that are positive to improve the cognitive decline may also potentially

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prevent the exacerbation of seizures and epilepsy. Physical exercise shows positive consequences on cognition in healthy individuals. Furthermore, several studies have demonstrated that physical exercise produced beneficial effects on seizure frequency and severity [5,6]. However, there is no direct evidence to determine the role and the potential mechanism of physical exercise on the cognitive impairment and the relationship of susceptibility to seizures.

Previous reports showed that modulation of normal hippocampal synapse may have an important role for cognitive improvement in epilepsy [7]. It may be a therapeutic strategy to restore normal synaptic activity in the hippocampus of an early phase of a mouse model of epilepsy. Additionally, the hippocampus is a vulnerable brain region that is usually damaged by excitotoxic agents such as pentylenetetrazole (PTZ), a noncompetitive $GABA_AR$ antagonist, that is widely used to reproduce epilepsy in animals with seizures [8]. However, whether the alterations of synaptic-related proteins such as postsynaptic density 95 (PSD95), Synapsin 1, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunit GluA1, and brain-derived neurotrophic factor (BDNF) in the hippocampus underlie the cognition deficits associated with epilepsy are still unknown.

γ-Aminobutyric acid (GABA) is one of the most inhibitory neurotransmissions in the brain to keep a balance between excitatory and inhibitory systems. It has been shown that dysfunction of the GABAergic system plays an important role in epilepsy, seizures, and epileptogenesis and that a focal GABAergic defect could be a primary epileptogenic mechanism [9–11]. In addition, alteration of synaptic $GABA_A$ receptors has been evidenced to participate in GABAergic inhibition and is associated with epilepsy [12,13]. Changes in GABAergic neuron numbers in CA3 (cornu ammonis 3) of the hippocampus may result in the reduction of inhibitory tonus leading to epileptic seizures in the entire brain. While the increase of GABA levels and activation of GABARs are evidenced to be protective against epilepsy in patients and in animal models [14]. Recent findings have demonstrated that exercise prevented GABAergic neuronal loss and increased glutamic acid decarboxylase (GAD67) expression in epileptic rats [15], suggesting that physical exercise as a novel intervention could oppose synaptic and GABAergic failure and cognitive impairment in patients with epilepsy.

According to the above evidence, we hypothesize that regular exercise buffers the cognitive dysfunction and decreases the susceptibility to seizures via normalization of hippocampal synaptic plasticity and GABAergic system. As such, the goal of the current investigation was to explore (i) whether sustained physical exercise improves the cognitive dysfunction in PTZ-induced epileptic rats; (ii) whether sustained physical exercise decreases the susceptibility to seizures in rats with epilepsy; and (iii) whether hippocampal synaptic plasticity and GABAergic function mediate the reversal effects of physical activity on seizures and cognition in PTZ-treated rats.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (weighed 220–240 g) were housed at a constant temperature (23 ± 2 °C) and maintained on a 12-h light/dark cycle (lights on, 07:30 h) with free access to food and water. All of the animal procedures were performed in accordance with the National Institutes of Health's 'Guide for the Care and Use of Laboratory Animals', and the procedures were approved by the Ethics of Animal Experiments of Jinan Central Hospital (permit number: SA-2017-021). All of the behavioral tests, physical exercise, and drug administrations were carried out in the active phase of animals. The investigation conforms to the Regulations on the Administration of Laboratory Animals approved by the State Council and published by the Ministry of Science and Technology of China (1998).

2.2. Drugs

Pentylenetetrazole was supplied from Sigma-Aldrich Chemical Company (Sigma, USA), and $GABA_AR$ antagonist bicuculline methiodide was purchased from Abcam (Abcam, USA). Pentylenetetrazole was dissolved in sterile saline, and was given at a dose of 35 mg/kg, intraperitoneally (i.p.), in a volume which did not exceed 2 ml/100 g body weight. Bicuculline methiodide was dissolved in saline and was infused into CA3 at a dose of 25 ng as described previously [16].

2.3. PTZ-induced seizure of epilepsy

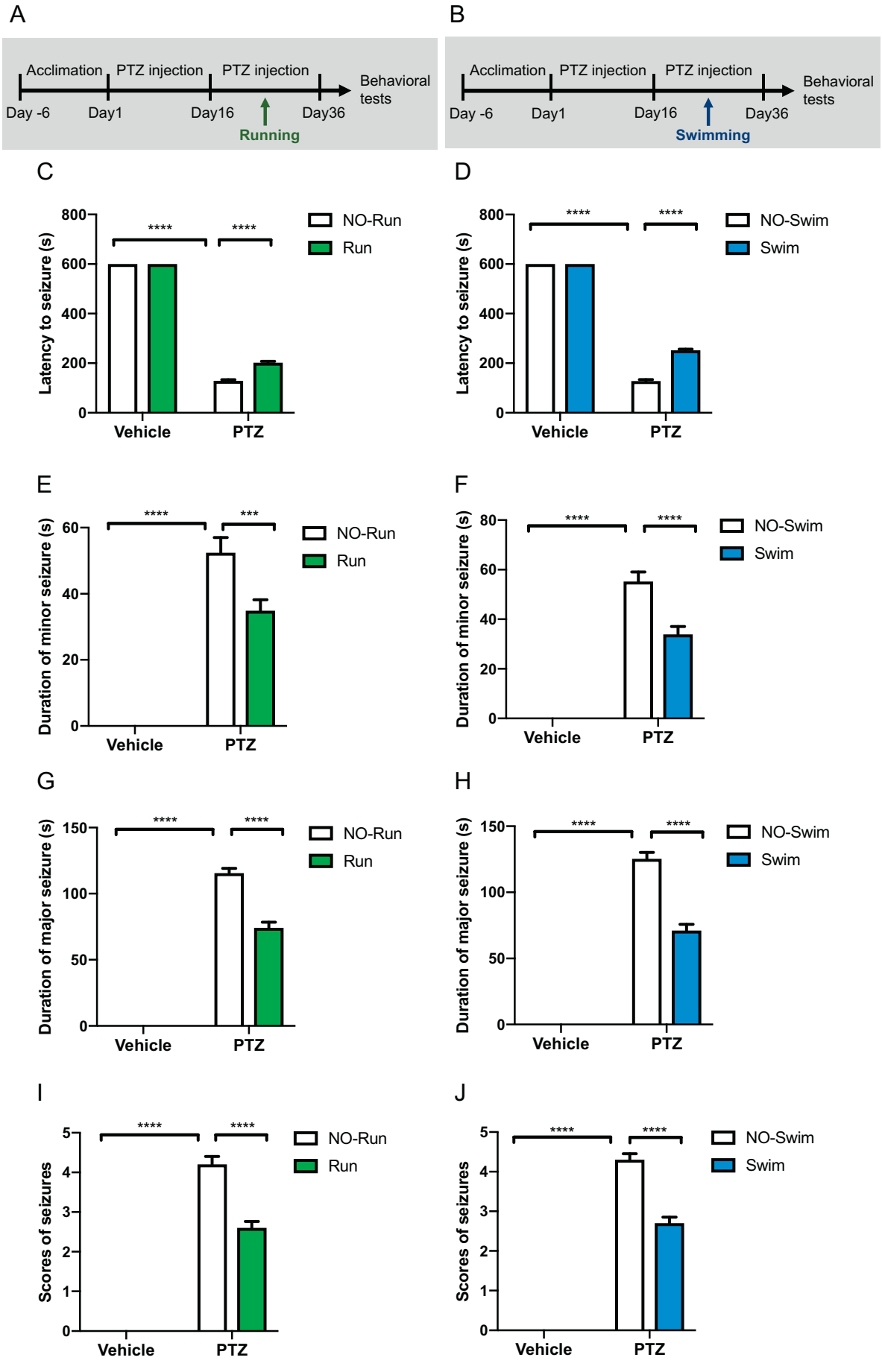
Rats were deprived of food but not water 12 h before the experiments to prevent aspiration of food. Rats received one injection of PTZ each day for seizure induction at a dose of 35 mg/kg, i.p., for the entire 36 days [17,18]. The control rats were given an intraperitoneal injection of normal saline solution. The epileptic seizure activity induced by PTZ was evaluated by latency to seizures (s), duration of the minor seizure onset (s), duration of the major seizure onset (s), and scores for the severity of seizures in 1 h after PTZ injection [19]. The minor seizure onset was termed as isolated myoclonic jerks and clonic seizures accompanied by facial and front extremity muscle clonus; while the major seizure following the minimal seizure is characterized by head, neck, and tail extension with the loss of the tonic flexor reflex and tonic flexion–extension following the protracted clonus [20]. The scores (0–6) were used to measure the severity of seizures after PTZ administration according to the following levels: 0: no changes in behavior;, 1: isolated myoclonic jerks and/or sudden behavioral arrest;, 2: only atypical minimal seizures;, 3: minimal seizures;, 4: major seizures without a tonic phase;, 5: completed tonic–clonic seizures while lying on the belly and/or pure tonic seizures; and 6: completed tonic–clonic seizures while lying on the side and/or wild jumping [21,22]. The performance of each rat was recorded by a video camera during the whole experimental procedures. The observers for the measurement of seizure scores were blind to the treatment of each group.

2.4. Physical exercise procedure

Rats were exposed to 15-min swimming exercise in a clear Plexiglas cylinder 25 cm in diameter and 65 cm high filled to a depth of 30 cm with 23–25 °C water. Rats were placed to swim 30 min prior to each injection of PTZ. A separate group of rats were exposed to daily 30-min voluntary wheel running similarly 30 min before each PTZ injection. The wheel channel (450 mm × 240 mm × 500 mm) was fixed in a heavy polycarbonate plastic base. The wheel was made of wire mesh with 450 mm in outer diameter, 300 mm in inner diameter, and 65 mm in width. The running time and distance were displayed in the operating system. Both swimming and running rats occurred between 8 am–10 pm and started from the 16th day until the end of the procedure on day 36.

2.5. Novel object recognition (NOR) test

The NOR (novel object recognition) test is widely used to assess cognitive impairment in several animal models for measurement of short-term/working memory [23,24]. On day 36, 2 h after the last injection of PTZ, the NOR test was performed. The procedure was conducted as previous descriptions with minor modifications [24–26]. In the training session, rats were exposed to two identical triangle objects for 10 min in a wood container (48 × 35 × 20 cm). For a 15-min interval, memory was assessed in a test session. Rats were subjected to two different objects, a familiar (triangle) and a novel (square) one in the same wood container. During the 3-min test session, familiar and novel objects were placed in positions identical to the training phase. The container and objects were cleaned with 70% alcohol, and air-dried after each animal exposure. Exploration time (s) was quantified by measuring the time animals


A
Acclimation
PTZ injection
PTZ injection
Behavioral tests
Day -6
Day1
Day16
Day36
Running
B
Acclimation
PTZ injection
PTZ injection
Behavioral tests
Day -6
Day1
Day16
Day36
Swimming
C
Latency to seizure (s)
NO-Run
Run
Vehicle
PTZ
D
Latency to seizure (s)
NO-Swim
Swim
Vehicle
PTZ
E
Duration of minor seizure (s)
NO-Run
Run
Vehicle
PTZ
F
Duration of minor seizure (s)
NO-Swim
Swim
Vehicle
PTZ
G
Duration of major seizure (s)
NO-Run
Run
Vehicle
PTZ
H
Duration of major seizure (s)
NO-Swim
Swim
Vehicle
PTZ
I
Scores of seizures
NO-Run
Run
Vehicle
PTZ
J
Scores of seizures
NO-Swim
Swim
Vehicle
PTZ

touched the object with their nose and was recorded by a camera. Finally, a recognition ratio was obtained as ratio of time spent for exploring of novel to familiar objects.

2.6. Passive avoidance test (PAT)

The PAT (passive avoidance test) was conducted on day 36 to test memory deficits after corresponding treatment (PTZ injection, physical activity, and $GABA_AR$ antagonist infusion) according to specific administration of each experiment. The experimental protocol was performed as previously described [27,28]. The passive avoidance apparatus consisted of a dark and a light compartment with same dimensions (20 × 20 × 30 cm). The two compartments were separated by a guillotine door. The dark compartment had a stainless-steel shock grid floor. During the acquisition trial, each animal was placed in the light chamber. After a 60-s habituation period, the guillotine door was opened, and the initial latency of the animals to enter the dark chamber was recorded. Rats with initial latency time more than 60 s were excluded from further experiments. After the rat had entered the dark chamber, the guillotine door was closed, and an electric foot shock (50 Hz; 2 mA; 1.5 s) was delivered to the floor grid using a stimulator for 3 s. Five seconds later, the animal was removed from the dark chamber and returned to its home cage. After 24 h, the retention latency time was measured in the same way as that of the acquisition trial without the foot shock. The latency time and the time in dark compartment, and the time in light compartment were recorded to a maximum of 600 s.

2.7. Open-field test (OPT)

The locomotor activity of rats was measured in the open-field test (OPT) as previously described. Briefly, the apparatus consisted of a 75 × 75 × 40 cm square arena divided into 25 equal squares (15 × 15 cm). A single rat was placed in the center of the apparatus, and the total distance (cm) was recorded by a connected computer according to the tracking system in 10 min. The OPT was conducted immediately after NOR on day 36.

2.8. Intracerebral cannula implantation and intracranial injections

Rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), and guide cannulae (23-gauge, Plastics One, USA) were implanted bilaterally 1 mm above the CA3 of the hippocampus. The stereotaxic coordinates for CA3 were anterior/posterior (AP): −3.8 mm, lateral (L): ±3.8 mm, and dorsal/ventral (DV): −3.2 mm [29,30]. Vehicle or bicuculline was intracranially microinjected using 10 ml Hamilton syringes (USA) that were connected via polyethylene-50 tubing to 30-gauge injectors (Plastics One). A total volume of 0.5 μl was infused into each side during 5 min. At the end of the experiments, cannula placements were assessed using Nissl staining. Subjects with misplaced cannulae were excluded from the statistical analysis.

2.9. Tissue sample preparation

Rats were killed 30 min after the final administration of PTZ and physical exercise exposure. Their brains were extracted, and bilateral hippocampal CA3 tissue punches were obtained from approximately 1 mm thick coronal sections cut using a freezing cryostat at −20 °C. The rostral faces of the coronal sections were approximately 3.8 mm from bregma [29,30]. Tissue punches were homogenized (10–15 s × 3; 5-s interval) with an electrical disperser (Wiggenhauser, Sdn Bhd) after being lysed with ice-cold RIPA (radio immunoprecipitation assay) lysis buffer with protease inhibitor (Beyotime Biotechnology, Beijing, China) for 30 min. Subsequently, the homogenate was subjected to 10,000 × *g* centrifugation at 4 °C for 10 min. The supernatant fractions were collected, and protein concentrations of all samples were determined using the BCA (bicinchoninic acid) assay kit (Beyotime Biotechnology). The protein concentration of each sample was equalized by adding RIPA lysis buffer.

2.10. Western blot assay

Samples with equal protein (~30 μg/well) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8% acrylamide/0.27% *N*,*N*′-methylenebisacryalamide resolving gel) for approximately 30 min at 80 V in stacking gel and approximately 1 h at 120 V in resolving gel. Proteins were transferred electrophoretically to Immobilon-P transfer membranes (Millipore, Bedford, MA, USA) at 0.25 A for 3 h. Membranes were washed with TBST (tris-buffered saline plus 0.05% Tween-20, pH 7.4) before dipping in blocking buffer (5% skimmed dry milk in TBST) overnight at 4 °C. Membranes were then incubated for 1 h at room temperature with anti-PSD95 (1:1000; Abcam, USA), anti-Synapsin 1 (1:2000; Bioss, China), anti-GluA1 (1:2000; Abcam, USA), anti-BDNF (1:2000; Abcam, USA), anti-GAD67 (1:2000; Bioss, China), anti-$GABA_A$ receptor (1:1000; Millipore, USA), and anti-β-actin antibody (1:2000; Sigma, USA) in TBST plus 5% bovine serum albumin. After the membrane was shaken in 4 × 6-min washes in TBST buffer, the blots were incubated for 45 min at room temperature with horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit or mouse IgG (immunoglobulin G); Santa Cruz Biotechnology and Vector Labs, respectively) diluted 1:5000 in 5% nonfat milk in TBST. The blots were then shaken in 4 × 6-min washes in TBST. Afterward, the blots were incubated with enhanced chemiluminescence (ECL) reagent (Thermo, Marina, CA, USA) for 1 min at room temperature and were directly imaged using ChemiDoc™ MP (Bio-Rad, CA, USA). Finally, the band intensities for target proteins were normalized to β-actin protein expressions with Image Lab Software V5.1 (Bio-Rad, CA, USA).

2.11. Data analysis

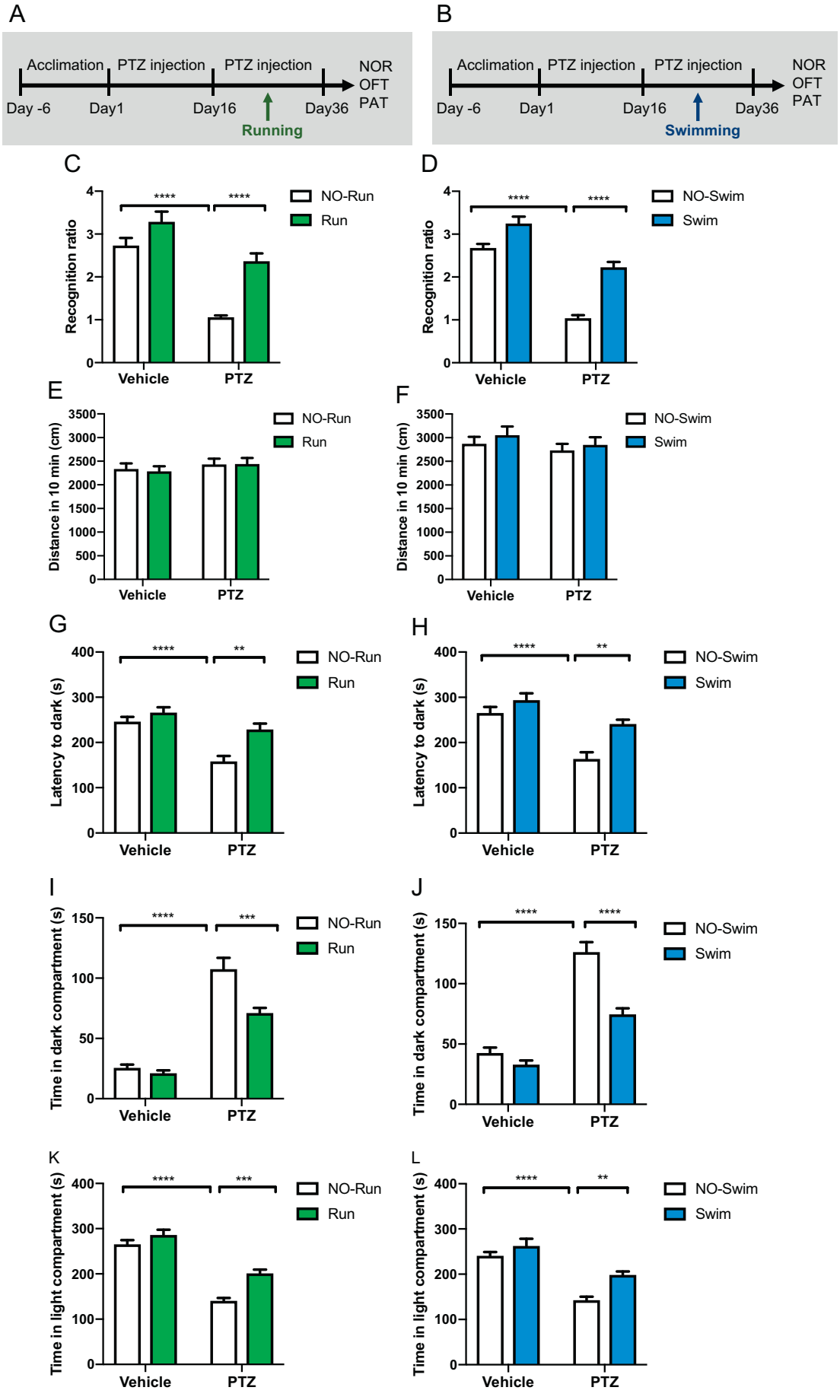
The data were expressed as mean ± SEM (standard error of the mean) and were analyzed using two-way analysis of variance (ANOVA) followed by Tukey's post hoc test (for details, see Results section). In the two-way ANOVA tests, to determine the effects of physical activity on the seizures and cognition induced by PTZ, the following two factors are involved: 1. PTZ and saline groups and 2. physical activity and no physical activity exposures; and to determine the role of GABAergic function in the reversal effects of physical activity, the following two factors are involved: 1. physical activity and no physical activity exposures and 2. bicuculline and vehicle treatments. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Physical activity reduces seizure frequency and duration in PTZ-treated rats

We selected daily running and swimming as two types of aerobic physical activity in rats. The effects of running and swimming exercises

Fig. 1. Physical activity reduces seizure frequency and duration in PTZ-treated rats. (A) Schematic of the experimental design for running exercise. (B) Schematic of the experimental design for swimming exercise. (C) The latency to seizure in rats treated with PTZ or vehicle with chronic running exposure. (D) The latency to seizure in rats treated with PTZ or vehicle with chronic swimming exposure. (E) The duration of minor seizure in rats treated with PTZ or vehicle with chronic running exposure. (F) The duration of minor seizure in rats treated with PTZ or vehicle with chronic swimming exposure. (G) The duration of major seizure in rats treated with PTZ or vehicle with chronic running exposure. (H) The duration of major seizure in rats treated with PTZ or vehicle with chronic swimming exposure. (I) The scores of seizures in rats treated with PTZ or vehicle with chronic running exposure. (J) The scores of seizures in rats treated with PTZ or vehicle with chronic swimming exposure. Data were expressed as mean ± SEM. $^{***}p < 0.001$, $^{****}p < 0.0001$, different from other experimental groups (n = 10 mice/group). PTZ, pentylenetetrazole.


A
Acclimation
PTZ injection
PTZ injection
NOR
OFT
PAT
Day -6
Day1
Day16
Day36
Running
B
Acclimation
PTZ injection
PTZ injection
NOR
OFT
PAT
Day -6
Day1
Day16
Day36
Swimming
C
Recognition ratio
NO-Run
Run
Vehicle
PTZ
D
Recognition ratio
NO-Swim
Swim
Vehicle
PTZ
E
Distance in 10 min (cm)
NO-Run
Run
Vehicle
PTZ
F
Distance in 10 min (cm)
NO-Swim
Swim
Vehicle
PTZ
G
Latency to dark (s)
NO-Run
Run
Vehicle
PTZ
H
Latency to dark (s)
NO-Swim
Swim
Vehicle
PTZ
I
Time in dark compartment (s)
NO-Run
Run
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J
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NO-Swim
Swim
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K
Time in light compartment (s)
NO-Run
Run
Vehicle
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L
Time in light compartment (s)
NO-Swim
Swim
Vehicle
PTZ

on the seizures induced by chronic PTZ administration were tested using two separate four groups of rats in a 2 (treatment: vehicle and PTZ) × 2 (physical activity: no running/swimming and running/swimming) factorial design (Fig. 1A, B). In the vehicle groups, the latency to seizures was as maximum as 600 s, suggesting no seizure symptom; while the latency to seizures in PTZ groups was significantly decreased ($p < 0.0001$). However, both running and swimming markedly increased the latency to seizures compared with rats without exposure to physical exercise (Fig. 1C, D), indicating the seizure has been alleviated. We analyzed the latency to seizures using two-way ANOVA, with the between-subjects factors treatment and physical activity. The statistical analysis revealed significant effects of treatment ($F_{(1, 36)} = 14{,}352$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 102.1$; $p < 0.0001$) and a significant treatment × physical activity interaction for running ($F_{(1, 36)} = 102.1$; $p < 0.0001$) (Fig. 1C). The statistical analysis also revealed significant effects of treatment ($F_{(1, 36)} = 11{,}075$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 252.9$; $p < 0.0001$) and a significant treatment × physical activity interaction for swimming ($F_{(1, 36)} = 252.9$; $p < 0.0001$) (Fig. 1D).

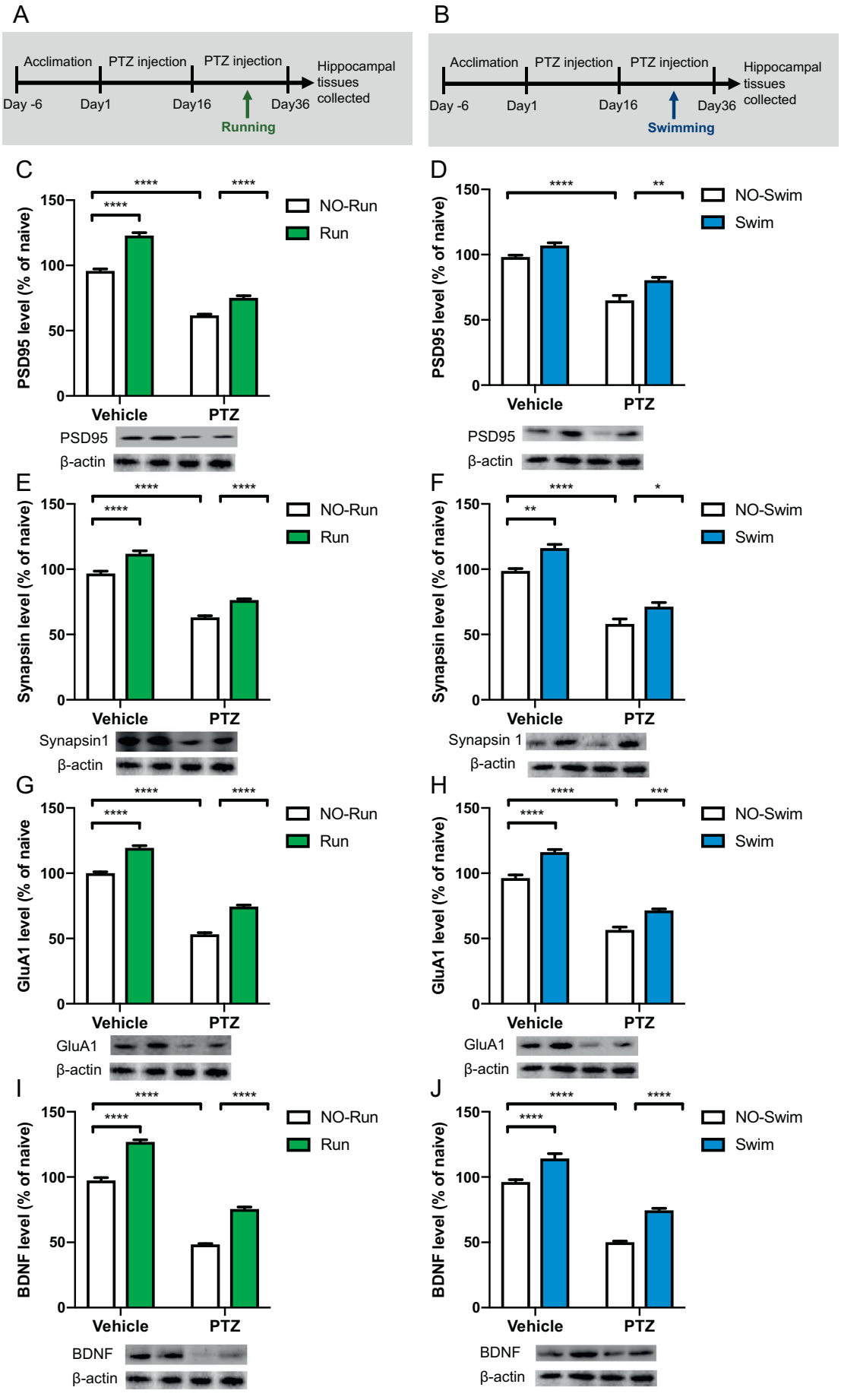
In the vehicle groups, the duration of minor (Fig. 1E, F) and major (Fig. 1G, H) seizures, and the scores of seizures (Fig. 1I, J) were as low as zero, indicating there is no seizure induction compared with PTZ-treated groups. However, both running and swimming markedly reversed these abnormal behaviors compared with rats without exposure to physical exercise (Fig. 1E–J). The two-way ANOVA showed revealed significant effects of treatment ($F_{(1, 36)} = 238.6$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 9.586$; $p < 0.01$) and a significant treatment × physical activity interaction for running ($F_{(1, 36)} = 9.586$; $p < 0.01$) on the duration of minor seizures (Fig. 1E). The statistical analysis also revealed significant effects of treatment ($F_{(1, 36)} = 310.9$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 17.76$; $p < 0.001$) and a significant treatment × physical activity interaction for swimming on the duration of minor seizures ($F_{(1, 36)} = 17.76$; $p < 0.001$) (Fig. 1F). The statistical analysis for the duration of major seizures revealed significant effects of treatment ($F_{(1, 36)} = 1174$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 55.85$; $p < 0.01$) and a significant treatment × physical activity interaction for running ($F_{(1, 36)} = 55.85$; $p < 0.01$) (Fig. 1G) and significant effects of treatment ($F_{(1, 36)} = 848.2$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 64.6$; $p < 0.01$) and a significant treatment × physical activity interaction for swimming ($F_{(1, 36)} = 64.6$; $p < 0.01$) (Fig. 1H). The statistical analysis for the scores of seizures revealed significant effects of treatment ($F_{(1, 36)} = 693.6$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 38.4$; $p < 0.01$) and a significant treatment × physical activity interaction for running ($F_{(1, 36)} = 38.4$; $p < 0.01$) (Fig. 1I) and significant effects of treatment ($F_{(1, 36)} = 1050$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 54.86$; $p < 0.01$) and a significant treatment × physical activity interaction for swimming ($F_{(1, 36)} = 54.86$; $p < 0.01$) (Fig. 1J). The post hoc analysis showed that the duration of minor and major seizures, and the scores of seizures in rats in the PTZ-trained group exposed to the physical exercise were lower than those rats without exercise, suggesting that chronic physical exercise activity has the potential to reduce the seizures in PTZ-induced epilepsy.

3.2. Physical activity improves cognitive impairment in PTZ-treated rats

Next, we determined whether chronic physical exercise could improve the cognitive deficits induced by repeated PTZ administration. We also used a 2 (treatment: vehicle and PTZ) × 2 (physical activity: no running/swimming and running/swimming) factorial design to detect the effects of chronic physical exercise on the cognition as measured in NOR and PAT (Fig. 2A, B). In the NOR test, we found that the recognition ratio was decreased in the PTZ groups compared to vehicle groups, suggesting a weak cognitive performance (Fig. 2C, D). However, rats that received running/swimming during PTZ-injection showed significant increases in recognition ratio in both vehicle and PTZ groups. The two-way ANOVA showed revealed significant effects of PTZ treatment ($F_{(1, 36)} = 53.94$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 27.83$; $p < 0.0001$) and a significant treatment × physical activity interaction for running ($F_{(1, 36)} = 4.531$; $p < 0.05$) on the recognition ratio (Fig. 2C). Similarly, the statistical analysis also revealed significant effects of PTZ treatment ($F_{(1, 36)} = 129.3$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 56.62$; $p < 0.001$) and a significant treatment × physical activity interaction for swimming on the recognition ratio ($F_{(1, 36)} = 6.933$; $p < 0.05$) in the NOR test (Fig. 2D). To exclude the possible impact of hyperactivity or hypoactivity in the spontaneous activity on the performance of rats in the NOR test, we measured the locomotion activity of rats in the OPT immediately after the NOR test. The data showed that there is no significant difference of total distance traveled between vehicle and PTZ treatment that was observed among running or swimming groups (Fig. 2E, F).

We further determine the role of chronic running and swimming on the cognition in the PAT. Rats in PTZ groups showed significant impairments of cognition as indicated by decreased latency to dark compartment (Fig. 2G, H), increased time spent in dark compartment (Fig. 2I, J), as well as reduced time in light compartment (Fig. 2K, L). However, rats exposed to chronic running or swimming exercise during PTZ induction procedure showed significant improvement of cognitive function in the PAT. Specifically, the two-way ANOVA showed revealed significant effects of PTZ treatment ($F_{(1, 36)} = 27.90$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 14.61$; $p < 0.001$) and a treatment × physical activity interaction for running ($F_{(1, 36)} = 4.485$; $p < 0.05$) on the latency to dark compartment (Fig. 2G). Likely, the statistical analysis also revealed significant effects of PTZ treatment ($F_{(1, 36)} = 33.25$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 15.6$; $p < 0.001$) for swimming on the latency to dark compartment (Fig. 2H). In the assessment of time spent in dark compartment, the statistical analysis also revealed significant effects of PTZ treatment ($F_{(1, 36)} = 143.3$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 13.91$; $p < 0.001$) and a significant treatment × physical activity interaction for running ($F_{(1, 36)} = 8.475$; $p < 0.01$) (Fig. 2I; and significant effects of PTZ treatment ($F_{(1, 36)} = 125.0$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 29.78$; $p < 0.0001$) and a significant treatment × physical activity interaction for swimming ($F_{(1, 36)} = 14.03$; $p < 0.001$) (Fig. 2J). According to the time spent in light compartment, the statistical analysis also revealed significant effects of PTZ treatment ($F_{(1, 36)} = 135.4$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 20.41$; $p < 0.001$) and a significant treatment × physical activity interaction for running ($F_{(1, 36)} = 4.942$; $p < 0.05$) (Fig. 2K); and significant effects of PTZ treatment ($F_{(1, 36)} = 59.15$; $p < 0.0001$) and physical activity ($F_{(1, 36)} = 13.54$; $p < 0.001$) (Fig. 2L). These data suggested that the cognition impaired by repeated PTZ treatment could be improved by regular exercise including both running and swimming in rats.

Fig. 2. Physical activity improves cognitive impairment in PTZ-treated rats. Physical activity reduces seizure frequency and duration in PTZ-treated rats. (A) Schematic of the experimental design for running exercise. (B) Schematic of the experimental design for swimming exercise. (C) The recognition ratio in rats treated with PTZ or vehicle with chronic running exposure in the NOR test. (D) The recognition ratio in rats treated with PTZ or vehicle with chronic swimming exposure in the NOR test. (E) The distance measured in OFT of rats treated with PTZ or vehicle with chronic running exposure. (F) The distance measured in OFT of rats treated with PTZ or vehicle with chronic swimming exposure. (G) The latency to dark in PAT of rats treated with PTZ or vehicle with chronic running exposure. (H) The latency to dark in PAT of rats treated with PTZ or vehicle with chronic swimming exposure. (I) The time in dark compartment in PAT of rats treated with PTZ or vehicle with chronic running exposure. (J) The time in dark compartment in PAT of rats treated with PTZ or vehicle with chronic swimming exposure. (K) The time in light compartment in PAT of rats treated with PTZ or vehicle with chronic running exposure. (L) The time in light compartment in PAT of rats treated with PTZ or vehicle with chronic swimming exposure. Data were expressed as mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, different from other experimental groups (n = 10 mice/group). PTZ, pentylenetetrazole; NOR, novel object recognition; OFT, open-field test; PAT, passive avoidance test.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Acclimation
PTZ injection
PTZ injection
Hippocampal tissues collected
Day -6
Day1
Day16
Day36
Running
B
Acclimation
PTZ injection
PTZ injection
Hippocampal tissues collected
Day -6
Day1
Day16
Day36
Swimming
C
PSD95 level (% of naive)
NO-Run
Run
Vehicle
PTZ
PSD95
β-actin
D
PSD95 level (% of naive)
NO-Swim
Swim
Vehicle
PTZ
PSD95
β-actin
E
Synapsin level (% of naive)
NO-Run
Run
Vehicle
PTZ
Synapsin1
β-actin
F
Synapsin level (% of naive)
NO-Swim
Swim
Vehicle
PTZ
Synapsin 1
β-actin
G
GluA1 level (% of naive
NO-Run
Run
Vehicle
PTZ
GluA1
β-actin
H
GluA1 level (% of naive)
NO-Swim
Swim
Vehicle
PTZ
GluA1
β-actin
I
BDNF level (% of naive)
NO-Run
Run
Vehicle
PTZ
BDNF
β-actin
J
BDNF level (% of naive)
NO-Swim
Swim
Vehicle
PTZ
BDNF
β-actin

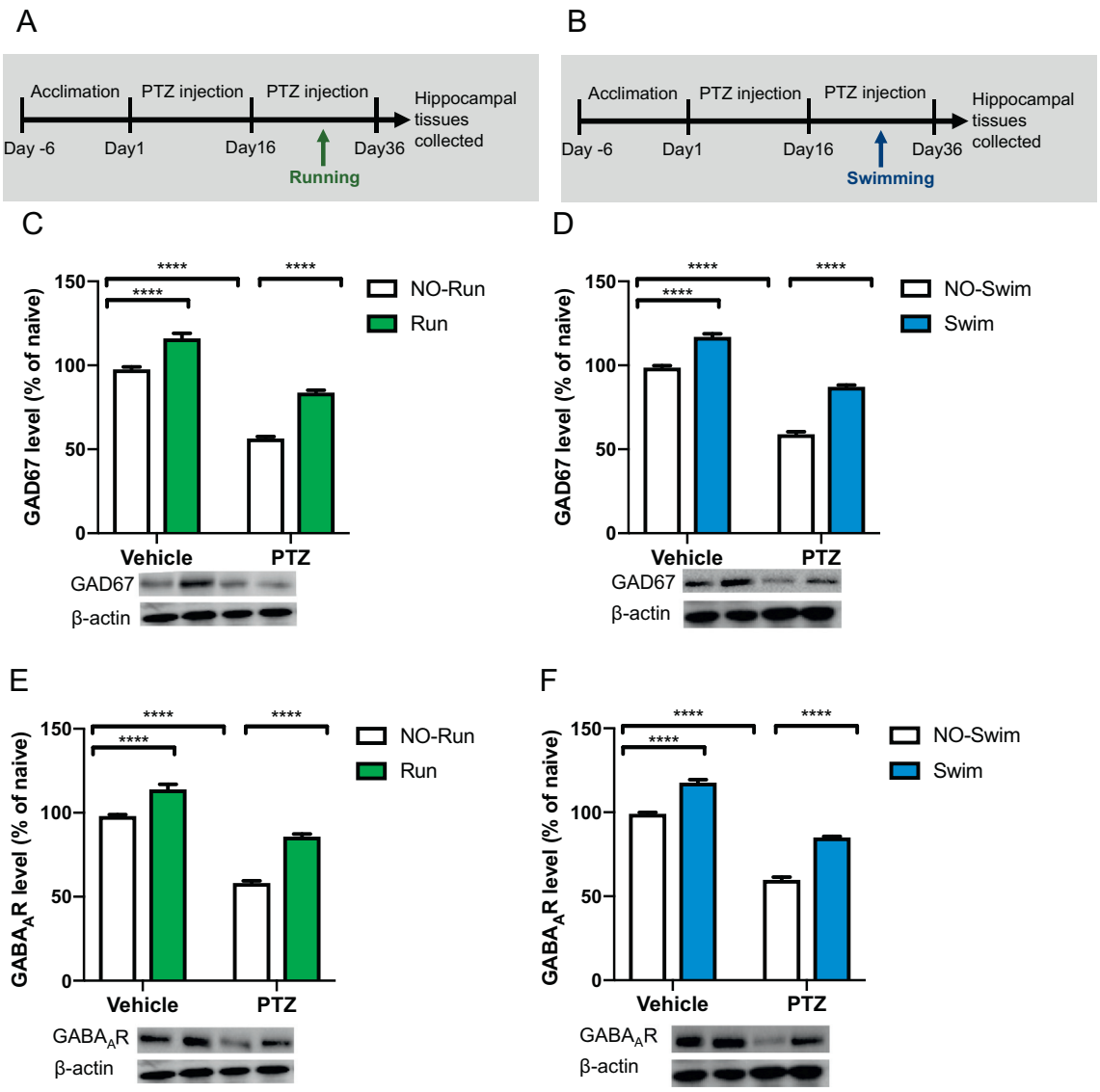
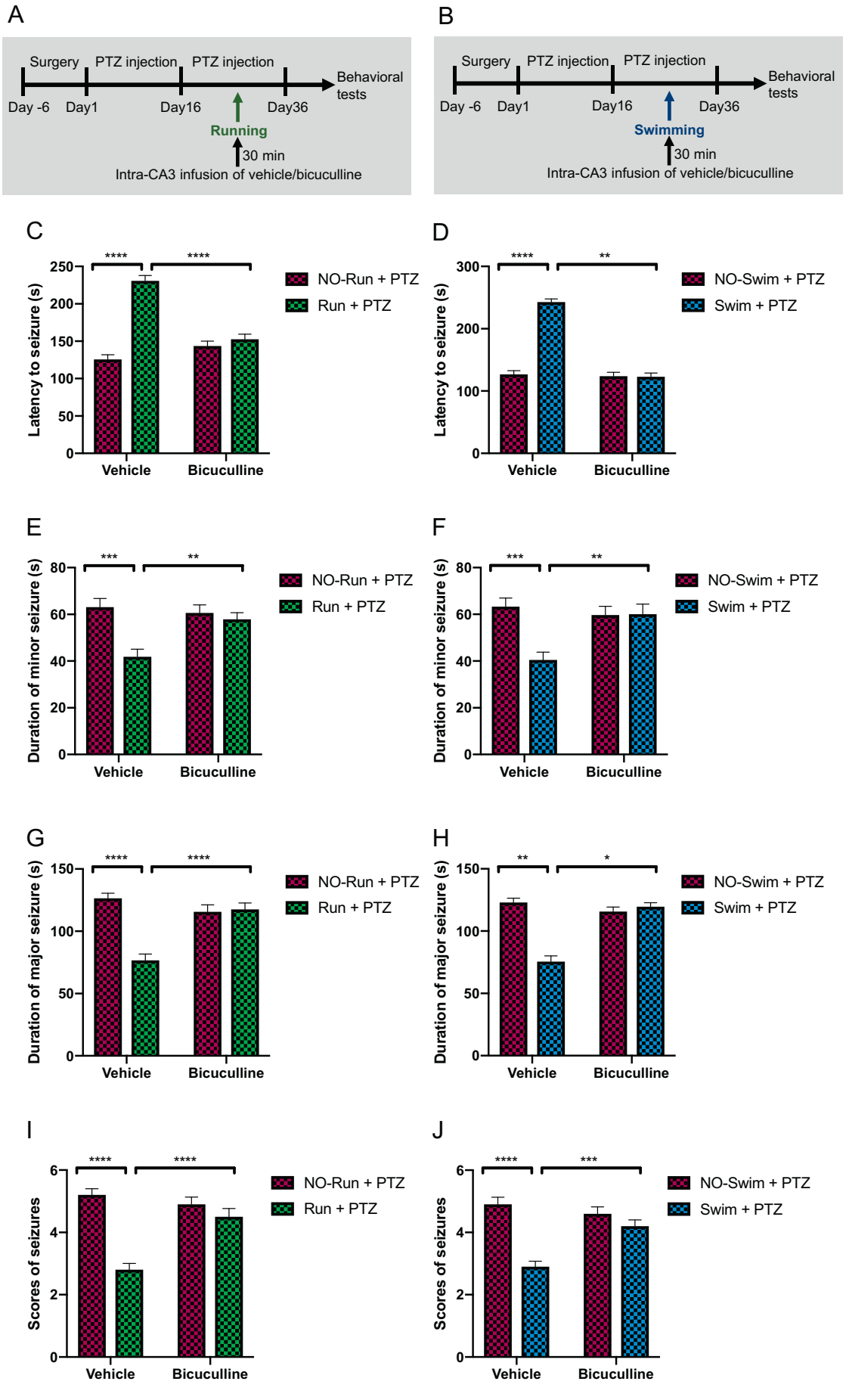


Fig. 4. Physical activity enhances hippocampal GAD67 levels and GABAergic function in PTZ-treated rats. (A) Schematic of the experimental design for running exercise. (B) Schematic of the experimental design for swimming exercise. (C) GAD67 levels of hippocampal CA3 in rats treated with PTZ or vehicle with chronic running exposure. (D) GAD67 levels of hippocampal CA3 in rats treated with PTZ or vehicle with chronic swimming exposure. (E) $GABA_AR$ levels of hippocampal CA3 in rats treated with PTZ or vehicle with chronic running exposure. (F) $GABA_AR$ levels of hippocampal CA3 in rats treated with PTZ or vehicle with chronic swimming exposure. The corresponding representative bands from Western blot were shown on the bottom of the bar. Data were expressed as mean ± SEM. $^{****}p < 0.0001$, different from other experimental groups (n = 6 mice/group). PTZ, pentylenetetrazole.

3.3. Physical activity alleviates hippocampal synaptic deficits in PTZ-treated rats

Chronic physical activity enhances the hippocampal synaptic plasticity and reverses the cognitive dysfunction in several brain disorders, but whether hippocampal synapses mediated in the beneficial effects of physical exercise in PTZ-treated epileptic rats are unknown. Therefore, we determined the functional role of hippocampal synaptic plasticity in the enhanced cognition of rats induced by chronic exposure to running/swimming. For this purpose, we measured synaptic-related proteins including PSD95, Synapsin 1, GluA1, and BDNF in hippocampal CA3 subregions of rats subjected to vehicle or PTZ with or without physical exercise (Fig. 3A, B). Western blot assays showed that PTZ treatment significantly decreased the levels of PSD95, Synapsin, GluA1, and BDNF compared to vehicle groups. However, rats that received chronic physical exercise (running or swimming) produced remarkably increases in these synaptic-related proteins relative to rats that received no physical exercise. The statistical analysis revealed significant effects of PTZ treatment ($F_{(1, 20)} = 644.4$; $p < 0.0001$) and physical activity ($F_{(1, 20)} = 17.87$; $p < 0.001$) and a significant treatment × physical activity

Fig. 3. Physical activity alleviates hippocampal synaptic deficits in PTZ-treated rats. (A) Schematic of the experimental design for running exercise. (B) Schematic of the experimental design for swimming exercise. (C) PSD95 levels of hippocampal CA3 in rats treated with PTZ or vehicle with chronic running exposure. (D) PSD95 levels of hippocampal CA3 in rats treated with PTZ or vehicle with chronic swimming exposure. (E) Synapsin 1 levels of hippocampal CA3 in rats treated with PTZ or vehicle with chronic running exposure. (F) Synapsin 1 levels of hippocampal CA3 in rats treated with PTZ or vehicle with chronic swimming exposure. (G) GluA1 levels of hippocampal CA3 in rats treated with PTZ or vehicle with chronic running exposure. (H) GluA1 levels of hippocampal CA3 in rats treated with PTZ or vehicle with chronic swimming exposure. (I) BDNF levels of hippocampal CA3 in rats treated with PTZ or vehicle with chronic running exposure. (J) BDNF levels of hippocampal CA3 in rats treated with PTZ or vehicle with chronic swimming exposure. The corresponding representative bands from Western blot were shown on the bottom of the bar. Data were expressed as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$, different from other experimental groups (n = 6 mice/group). PTZ, pentylenetetrazole.


A
Surgery
PTZ injection
PTZ injection
Behavioral tests
Day -6
Day1
Day16
Day36
Running
30 min
Intra-CA3 infusion of vehicle/bicuculline
B
Surgery
PTZ injection
PTZ injection
Behavioral tests
Day -6
Day1
Day16
Day36
Swimming
30 min
Intra-CA3 infusion of vehicle/bicuculline
C
Latency to seizure (s)
NO-Run + PTZ
Run + PTZ
Vehicle
Bicuculline
D
Latency to seizure (s)
NO-Swim + PTZ
Swim + PTZ
Vehicle
Bicuculline
E
Duration of minor seizure (s)
NO-Run + PTZ
Run + PTZ
Vehicle
Bicuculline
F
Duration of minor seizure (s)
NO-Swim + PTZ
Swim + PTZ
Vehicle
Bicuculline
G
Duration of major seizure (s)
NO-Run + PTZ
Run + PTZ
Vehicle
Bicuculline
H
Duration of major seizure (s)
NO-Swim + PTZ
Swim + PTZ
Vehicle
Bicuculline
I
Scores of seizures
NO-Run + PTZ
Run + PTZ
Vehicle
Bicuculline
J
Scores of seizures
NO-Swim + PTZ
Swim + PTZ
Vehicle
Bicuculline

interaction for running ($F_{(1, 20)} = 159.5$; $p < 0.01$) on PSD95 (Fig. 3C); and significant effects of PTZ treatment ($F_{(1, 20)} = 148.7$; $p < 0.0001$) and physical activity ($F_{(1, 20)} = 24.70$; $p < 0.0001$) for swimming on PSD95 (Fig. 3D). The statistical analysis revealed significant effects of PTZ treatment ($F_{(1, 20)} = 463.3$; $p < 0.0001$) and physical activity ($F_{(1, 20)} = 78.04$; $p < 0.0001$) on Synapsin 1 (Fig. 3E); and significant effects of PTZ treatment ($F_{(1, 20)} = 214.4$; $p < 0.0001$) and physical activity ($F_{(1, 20)} = 27.60$; $p < 0.0001$) for swimming on Synapsin 1 (Fig. 3F). The statistical analysis revealed significant effects of PTZ treatment ($F_{(1, 20)} = 1318$; $p < 0.0001$) and physical activity ($F_{(1, 20)} = 258.6$; $p < 0.0001$) for running on GluA1 (Fig. 3G); and significant effects of PTZ treatment ($F_{(1, 20)} = 438.4$; $p < 0.0001$) and physical activity ($F_{(1, 20)} = 74.29$; $p < 0.0001$) for swimming on GluA1 (Fig. 3H). The statistical analysis revealed significant effects of PTZ treatment ($F_{(1, 20)} = 1008$; $p < 0.0001$) and physical activity ($F_{(1, 20)} = 321.0$; $p < 0.0001$) for running on BDNF (Fig. 3I); and significant effects of PTZ treatment ($F_{(1, 20)} = 400.2$; $p < 0.0001$) and physical activity ($F(1, 20) = 97.60$; $p < 0.0001$) for swimming on BDNF (Fig. 3J).

3.4. Physical activity enhances hippocampal GAD67 levels and GABAergic function in PTZ-treated rats

γ-Aminobutyric acid-ergic system is known to trigger the seizures in epilepsy through alterations of synaptic GAD67 and $GABA_A$ receptors protein levels. The alleviated seizures and improved cognition in PTZ-treated rats after exposure to chronic running or swimming may be mediated by enhancement of GABAergic function. The possibility was determined in 4 groups of rats (vehicle or PTZ injection; with or without physical activity) using Western blot assay of GAD67 and $GABA_AR$ levels (Fig. 4A, B). The results showed that compared to vehicle group, chronic PTZ injection significantly decreased the levels of GAD67 and $GABA_AR$ in hippocampal CA3 regions. However, chronic physical exercise (both running and swimming) increased the levels of GAD67 and $GABA_AR$ proteins. The statistical analysis revealed significant effects of PTZ treatment ($F_{(1, 20)} = 372.3$; $p < 0.0001$) and physical activity ($F_{(1, 20)} = 145.4$; $p < 0.0001$) and a significant physical activity × PTZ interaction ($F_{(1, 20)} = 5.309$; $p < 0.05$) for running on GAD67 (Fig. 4C); and significant effects of PTZ treatment ($F_{(1, 20)} = 584.0$; $p < 0.0001$) and physical activity ($F_{(1, 20)} = 261.2$; $p < 0.0001$) and a significant physical activity × PTZ interaction ($F_{(1, 20)} = 12.22$; $p < 0.01$) for swimming on GAD67 (Fig. 4D). This finding is consistent with previous evidence that exercise increased GAD67 expression in epileptic rats [15]. The statistical analysis revealed significant effects of PTZ treatment ($F_{(1, 20)} = 342.9$; $p < 0.0001$) and physical activity ($F_{(1, 20)} = 139.4$; $p < 0.0001$) and a significant physical activity × PTZ interaction ($F_{(1, 20)} = 10.14$; $p < 0.01$) for running on $GABA_AR$ (Fig. 4E); and significant effects of PTZ treatment ($F_{(1, 20)} = 806.9$; $p < 0.0001$) and physical activity ($F_{(1, 20)} = 297.7$; $p < 0.0001$) and a significant physical activity × PTZ interaction ($F_{(1, 20)} = 6.741$; $p < 0.05$) for swimming on $GABA_AR$ (Fig. 4F). We also found that chronic physical exercise increased the GAD67 ($p < 0.01$ and $p < 0.0001$, respectively) and $GABA_AR$ ($p < 0.0001$ in both running and swimming groups) levels in rats of vehicle groups.

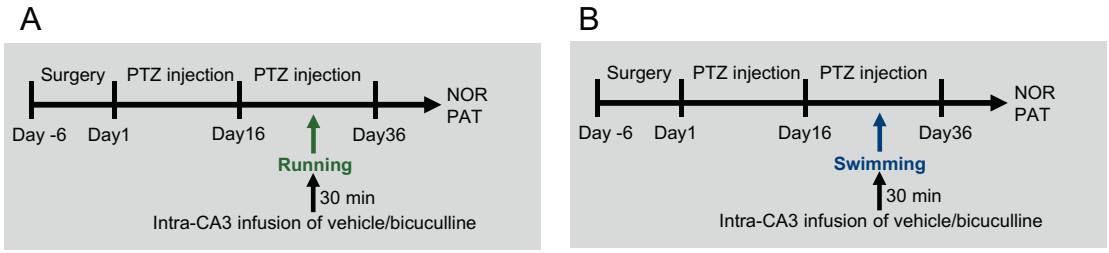
3.5. Antagonism of hippocampal CA3 $GABA_AR$ blocks the reversal effects of physical activity on seizures in PTZ-treated rats

In this part, to further determine the role of CA3 GABAergic system in the reversal effects of chronic physical activity on the epileptic seizures, $GABA_A$ receptor antagonist bicuculline was infused into the CA3 30 min before daily physical exercise procedure during the PTZ-induction (Fig. 5A, B). Pretreatment with bicuculline in the CA3 attenuated exercise-induced improvement in seizures induced by PTZ treatment in both running and swimming groups as measured in latency to seizure (Fig. 5C, D), duration of minor seizure (Fig. 5E, F), duration of major seizure (Fig. 5G, H), and scores of seizures (Fig. 5I, J). Data were analyzed using the factors physical activity (no running/swimming and running/swimming) and bicuculline dose (0 and 50 ng/side). The statistical analysis revealed significant effects of running ($F_{(1, 36)} = 72.35$; $p < 0.0001$) and bicuculline ($F_{(1, 36)} = 20.18$; $p < 0.0001$) and a significant running × bicuculline interaction ($F_{(1, 36)} = 51.52$; $p < 0.0001$) on the latency to seizure (Fig. 5C). For the swimming, the statistical analysis also revealed significant effects of swimming ($F_{(1, 36)} = 95.09$; $p < 0.0001$) and bicuculline ($F_{(1, 36)} = 107.7$; $p < 0.0001$) and a significant swimming × bicuculline interaction ($F_{(1, 36)} = 98.76$; $p < 0.0001$) on the latency to seizure (Fig. 5D). In the measurement of duration of minor seizure, the statistical analysis revealed significant effects of running ($F_{(1, 36)} = 13.04$; $p < 0.001$) and bicuculline ($F_{(1, 36)} = 4.187$; $p < 0.05$) and a significant running × bicuculline interaction ($F_{(1, 36)} = 7.831$; $p < 0.01$) (Fig. 5E); the statistical analysis also revealed significant effects of swimming ($F_{(1, 36)} = 8.825$; $p < 0.01$) and bicuculline ($F_{(1, 36)} = 4.407$; $p < 0.05$) and a significant swimming × bicuculline interaction ($F_{(1, 36)} = 9.302$; $p < 0.01$) (Fig. 5F). As shown in the duration of major seizure, the statistical analysis revealed significant effects of running ($F_{(1, 36)} = 22.62$; $p < 0.0001$) and bicuculline ($F_{(1, 36)} = 8.985$; $p < 0.001$) and a significant running × bicuculline interaction ($F_{(1, 36)} = 26.79$; $p < 0.0001$) (Fig. 5G); and for the swimming, the statistical analysis also revealed significant effects of swimming ($F_{(1, 36)} = 33.83$; $p < 0.0001$) and bicuculline ($F_{(1, 36)} = 24.55$; $p < 0.0001$) and a significant swimming × bicuculline interaction ($F_{(1, 36)} = 47.52$; $p < 0.0001$) (Fig. 5H). In the scores of seizures' test, the statistical analysis revealed significant effects of running ($F_{(1, 36)} = 37.94$; $p < 0.0001$) and bicuculline ($F_{(1, 36)} = 9.484$; $p < 0.01$) and a significant running × bicuculline interaction ($F_{(1, 36)} = 19.35$; $p < 0.0001$) (Fig. 5I); and for the swimming, the statistical analysis also revealed significant effects of swimming ($F_{(1, 36)} = 32.81$; $p < 0.0001$) and bicuculline ($F_{(1, 36)} = 5.696$; $p < 0.05$) and a significant swimming × bicuculline interaction ($F_{(1, 36)} = 14.58$; $p < 0.001$) (Fig. 5J).

3.6. Antagonism of hippocampal CA3 $GABA_AR$ blocks the beneficial effects of physical activity on cognition in PTZ-treated rats

Subsequently, we determined whether antagonism of the GABAergic system affects exercise-induced improvement of cognition. Bicuculline or its vehicle (saline) was infused into the CA3 30 min before daily exercise exposure (Fig. 6A, B). Rats in exercise groups showed improved cognition compared with rats that received no exercise of PTZ-treated group. However, CA3 infusion of bicuculline blocked the positive effects of exercise on cognitive performance in epileptic rats induced by PTZ in both NOR (Fig. 6C, D) and PAT tests (Fig. 6E–J). The statistical

Fig. 5. Antagonism of hippocampal CA3 $GABA_AR$ blocks the reversal effects of physical activity on seizures in PTZ-treated rats. (A) Schematic of the experimental design for running exercise in PTZ-induced seizure. (B) Schematic of the experimental design for swimming exercise in PTZ-induced seizure. (C) The latency to seizure in rats received intra-CA3 microinjection of $GABA_AR$ antagonist bicuculline or vehicle with chronic running exposure. (D) The latency to seizure in rats. The latency to seizure in rats received intra-CA3 microinjection of $GABA_AR$ antagonist bicuculline or vehicle with chronic swimming exposure. (E) The duration of minor seizure in rats received intra-CA3 microinjection of $GABA_AR$ antagonist bicuculline or vehicle with chronic running exposure. (F) The duration of minor seizure in rats received intra-CA3 microinjection of $GABA_AR$ antagonist bicuculline or vehicle with chronic swimming exposure. (G) The duration of major seizure in rats received intra-CA3 microinjection of $GABA_AR$ antagonist bicuculline or vehicle with chronic running exposure. (H) The duration of major seizure in rats received intra-CA3 microinjection of $GABA_AR$ antagonist bicuculline or vehicle with chronic swimming exposure. (I) The scores of seizures in rats received intra-CA3 microinjection of $GABA_AR$ antagonist bicuculline or vehicle with chronic running exposure. (J) The scores of seizures in rats received intra-CA3 microinjection of $GABA_AR$ antagonist bicuculline or vehicle with chronic swimming exposure. Data were expressed as mean ± SEM. $^{***}p < 0.001$, $^{****}p < 0.0001$, different from other experimental groups (n = 10 mice/group). PTZ, pentylenetetrazole.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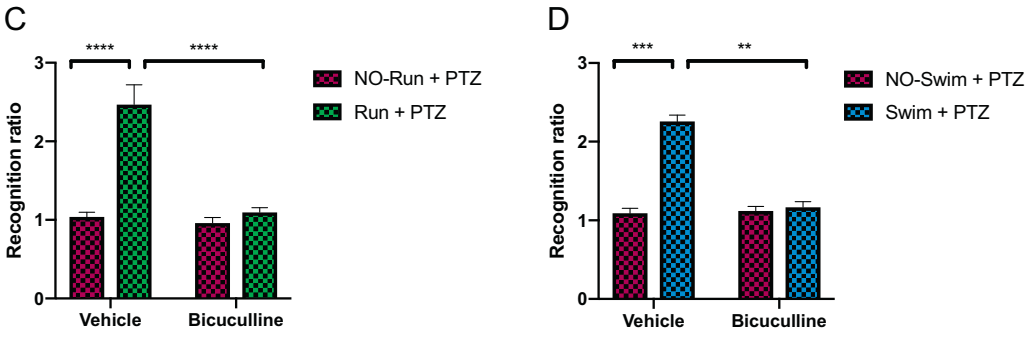
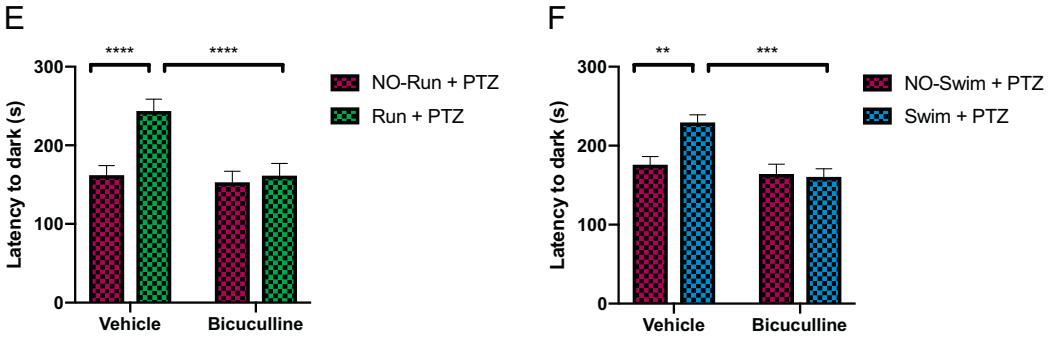
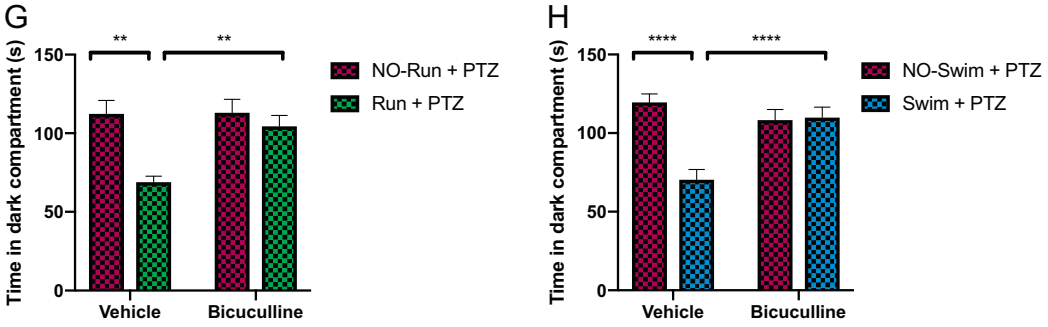
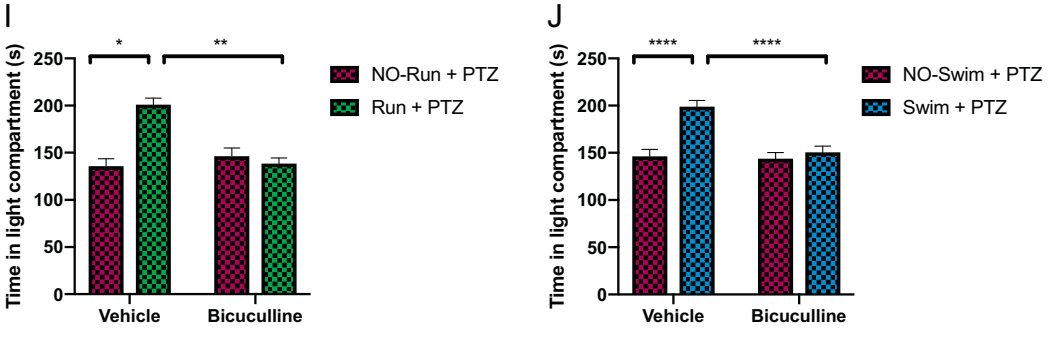
Surgery
PTZ injection
PTZ injection
NOR
PAT
Day -6
Day1
Day16
Day36
Running
30 min
Intra-CA3 infusion of vehicle/bicuculline
B
Surgery
PTZ injection
PTZ injection
NOR
PAT
Day -6
Day1
Day16
Day36
Swimming
30 min
Intra-CA3 infusion of vehicle/bicuculline


C
Recognition ratio
NO-Run + PTZ
Run + PTZ
Vehicle
Bicuculline
D
Recognition ratio
NO-Swim + PTZ
Swim + PTZ
Vehicle
Bicuculline


E
Latency to dark (s)
NO-Run + PTZ
Run + PTZ
Vehicle
Bicuculline
F
Latency to dark (s)
NO-Swim + PTZ
Swim + PTZ
Vehicle
Bicuculline


G
Time in dark compartment (s)
NO-Run + PTZ
Run + PTZ
Vehicle
Bicuculline
H
Time in dark compartment (s)
NO-Swim + PTZ
Swim + PTZ
Vehicle
Bicuculline


I
Time in light compartment (s)
NO-Run + PTZ
Run + PTZ
Vehicle
Bicuculline
J
Time in light compartment (s)
NO-Swim + PTZ
Swim + PTZ
Vehicle
Bicuculline

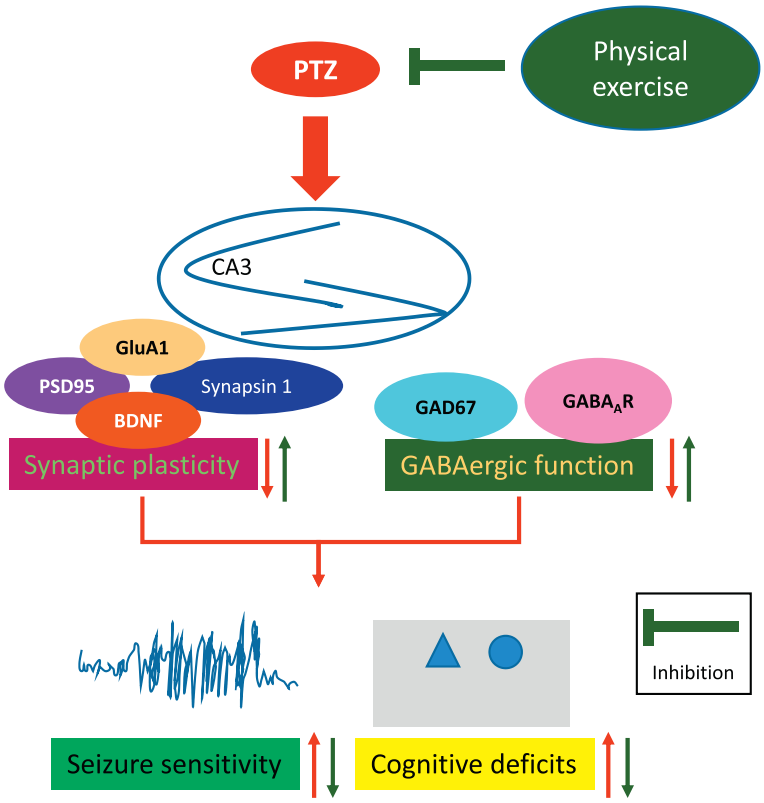


Fig. 7. Model illustrating the cellular pathways for the cognition and seizures regulation of physical activity. Repeated PTZ administration induced reductions of synaptic-related proteins including PSD95, Synapsin 1, GluA1, and BDNF, and decrease of GAD67 and GABA$_A$R expression in hippocampal CA3, leading to a dysfunction of synaptic plasticity and GABAergic system. As a result, rats that receive chronic PTZ treatment showed increased sensitivity to seizures and impaired cognition. However, regular physical exercise increased synaptic-related proteins and GABAergic function. Subsequently, the seizure frequency and cognitive performance were also reversed by physical exercise. Green line is the response triggered by PTZ treatment; red line is the action of physical exercise, up-head arrows mean upregulation; down-head arrows mean downregulation.

analysis revealed significant effects of running ($F_{(1, 36)} = 33.18$; $p < 0.0001$) and bicuculline ($F_{(1, 36)} = 28.53$; $p < 0.0001$) and a significant running × bicuculline interaction ($F_{(1, 36)} = 22.73$; $p < 0.0001$) on the recognition ratio (Fig. 6C); and for the swimming, the statistical analysis also revealed significant effects of swimming ($F_{(1, 36)} = 77.35$; $p < 0.0001$) and bicuculline ($F_{(1, 36)} = 58.76$; $p < 0.0001$) and a significant swimming × bicuculline interaction ($F_{(1, 36)} = 65.84$; $p < 0.0001$) on the recognition ratio of NOR test (Fig. 6D). The statistical analysis revealed significant effects of running ($F_{(1, 36)} = 9.874$; $p < 0.01$) and bicuculline ($F_{(1, 36)} = 10.09$; $p < 0.01$) and a significant running × bicuculline interaction ($F_{(1, 36)} = 6.457$; $p < 0.05$) on the latency to dark compartment (Fig. 6E); and for the swimming, the statistical analysis also revealed significant effects of swimming ($F_{(1, 36)} = 5.531$; $p < 0.05$) and bicuculline ($F_{(1, 36)} = 14.30$; $p < 0.001$) and a significant swimming × bicuculline interaction ($F_{(1, 36)} = 7.289$; $p < 0.05$) on the latency to dark compartment of PAT (Fig. 6F). The statistical analysis revealed significant effects of running ($F_{(1, 36)} = 13.05$; $p < 0.001$) and bicuculline ($F_{(1, 36)} = 6.334$; $p < 0.05$) and a significant running × bicuculline interaction ($F_{(1, 36)} = 5.788$; $p < 0.05$) on the time in dark compartment (Fig. 6G); and for the swimming, the statistical analysis also revealed significant effects of swimming ($F_{(1, 36)} = 13.94$; $p < 0.001$) and bicuculline ($F_{(1, 36)} = 4.950$; $p < 0.05$) and a significant swimming × bicuculline interaction ($F_{(1, 36)} = 15.89$; $p < 0.001$) on the time in dark compartment of PAT test (Fig. 6H). The statistical analysis revealed significant effects of running ($F_{(1, 36)} = 14.77$; $p < 0.001$) and bicuculline ($F_{(1, 36)} = 12.02$; $p < 0.01$) and a significant running × bicuculline interaction ($F_{(1, 36)} = 24.03$; $p < 0.0001$) on the time in light compartment (Fig. 6I), and for the swimming, the statistical analysis also revealed significant effects of swimming ($F_{(1, 36)} = 18.75$; $p < 0.001$) and bicuculline ($F_{(1, 36)} = 14.09$; $p < 0.001$) and a significant swimming × bicuculline interaction ($F_{(1, 36)} = 11.46$; $p < 0.01$) on the time in light compartment of PAT (Fig. 6J). These data showed that the

Fig. 6. Antagonism of hippocampal CA3 GABA$_A$R blocks the beneficial effects of physical activity on cognition in PTZ-treated rats. (A) Schematic of the experimental design for running exercise in PTZ-induced seizure. (B) Schematic of the experimental design for swimming exercise in PTZ-induced seizure. (C) The recognition ratio in rats received intra-CA3 microinjection of GABA$_A$R antagonist bicuculline or vehicle with chronic running exposure in the NOR test. (D) The recognition ratio in rats received intra-CA3 microinjection of GABA$_A$R antagonist bicuculline or vehicle with chronic swimming exposure in the NOR test. (E) The latency to dark in PAT of rats received intra-CA3 microinjection of GABA$_A$R antagonist bicuculline or vehicle with chronic running exposure. (F) The latency to dark in PAT of rats received intra-CA3 microinjection of GABA$_A$R antagonist bicuculline or vehicle with chronic swimming exposure. (G) The time in dark compartment in PAT of rats received intra-CA3 microinjection of GABA$_A$R antagonist bicuculline or vehicle with chronic running exposure. (H) The time in dark compartment in PAT of rats received intra-CA3 microinjection of GABA$_A$R antagonist bicuculline or vehicle with chronic swimming exposure. (I) The time in light compartment in PAT of rats received intra-CA3 microinjection of GABA$_A$R antagonist bicuculline or vehicle with chronic running exposure. (J) The time in light compartment in PAT of rats received intra-CA3 microinjection of GABA$_A$R antagonist bicuculline or vehicle with chronic swimming exposure. Data were expressed as mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, different from other experimental groups (n = 10 mice/group). PTZ, pentylenetetrazole; NOR, novel object recognition; OFT, open-field test; PAT, passive avoidance test.

reversal effects of physical activity on the cognitive impairment induced by repeated PTZ administration is blocked by specific antagonism of $GABA_AR$ in CA3 of hippocampus.

4. Discussion

We found that chronic physical activity such as running and swimming produced a significant reduction of seizure frequency and duration in PTZ-treated epileptic rats. Simultaneously, both running and swimming improved cognitive function as measured by enhanced performance in object recognition test and PAT in rats with epilepsy induced by PTZ. Furthermore, PTZ reduced the levels of synaptic-related proteins and $GABA_AR$ in hippocampal CA3, which was also reversed by regular physical exercise in rats. Finally, specific antagonism of hippocampal CA3 $GABA_AR$ blocked the protective effects of physical activity on both seizures and cognition in PTZ-treated rats. These findings suggest that CA3 synaptic plasticity and GABAergic function mediate the beneficial effects of physical activity on seizure and cognition in epileptic rats. Overall, our study reveals a potential implication that physical activity may be a nonpharmacological treatment to improve cognitive performance of individuals with epilepsy.

Given the evidence that people with epilepsy had worse physical activity [31], clinical professionals encouraged these individuals with epilepsy to increase physical activities so as to improve health outcomes. Similarly, a cross-sectional study in individuals diagnosed as having epilepsy showed that the significant improvement in health conditions including quality of life and mental health was associated with greater physical activity [32]. Physical activity is well known for its beneficial effects on improving both physical and psychological quality of life. In particular, regular participation in physical activity contributed to brain physiology and cognition enhancement. Higher levels of exercise training are strongly associated with larger hippocampal volume, and better performance on memory function [33–35]. Human clinical trials and rodent models have consistently shown that the hippocampus is unique among brain regions as displaying the most sensitive response to exercise and plays a critical role in improvements in hippocampal-dependent memory, learning, and cognitive function driven by regular physical activity [33,36,37]. Increases in hippocampal neurotrophic factors, such as BDNF protein levels following repeated exercise have also been established [38]. The number of activated neurons in the DG (dentate gyrus), CA1, and CA3 regions of the hippocampus was increased in rodents after running exercise [39,40].

Our findings that chronic exercise enhances the expression of BDNF in the hippocampal CA3 are consistent with previous studies showing that exercise upregulates BDNF and improves cognitive function in naïve animals [41]. Similarly, we also found here that chronic exercise increases synaptic-related proteins (PSD95, Synapsin 1, GluA1, and BDNF) in PTZ-treated rats. The CA3 hippocampus plays a critical role in the pathological conditions of seizure activity with neuronal hyperexcitability in this region [42,43]. For example, the intrinsic firing response gain of CA3 pyramidal cells and the hippocampal plasticity is closely associated with the status epilepticus induced by pilocarpine [44]. The inhibitory neurotransmitter GABA critically regulates information processing by modifying neuronal excitability and synaptic plasticity; while, the dysregulation of GABAergic transmission is involved in the pathogenesis of several disorders, including cognitive dysfunction. The loss of GABAergic interneurons and GABA lesions has also been described in molecular pathways underlying epilepsy, suggesting a useful therapeutic target against epilepsy [45]. There is also growing evidence that tonic currents play an important role in epilepsy, memory, and cognition. Tonic inhibition (a persistent $GABA_A$ receptor activation) is modulated by GABA uptake and $GABA_A$ receptor expression [46]. In the hippocampus, tonic $GABA_A$ receptor-mediated currents modulate neuronal development and synapse formation [47]. Therefore, interventions specifically focus on the extrasynaptic receptors involved in tonic inhibition could be a novel direction to explore the pathological processes of these disorders.

The deficits of neuronal migration, formation, and the maturation of GABAergic synaptic connections result in network hyperexcitability and seizure activity [48]. γ-Aminobutyric acid-ergic neuron loss has been observed in the hippocampal formation in pilocarpine-induced seizures and models of temporal lobe epilepsy [49,50]. Moreover, impaired GABAergic inhibitory input is possibly related to the hypersynchronous firing and subsequent epilepsy-like symptoms in animals [51,52]. The morphological changes in GABAergic neurons and GABAergic inhibition can also lead to epilepsy [53]. The $GABA_AR$ is a major therapeutic target for the treatment of epilepsy, and the dysfunction of $GABA_AR$ is attributed to the induction of seizures [54]. The reductions of $GABA_AR$ expression levels and number of synapses may change the excitatory/inhibitory balance in neurons expressing GABAergic synapses [55]. Synaptic $GABA_ARs$ mainly comprised α, β, and γ subunits. The α4 $GABA_ARs$ are also necessary for the dendritic morphology of CA3 pyramidal cells as evidenced by a recent finding that synaptic pruning was prevented in α4 $GABA_AR$ knockout mice [56]. In the current investigation, CA3 $GABA_AR$ antagonist bicuculline damaged the protective effects of exercise on both seizure and cognition possibly due to the morphological integrity of CA3 that was impaired by $GABA_AR$ antagonism. Long-term voluntary exercise increases GAD67 mRNA (messenger RNA) in hippocampus, suggesting that regular exercise enhances GABA synthesis [57]. In our PTZ-treated rats, we found that chronic physical exercise (both running and swimming) increased the levels of hippocampal CA3 GAD67 proteins which were decreased by PTZ injection. Interestingly, a recent research showed that overexpression of GAD67 in the hippocampal CA3 region in mice model of temporal lobe epilepsy significantly reduced seizure generation with a long-lasting effects for 14 weeks [58].

The abnormalities of CA3 synapses, such as the reduction of neuronal size and dendritic spines, have been reported in schizophrenia, mood disorders, and Alzheimer's disease [59–61], suggesting that CA3 hippocampus may potentially underlie the cognitive impairments in these disorders. There is increasing evidence that GABAergic neuronal loss and deficits of hippocampal GABA-mediated neurotransmission have been implicated in epilepsy [62], suggesting that normalization of GABA interneurons is a promising strategy for reducing the seizures and behavioral deficits in epileptic animals. In contrast to the findings from normal animals without seizures that $GABA_A$ receptor antagonist bicuculline improves learning and memory and enhances BDNF expression in the hippocampus [63], our current results showed that intra-CA3 bicuculline microinjection did not alter the cognition of naive rats receiving no exercise but did reverse the beneficial effects of physical activity on both cognitive and synaptic proteins in PTZ-treated rats. We explained this discrepancy due to the possibility that there is a distinct pathophysiological process between control and epilepsy models underlying the GABAergic system and BDNF pathway. Because that PTZ-induced seizure rats produced reductions of both GAD67 and $GABA_AR$ protein levels as well as alleviated the seizure and enhanced cognition simultaneously, highlighting our hypothesis that there is a strong link between GABAergic system and recovery of cognitive performance in epileptic rats received long-term physical exercise.

Although significant increases in BDNF levels were observed in the hippocampus of the kindled rats induced by PTZ as supported by numerous evidence, there are some research that did not reveal the similar findings. For example, Nanda and Mack showed that PTZ-induced seizure did not increase BDNF protein levels in rat hippocampus [64]. In addition, BDNF protein level was decreased in the granule cell layer and neurons of the hilus 3 h after acute PTZ (50 mg/kg)-induced epileptic seizures in rats [65]. There are possible explanations to the discrepancy among previous studies and our study on the BDNF protein changes after PTZ injection. First, the doses and duration of the PTZ used are quite different, leading to different patterns of BDNF expression within the hippocampus. In our experiment, rats were treated with daily PTZ (35 mg/kg, i.p.) for 36 days to induce chronic epilepsy.

While BDNF has been demonstrated to increase in the rat hippocampus following seizures triggered by the repeated injections of PTZ at the dose of 30 mg/kg or hippocampal, BDNF concentrations were increased in mice that received PTZ on alternate days for 21 days [65,66]. These findings suggested that specific types of PTZ administration including the dose and time course can regulate BDNF expression differently. Second, the selected brain regions are distinct because the patterns of BDNF protein expression are dependent on tissue specific factors [64]. We used the subregion CA3 of hippocampus while others chose the whole part of hippocampus without separation of even DV part. Pentylenetetrazole primarily acts as a noncompetitive $GABA_A$ receptor antagonist and increases glutamate release through reduction of the inhibitory GABAergic input [67]. Previous studies reported that the establishment of functional GABAergic synapse in the developing rat hippocampus is induced in CA3 pyramidal neurons and BDNF endogenous TrkB (tyrosine kinase receptor B) receptor is also involved in this process [68]. Furthermore, the balance of glutamatergic and GABAergic system in CA3 plays an important role in reversal of memory deficit [69]. Consistently, targeted regulation of GABA signaling in CA3 reversed memory deficits in mice by improving cognitive function [70]. As the main point of the current study is concentrated on the cognitive dysfunction induced by chronic PTZ injection, we exclusively analyzed that the BDNF levels on cognition in those animals were impaired by PTZ. Therefore, we hypothesize that PTZ might induce a different change trend of hippocampal CA3 BDNF protein in vulnerable individuals with cognitive deficits affected by long-term PTZ administration. Similarly, those animals that demonstrated significant cognitive impairment after PTZ injection also showed reduced PSD95, GAD67, GluA1, and $GABA_A$ receptor levels. However, we found here that chronic physical exercise, which has been shown to enhance these synaptic-related proteins, is beneficial to improve the negative outcomes on memory in PTZ-treated rats. A reasonable explanation for the contrary result from previous studies is that the samples we used here are specific, suggesting that different behavioral phenotypes induced by PTZ might lead to different molecular changes. Further studies are required to investigate the mechanisms underlying the functions of these proteins in impaired cognition in PTZ-induced seizure.

5. Conclusion

In summary, the present data showed that chronic physical exercise reduced the frequency of seizures and improved the cognitive function in a rat model of chronic epilepsy through normalization of hippocampal synaptic plasticity and GABAergic function (Fig. 7). Our findings suggest that chronic physical exercise has beneficial effects to control seizure through enhancement of cognition and highlights the possibility to translate into reduced seizure recurrence in people with epilepsy.

Author contributions

LXY and CW designed the experiments. LXY, CY, and WL performed the animal model and epileptic behavioral experiments. WL, CY, and LXY carried out exercise training experiments and cognitive measurements. CW performed intracranial implantation surgery, microinjections, and Western blot experiments. CY and WL analyzed the data. LXY and CW wrote the manuscript. CY and WL reviewed and edited the manuscript.

Declaration of Competing Interest

The authors declare no competing interests.

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