Combination Effects of Forced Mild Exercise and GABA
Receptor Agonist on Spatial Learning, Memory, and Motor Activity in Striatum Lesion Rats

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ABSTRACT. Basal ganglia (BG) lesions cause impairments of different mammalian’s movement and cognition behaviors. Motor circuit impairment has a dominant role in the movement disorders. An inhibitory factor in BG is GABA neurotransmitter, which is released from striatum. Lesions in GABAergic neurons could trigger movement and cognition disorders. Previous evidence showed that GABAB receptor agonist (Baclofen) administration in human improves movement disorders and exercise can improve neurodegenerative and cognitive decline; however, the effects of both Baclofen and mild forced treadmill exercise on movement disorders are not well known. The main objective of this study is to investigate the combined effects of mild forced treadmill exercise and microinjection of Baclofen in the internal Globus Pallidus on striatum lesion-induced impairments of spatial learning and motor activity. We used Morris water maze and open field tests for studying spatial learning, and motor activity, respectively. Results showed that mild exercise and Baclofen microinjection could not completely affect the spatial learning, and motor activity impairments while the combination of them could alleviate spatial learning, and motor activity impairments in striatum lesion animals. Our results suggest that striatum lesion-induced memory and motor activity impairments can improve with combination interaction of GABAB receptor agonist and exercise training.

Keywords: basal ganglia; treadmill exercise; baclofen; spatial memory and learning; motor activity dysfunction; ibotenic acid

1. Introduction

Basal ganglia (BG) is main role in neurodegenerative diseases and motor behaviors (Packard & Knowlton, 2002). BG includes components of circuits called cortical-thalamus (Tarsy, Vitek, & Lozano, 2002). These circuits originate from specific cortical areas, pass through separate portions of the BG and thalamus, and project back to the frontal cortical area, which took origin from them (Albin, Young, & Penney, 1989). The BG’s pathways play key roles in the movement disorders such as Parkinson’s disease (PD), Chorea, and Huntington. The prevailing functional model of BG is based on activation of the striatal direct pathway that facilitates movement and activation of the indirect pathway that inhibits movement (Alexander, DeLong, & Strick, 1986; DeLong, 1990). So, dysfunction of these pathways causes movement disorders or lesions in the selective movement programs and initiation of movement. In addition, behavioral studies in humans and rats demonstrate a critical role of BG in habit learning (McDonald & White, 1993; Packard & Knowlton, 2002) and lesions of the medial and dorsal striatum impair spatial navigation (Devan, McDonald, & White, 1999; Whishaw, Mittleman, Bunch, & Dunnett, 1987) because of its related functional relationship with higher-order memory systems (e.g., the hippocampus) (Packard & Goodman, 2013).

In a direct pathway, striatum (Str) releases gamma-aminobutyric acid (GABA) neurotransmitter to inhibit GABAergic neurons of the internal Globus Pallidus (GPI) which projects GABAergic outputs to many nuclei of the BG including Str, STN, entopeduncular nucleus, and substantia nigra (SN) (Bevan, Booth, Eaton, & Bolam, 1998; Hazrati, Parent, Mitchell, & Haber, 1990; Kita, 1994; Kita & Kita, 1994, 2001). In indirect pathways, cortical activation results in the activation of the GABAergic striatum, followed by enhanced inhibition of GABAergic neurons in the Globus Pallidus external (GPe). Therefore, a direct pathway excites thalamus while an indirect one results in inhibition of thalamic activity (Galvan, Devergnas, & Wichmann, 2015). Block, Kunkel, and Schwarz (1993) showed that injection of quinolinic acid (QA) into the striatum of rats induced deficits in spatial learning and motor performance similar to those observed in Huntington's disease (Block et al., 1993). However, Lee et al. (2011) investigated sensory processing in the barrel cortex neurons of an animal model of Alzheimer’s disease (AD) by infusion of ibotenic acid (IA) into nucleus basalis of Meynert (NBM). They showed that IA infusion into NBM significantly reduced memory and sensory processing, and contact discrimination in AD animals (Goshadrou, 2011). Several studies have described that lesions of GP alone or in combination with SN cause motor activity deficits (Mink, 1996). Output of both
direct and indirect pathways through GPi and SNr, projects to the thalamus and brainstem (Walter & Vitek, 2012). GABAergic receptors are involved in the modulation of GABAergic transmission in the GPe and GPi. It is possible that changes in the function or localization of these receptors contribute to the alterations of GABAergic transmission (Galvan et al., 2011). Previous studies suggested that GABA_A receptor in the GP plays an important role in the regulation of movement by modulating of GABAergic inputs at a presynaptic site (Chen, Chan, & Yung, 2002) and microinjection of Baclofen in the GPe and GPi leads to a significant increase in the proportion of spikes in parkinsonian animals (Galvan et al., 2011). So, striatum dysfunction disturbs inhibitory inputs to the GPi and other nuclei. In addition, human’s research indicated Baclofen to be an effective treatment for spasticity in cerebral palsy, dystonia, and other various movement disorders (Kraus et al., 2017). However, studies showed that GABAergic transmission in BG has an effective role in memory processes. For example, post-training infusions of the GABA receptor antagonist picrotoxin into the SN and dorsal striatum impairs memory (Kim & Routtenberg, 1976; Salado-Castillo, del Guante, Alvarado, Quirarte, & Prado-Alcalá, 1996).

In addition, studies suggested that exercise have beneficial effects on brain function in animal models and in a growing number of clinical studies on humans (Lin & Kuo, 2013). Exercise influences brain function as regulations of the secretion of neurotrophic factors, vasculotrop factors, inflammatory mediators, and regulations on neurotrophic factors and neurotransmitters (Lin & Kuo, 2013). In this study, we hypothesized that striatum-lesion decreases direct pathway activity leads to the decreased inhibition of GPI neurons and demotes movement through inhibition of movement-related thalamocortical neurons. The striatum is the primary input center of the BG, so striatum lesion induced GPI output impairment. Thus, we injected Baclofen in the GPI, whether it helps to alleviate this impairment. The present study determines whether mild forced treadmill exercise and Baclofen microinjection into GPi facilitate impairments of spatial learning, memory, and motor activity in rats with striatum lesions induced by IA infusion.

### 2. Material and methods

#### 2.1. Subjects

In this study, 96 young male Wistar rats weighing 200–250 g at the time of surgery were ordered from Pasteur Institute of Iran. They were housed in large cages (eight per cage) and kept on a 12 h light/dark cycle (lights on at 7:00 am and off at 7:00 pm) at room temperature (22 ± 2 °C). Standard animal chow and water were freely available. After surgery and before testing, each animal was first handled (10 min) daily for a week. Declaration of Helsinki and the internationally accepted principles on procedures for animal experimentation were followed. All efforts were made to minimize the animals’ suffering (Baker & Ragozzino, 2014; Care, Animals, & Resources, 1985).

#### 2.2. Drugs

The following drugs were used in this study: Ibotenic Acid purchased from Sigma-Aldrich, St. Louis, MO, USA. Baclofen purchased from Pars Darou Company, Iran.

#### 2.3. Experimental design

After one week of adaptation to colony room condition, each rat was randomly divided into eleven groups (n = 8) (Baker & Ragozzino, 2014):

- **C-untrained group** = “Control-untrained” group: this group is as a control group for any non-exercise effects of treadmill running. Rats were placed on top of the treadmill apparatus without turning it on for a time period equivalent to exercise training.

- **Treadmill group** = “T” group: rats were trained at a speed (10 min at 4 m/min, 10 min at 7 m/min, and 10 min at 10 m/min) with 0° of inclination on the treadmill running 7 days in a week, for 4 weeks.

- **Str (Ibotenic Acid) group** = “Str (IA)” group: rats received bilaterally IA (5 μg/1 μl) into the striatum.

- **Str (normal saline) group** = “Str (NS)” group: rats received normal saline (1 μl) bilaterally into the striatum.

- **“Str (IA) +T” group** = rats received bilaterally IA (5 μg/1 μl) into the striatum then animals were placed on a treadmill and run the protocol.

- **“Str (NS) +T” group** = rats received normal saline (1 μl) bilaterally injection into the striatum then animals placed on a treadmill and run the protocol.

- **GPi (Baclofen) group** = “GPi (Bac)” group: Rats received Baclofen (0.05 μg/0.5 μl for each side) bilaterally into the GPi.

- **“GPi (NS)” group** = rats received normal saline (1 μl) bilaterally into the GPi.

- **“Str (NS) + GPi (Bac)” group** = Rats received normal saline (1 μl) bilaterally into the striatum then Baclofen (0.05 μg/0.5 μl for each side) was injected bilaterally into the GPi.

- **“Str (IA) + GPi (Bac)” group** = rats received IA (5 μg/1 μl) bilaterally into the striatum then Baclofen (0.05 μg/0.5 μl for each side) was injected bilaterally into the GPi.

- **“Str (IA) + GPi (Bac) + T” group** = IA was injected bilaterally into the striatum then Baclofen (0.05 μg/
0.5 µl for each side) was injected bilaterally into the GPi and placed on a treadmill and run the protocol.
All behavioral and open field tests were carried out to evaluate the capacity of learning and spatial memory and motor activity for each group.

2.4. Surgical and microinjection method

Rats were anesthetized with a combination of ketamine (100 mg/kg of body weight (b.w.) and xylazine intraperitoneally (10 mg/kg b.w. IP). A midline incision was done in the skin overlying the skull. Small holes were drilled using a size 1/2 burrs in a dental drill set up on a stereotaxic arm into striatum at coordinates: AP = +0.35, ML = ± 3.05 from bregma and DV = 5.5 from dura using a stereotactic atlas (Thomsen et al., 2015). Ibotenic Acid (Sigma-Aldrich) was injected bilaterally via a 1 µl Hamilton Syringe in doses of (5 µg/1 µl) in the striatum (ventral lateral). Hamilton syringe mounted on top of the hole and push slightly forward in target place of striatum during 3 min. The syringe was left to diffuse for a further 2 min before removal and then pulled slowly to the backstop. Sham animals received bilaterally an equal volume of normal saline into the striatum. Finally, the wound was closed with simple sutures. According to a standard stereotaxic atlas (Paxinos, Watson, & Emson, 1980), a guide cannula constructed from stainless steel was implanted into the GPi (2.4 mm posterior, ±2.8 mm lateral from the bregma, 6.8 mm ventral from the skull surface), on either side. The cannula was fixed to the skull with stainless steel screws and dental acrylic. Stainless steel styles were used to keep the cannula sealed.

Baclofen was administrated into the GPi bilaterally through the guide cannulas (23 gages) using injection needles (30 gauges) connected by polyethylene tubing to 10.0-µl Hamilton microsyringe after recovery days. Afterward, 0.05 µg doses of Baclofen dissolved in 0.5 µl normal saline were injected during 3 min in cannulas for each side (with an infusion pump at the speed of 0.25 µl/ min). The injector was left in place for another 2 min before it was slowly withdrawn, this dose has not been shown memory impairment (Chen et al., 2002; Nourjah, Rostami, & Barzegar, 2006). Baclofen was administrated every day before behavioral test. For exercise and drug components group, baclofen microinjection was administrated and then animals were placed on a treadmill exercise program.

2.5. Treadmill exercise regimen (exercise paradigm)

A six-lane motorized rodent treadmill was utilized for exercise training. All the training rats were allowed to adapt to treadmill running for 10 min on 2 consecutive days (first day at 5 m/min; a second day at 8 m/min) (Kim et al., 2002). Animals were trained at a speed (10 min at 4 m/min, 10 min at 7 m/min, 10 min at 10 m/min) with 0° of inclination (Whishaw et al., 1987). If the rat refused to run, it could be motivated by a transient and light electric stimulation from the grid at the beginning of the treadmill platform. After the exercise, rats were returned to their cages with free access to food and water. Figure 1 illustrates the experimental design of this study.

2.6. Behavioral assessment

2.6.1. Morris water maze

Morris water maze (MWM) test was performed to assess the hippocampus-dependent spatial learning and memory ability of rats as described previously (Vorhees & Williams, 2006). In order to familiarize to the pool, rats were allowed to perform a 60-s swimming without the platform 24 h before starting the Morris test (Habituation).

2.6.1.1. Apparatus. Briefly, it consisted of a dark circular pool (140 cm in diameter and 22 cm high) filled with
water (20 ± 1°C) to a depth of 25 cm. The pool was located in a room containing various prominent visual cues. The pool was divided into four equal quadrants Northeast (NE), Northwest (NW), Southeast (SE), and Southwest (SW). A transparent circular-shaped platform was submerged one cm beneath the water surface in the Northwest quadrant (target quadrant) of the pool. The maze was located in a room with sample surrounding visual cues. It is critical that the cues are not moved during testing, as these are the animal’s navigational reference signs for placing the goal, independent of start location. The position of the animal was tracked by a camera linked to a computer equipped with EthoVision software (EthoVision XT7, Netherland) that was mounted above the center of the pool (Vorhees & Williams, 2006).

2.6.1.2. Acquisition phase. All behavioral tests were done between 12.00 pm, midday to 14.00 pm. The learning trials were conducted over four days, with four trials per day (Whishaw et al., 1987). The animal is placed randomly in the desired start position in the maze, facing the tank wall and the platform was placed in the same position over the days. The animal is released into the water at water level (not dropped) until the platform was found and 1 min waiting interval between the four trials for each rat. If an animal fails to find the hidden platform in a maximal time of 90 s, were gently guided to the platform and allowed to rest for 20 s (Akbari, Naghdi, & Motamed, 2006; Azimi, Gharakhanlou, Naghdi, Khodadadi, & Heysieattalab, 2018; Naghdi, Oryan, & Etemadi, 2003; Narenji, Naghdi, Azadmanesh, & Edalat, 2015; Vorhees & Williams, 2006; Wang & Wang, 2016). A camera installed over the pool was used to record the swimming trace for later analysis. Dependent measures of performance on learning trials in the MWM begin with latency (s) (time from start to find hidden platform). Latency can be obtained with tracking software. Distance (cm) (path length that animal pass to find hidden platform) is also widely used.

2.6.1.3. Probe test. At least 24 h later, the probe trials that assess reference memory (memory consolidation) was taken and removed from the platform. Rats were permitted to swim freely in the pool for 90 s (Vorhees & Williams, 2006). The percentage of time spent in the target quadrant was measured as spatial memory retention (Vorhees & Williams, 2006).

2.6.1.4. Visible platform task. Visible platform (Cued Trial) test (one block of four trials per rat) one hour after the probe trial was performed to assess the rats’ visual-motor coordination like last methods of articles (Ghonsulakandi, Sheik, Shasaltaneh, Chopani, & Naghdi, 2017; Lee et al., 2011; Narenji et al., 2015). The animal was placed in the pool and given 90 s to reach the platform that placed in the opposite of the target quadrant (southeast quadrant), which was identified by a visible white platform above the surface (approximately 1.5 cm) of the water and placed in the opposite of the target quadrant (southeast quadrant). (Amiri & Naghdi, 2018; Azimi et al., 2018; Khodadai et al., 2018; Kitamura et al., 2017; Lee et al., 2011; Wang & Wang, 2016).

2.6.2. Open field apparatus

One day after finishing MWM, we evaluated the effects of IA-induced striatum lesion and Baclofen injection and Treadmill training on motor activity according to the animals’ performance in the open field. The apparatus, made of wood, was covered with dark polyethylene. The arena had a floor of 100 cm 100 cm and walls, 40 cm high. Its floor is divided into 12 parts. Each rat was placed in the center of the open field and a video camera system (Ethovision 1.6 Noldus, Waninggen) recorded its movements for a period of 10 min in dark room as MWM test. For each rat open filed arena cleaned by alcohol 70% and waiting for drying then using for another rat. The distance was used to evaluate the motor activity (Lee et al., 2011).

2.7. Histology

Following behavioral experiments, the rats were deeply anesthetized then microinjection sites received 1% methylene-blue solution bilaterally (0.3 µl/methylene-blue, per side), as described above. Rats were then decapitated; their brains were moved and placed in formaldehyde (10%). After several days, the brains were sliced for verification of injection sites. The data from animals with correct bilateral injection sites were included in the statistical analyses (Figure 2).

2.8. Statistical analysis

The results were expressed as means ± SEM. We used two-way repeated measures ANOVA (GROUP × DAY) to determine whether all groups reduced their latencies/distances traveled over the course of acquisition, or whether there was a GROUP × DAY interaction. Differences within or between normally distributed data were analyzed by one-way analysis of variance (one-way ANOVA), followed by Tukey’s post hoc test. The dependent variables were the SEM of “travel distance and escape latency” in MWM test, “percentage of time spent in target quadrant” in probe test, and “total distance” in the open-field test. All graphs were prepared using Graph Pad Prism 6.0 (Graph Pad Software Inc.). Statistically significant differences were accepted at P < 0.05.
3. Results

3.1. Effect of treadmill running on striatum lesion-induced spatial learning and motor activity deficits

Our results showed that there was no significant difference in traveled distance and escape latency parameters between treadmill “T” and “C-untrained” groups ($F_{(5, 42)} = 6.88, P = 0.92; F_{(5, 42)} = 30.37, P = 0.91$, respectively). However, there was no statistically significant difference between above both groups in percentage of time spent in target quadrant ($F_{(5, 42)} = 10.55, P = 0.91$) and open field test ($F_{(5, 42)} = 11.40, P = 0.25$, Total distance parameter).

Also, there was no significant difference between sham and control groups in the acquisition and probe phases of MWM and open field tests ($P > 0.05$). Thus, in our protocol, the treadmill had no significant effect in the acquisition and probe phases of MWM and open field tests.

In the acquisition phase, learning was measured by escape latency and traveled distance from day 1 to day 4. Two-way ANOVA with repeated measures revealed that main effect for day (within-subjects factor) (Figure 3a, b; Traveled distance: $F_{(3, 42)} = 241.4, P < 0.0001$; Escape latency: $F_{(3, 42)} = 156.00$, $P < 0.0001$).

![Figure 2](image-url)
P < 0.0001), and treatment (between-subjects factor) (Travel distance: \( F_{(5, 42)} = 5.48, P = 0.0006; \) Escape latency: \( F_{(5, 42)} = 17.40, P < 0.0001 \)) were statistically significant. However, days \( \times \) treatment interaction was not significant for traveled distance (\( F_{(15, 42)} = 1.63, P = 0.08 \)) or escape latency (\( F_{(15, 42)} = 1.64, P = 0.08 \)). One-way ANOVA followed by post hoc Tukey’s HSD test showed that travel distance and escape latency significantly increased in the “Str (IA)” group as compared to “Str (NS)” group (Figure 3a, b; Travel distance: \( P = 0.01 \) on day 1, \( P = 0.02 \) on day 2, \( P = 0.007 \) on day 3; Escape latency: \( P < 0.0001 \) on day 1, \( P < 0.0001 \) on day 2, \( P = 0.0006 \) on day 3, \( P = 0.003 \) on day 4). Thus, IA microinjection into striatum impairs spatial learning and memory. However, travel distance (\( F_{(5, 42)} = 6.88, P = 0.92 \)) and escape latency (\( F_{(5, 42)} = 30.37, P = 0.15 \)) had no significant different between “Str (IA)” and “Str (IA) + T” groups. Thus, mild treadmill exercise had not significant effect on IA-induced spatial learning and memory deficit. In addition, our data showed a significant increase in “Str (IA) + T” group as compared to “T” group (Figure 3a, b; Travel distance: \( P = 0.02 \) on day 1, \( P = 0.03 \) on day 2, \( P = 0.02 \) on day 3; Escape latency: \( P < 0.0001 \) on day 1, \( P = 0.0001 \) on day 2, \( P = 0.0009 \) on day 3, \( P = 0.05 \) on day 4). However, there was no statistically significant difference in travel distance and escape latency between “Str (NS)” and “Str (NS) + T” groups [Travel distance: \( F_{(5, 42)} = 6.88, P = 0.97 \) and Escape
latency: \(F(5, 42) = 30.37, P = 0.67\) and also “Str (NS) +T” and “T” groups [Travel distance: \(F(5, 42) = 6.88, P = 0.99\) and Escape latency: \(F(5, 42) = 30.37, P = 0.98\)]. Thus, microinjection normal saline into striatum alone or treadmill exercise had no significant effect on spatial memory.

In the probe test, the ability of spatial memory was assessed on day 5. One-way ANOVA showed that there was a significant difference among the groups on the percentage time spent in the targeted quadrant (Figure 3c; \(F(5, 42) = 10.55, P = 0.0001\)). Post hoc analysis showed that percentage of time spent in target quadrant significantly decreased in “Str (IA)” group as compared to “Str (NS)” group \((F(5, 42) = 10.55, P = 0.003)\). In addition, percentage of time spent in target quadrant increased significantly in “Str (NS) +T” group as compared to “Str (IA)+T” group \((F(5, 42) = 10.55, P = 0.01, \text{ Figure 3c})\). However, there was no significant difference in the percentage of time spent in target quadrant between “Str (IA)” and “Str (IA)+T” groups \((F(5, 42) = 10.55, P = 0.093)\). Thus, our data showed that IA microinjection into striatum significantly impaired spatial memory while treadmill exercise could not ameliorate IA-induced spatial memory impairment (Figure 3c).

Striatum lesion-induced by IA infusion significantly decreased an average of total distance in “Str (IA)” group as compared to “Str (NS)” group \((F(5, 42) = 11.40, P < 0.0001)\). Thus, IA microinjection in to the Str induced motor activity deficit. However, analysis of our open field test by using Tukey’s HS post-test disclosed a significant decrease in an average of total distance in “Str (IA)” group as compared to “Str (IA)+T” group \((F(5, 42) = 11.40, P < 0.004, \text{ Figure 3d})\). Accordingly, it seems that mild treadmill exercise ameliorated striatum lesion-induced motor activity deficit.

All animals learned the location of the hidden platform after 4 days of training. In order to, our results showed that moderate treadmill exercise had no statistically significant effect on probe test of IA-induced spatial memory deficit rats. Subsequently, visual ability of rats was assessed in the visible platform test. Results showed that there was no significant difference in visual-motor coordination and they were capable to find the platform according to visual cues (Figure 3e).

Taken together, MWM data indicated that microinjection of IA into the striatum impaired spatial learning and motor activity. Nevertheless, mild treadmill exercise had no significant effect on IA-induced spatial learning and memory deficit but it could improve IA-induced motor activity impairment.

### 3.2. Effect of Baclofen microinjection in the GPi on striatum lesion-induced spatial learning and motor activity deficits

Two-way ANOVA with repeated measures revealed that main effect for day (within-subjects factor) was statistically significant (Figure 4a,b; Travelled distance: \(F_3, 42) = 250.0, P < 0.0001\); Escape latency: \(F_3, 42) = 124.2, P < 0.0001\), as was the main effect of treatment (between-subjects factor) (Travelled distance: \(F_5, 42) = 6.54, P = 0.0001\); Escape latency: \(F_5, 42) = 21.72, P < 0.0001\)). However, days x treatment interaction was not significant for travelled distance \((F_{15, 42}) = 1.64, P = 0.07\) or escape latency \((F_{15, 42}) = 0.59, P = 0.87\). One way ANOVA followed by post hoc Tukey’s HSD test showed that travel distance and escape latency decreased in “GPi (Bac)” group compared to “GPi (NS)” group but this reduction was not significantly different \((F(5, 42) = 8.80, P = 0.02, \text{ Figure 4a, b})\). In addition, percentage of time spent in target quadrant and total distance were not significantly different in “GPi (Bac)” group compared to “GPi (NS)” group \((F(5, 42) = 12.14, P = 0.01, \text{ Figure 4b})\). Therefore, usage dose of Baclofen alone had no effect on spatial learning and memory. Moreover, there was no significant difference among “GPi (NS)” and control groups in MWM, probe, and open field tests \((P > 0.05)\). Striatum lesion induced by IA infusion increased significantly travel distance and escape latency in “Str (IA)+GPi (Bac)” and “Str (IA)” groups compared to other groups \((F(5, 42) = 8.80, P < 0.0001, F(5, 42) = 23.42, P < 0.0001)\). However, Baclofen microinjection could not decrease significantly travel distance and escape latency \((F(5, 42) = 8.80, P = 0.98, F(5, 42) = 23.42, P = 0.61 \text{ respectively})\) in “Str (IA)+GPi (Bac)” group compared to “Str (IA)” group (Figure 4a, b). Analysis of data showed that no statistically significant difference between “Str (NS)” and “Str (NS)+GPi (Bac)” groups in travel distance and escape latency. In addition, results showed a significant escape latency increase in “Str (IA)+GPi (Bac)” group as compared to “Str (NS)+GPi (Bac)” group (Figure 4b, Escape latency: \(P = 0.03 \text{ on day 1})\).

In the probe test, analysis of data showed no significant difference between “Str (NS)” and “Str (NS)+GPi (Bac)” groups \((P > 0.05)\), one-way ANOVA in the percentage of time spent in target quadrant. Moreover, our results showed that no significant difference between “Str (NS)+GPi (Bac)” and “Str (IA)+GPi (Bac)” groups \((F(5, 42) = 12.14, P = 0.05, \text{ Figure 4c})\). Therefore, IA-induced striatum lesion animals received Baclofen microinjection in the GPi showed no significant difference in the percentage of time spent in target quadrant as compared to IA-induced lesion animals \((F(5, 42) = 12.14, P = 0.07)\). Analysis using Tukey’s HS post-test disclosed a significant increase in average of total distance in “Str (IA)+GPi (Bac)” group compared to “Str (IA)” group in open field test \((F(5, 42) = 24.30, P < 0.0001)\). In this regard, it seems that Baclofen microinjection in the
GPi had no effect on the spatial learning and memory but it ameliorated motor activity impairment. However, results showed that there was no significant difference in visual-motor coordination and they were capable to find the platform according to visual cues (Figure 4e).

### 3.3 Effect of combination baclofen and mild treadmill exercise on striatum lesion-induced spatial learning, memory, and motor activity deficits

Two-way ANOVA with repeated measures revealed that main effect for day (within-subjects factor) was statistically significant (Figure 5a, b; Traveled distance: $F(3, 28) = 8.12, P < 0.0001$ and Escape latency: $F(3, 28) = 13.75, P < 0.0001$), as was the main effect of treatment (between-subjects factor) (Traveled distance: $F(3, 28) = 15.94, P < 0.0001$). However, day $\times$ treatment interaction was not significant for traveled distance ($F(9, 28) = 3.09, P = 0.002$) or escape latency ($F(9, 28) = 1.22, P = 0.28$). One-way ANOVA followed by post hoc Tukey’s HSD test showed significant differences between “Str (IA) + GPi (Bac)” and the other groups in the mean traveled distance ($F(3, 28) = 155.2, P < 0.0001$; Escape latency: $F(3, 28) = 54.97, P < 0.0001$), as was the main effect of treatment (between-subjects factor) (Traveled distance: $F(3, 28) = 6.15, P = 0.002$; Escape latency: $F(3, 28) = 15.94, P < 0.0001$). However, day $\times$ treatment interaction was not significant for traveled distance ($F(9, 28) = 3.09, P = 0.002$) or escape latency ($F(9, 28) = 1.22, P = 0.28$). Our results

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**FIGURE 4.** Baclofen microinjection in the GPi had no effect on IA-induced spatial learning and memory deficit but it improved motor activity in striatum lesion rats. In acquisition phase, Baclofen microinjection had no significant effect on the mean values of distance to find hidden platform (a) and escape latency (b) and time spent in target quadrant in probe test (c) but alleviated striatum lesion-induced motor activity impairment and produced a significant increase in total distance traveled between “Str (IA)” and “Str (IA) + GPi (Bac)” groups (d). Data presented as mean±SEM. * $P < 0.05$ versus the Str (NS) + GPi (Bac) group, # $P < 0.001$ versus the Str(IA) group.
showed that travel distance and escape latency significantly decreased in “Str (IA) + GPi (Bac) + T” group as compared to “Str (IA)” group (Figure 5a, b; Traveled distance: $P < 0.0001$ on day 1, $P = 0.0001$ on day 2, $P = 0.001$ on day 3, $P = 0.05$ on day 4; Escape latency: $P < 0.0001$ on day 1, $P < 0.0001$ on day 2, $P < 0.001$ on day 3). Travel distance and escape latency significantly declined in “Str (IA) + GPi (Bac) + T” group as compared to “Str (IA) + T” group (Figure 5a, b; Traveled distance: $P = 0.007$ on day 1, $P = 0.01$ on day 2, $P = 0.01$ on day 3; Escape latency: $P = 0.0001$ on day 1, $P = 0.01$ on day 2, $P < 0.003$ on day 3). However, travel distance and escape latency significantly declined in “Str (IA) + GPi (Bac)” group as compared to “Str (IA)” group (Figure 5a, b; Traveled distance: $P = 0.003$ on day 1, $P = 0.02$ on day 2; Escape latency: $P < 0.0001$ on day 1, $P = 0.01$ on day 2, $P = 0.0003$ on day 3, $P < 0.001$ on day 4).

Our results showed that a significant difference in the percentage of time spent in target quadrant between “Str (IA) + GPi (Bac) + T” and the other groups ($F_{(3, 28)} = 9.28, P = 0.0002$); and this parameter increased significantly in “Str (IA) + GPi (Bac) + T” group compared to “Str (IA)” group ($F_{(3, 28)} = 9.28, P = 0.0002$, Figure 5c). Average of total distance significantly increased in lesion animals treated with Treadmill and Baclofen microinjection in the GPi compared to IA-induced lesion animals in the open field test ($F_{(3, 28)} = 23.93, P = 0.0006$, Figure 5d). Thus, our results indicated that interaction of mild treadmill exercise and usage dose of Baclofen microinjection...
in the GPi alleviated striatum lesion-induced spatial memory and motor activity deficits. However, results showed that there was no significant difference in visual-motor coordination and they were capable to find the platform according to visual cues (Figure 5e).

4. Discussion

It has been shown that lesions of BG cause impairments in the acquisition of various types of behaviors like motor functions and also learning and memory processes (Packard & Knowlton, 2002). These impairments seem to pursue with the disturbed regulation of GABAergic circuit of BG. However, most of the available documents showed that physical exercise has a protective effect on brain health and cognitive function under normal or disease conditions such as neurodegenerative diseases (Cassilhas, Tufik, & de Mello, 2016). Thus, we investigated combination effects of Baclofen microinjection in the GPi and mild forced treadmill exercise on striatum lesion-induced impairments of spatial learning and motor activity. Initially, in this experiment, we showed that impairments of spatial learning and memory and motor activity after striatum lesion-induced by IA injection (Figure 6). Then, a combination of both mild forced treadmill exercise and Baclofen microinjection could alleviate BG’s dysfunctions in the motor activity and spatial learning and memory.

The results of MWM test showed that distance and escape latency to find hidden platform significantly decreased in all groups in four training days. Comparison of MWM and probe tests showed that there was no significant difference between Str (IA) and Str (IA) + T groups. Therefore, mild forced treadmill exercise could not significantly improve spatial learning and memory impairment in striatum lesion groups. This result is inconsistent with previous studies for the duration of exercise. It has been reported that forced running paradigm for 5 weeks is effective in enhancing spatial learning and memory by increasing in the number of cholinergic neurons in the horizontal diagonal band of Broca in the septum (Ang, Dawe, Wong, Moochhala, & Ng, 2006). De Senna et al. has shown that exercise for 12 weeks improves short-term memory retention, enhances motor performance in diabetic rats, and affects important structural components of the striatal blood-brain barrier (de Senna et al., 2015). Overall, our findings suggest that exercise program could not improve impairment induced in striatum lesion by IA injection. However, further research is needed to consider various intensity and period of treadmill exercise and another types of exercise in this field, because this kind of exercise has a forced potential and animals may exposure stress during running. Results of open field test demonstrated a significant difference between Str (IA) and Str (IA) + T groups. So, mild forced treadmill exercise could improve motor activity consistent with previous studies (Loprinzi, Herod, Cardinal, & Noakes, 2013).

Consistent with our findings, positive effects of exercise on improving motor control and functional mobility have been documented in patients with PD. For example, intensive and challenging task-specific and functional mobility training may increase neuroplasticity in the BG system, which is responsible for active motor control in PD patients. Neuroprotective and neurorestorative capacities after intensive exercise have also been reported by animal studies. Furthermore, the exercise-induced benefits on overall brain health, including increased blood flow and trophic factors and a stronger immune system, may help address the environmental need for neuroplasticity in the damaged brain. Therefore, the improvements in motor activity after treadmill training in our study may also be attributed to the mechanisms mentioned above. Studies in both humans and animals have shown that physical activity can increase a wide range of neurotrophic factors (Fisher et al., 2008). Exercise-induced induction of neurotrophic factors occurs in a number of brain regions involved in motor control (Fisher et al., 2013). In addition, neurotrophic factors can promote a wide range of effects including synaptogenesis at dendritic sites (local translation effects); i.e., activation of kinases, including the MAPK cascades; induction of gene expression at the level of the cell nucleus; the regulation of presynaptic neurotransmitter release; and increased neurogenesis. These neurotrophic-mediated mechanisms may play a key role in exercise-induced neuroplasticity and alleviate motor activity impairment after striatum lesion.

FIGURE 6. The effects of Baclofen injection in GPi and force treadmill exercise in direct and indirect pathways in animal model of Str lesion-induced by injection of IA.
In the second part of our experiment, we injected a specific dose of Baclofen in normal rats and our results showed that there was no significant difference between GPi (Bac) and GPi (NS) groups. However, Baclofen microinjection into GPi could not improve significantly spatial learning and memory in striatum lesion animals. Probably, this dose of the drug was not sufficient to improve spatial learning and memory as discussed in previous foundlings (McNamara & Skelton, 1996). Our findings were inconsistent with those of Luo et al. (2016), who illustrated that Baclofen treatment relieves spatial working memory deficits in rats (Luo et al., 2016). This conflict may refer to the Baclofen’s injection area in the brain and amount dose was used for micro-injection of baclofen. Results of open field test founded a significant difference between Str (IA) and Str (IA) + GPi (Bac) groups. So, Baclofen microinjection in GPi could improve significantly motor activity. These results are consistent with previous works that revealed stimulation of GABAergic neurons with the GABA_B receptor agonist, baclofen, increases ubiquitin-proteosome system (UPS) function and cell survival in vitro and in vivo models of HD (Kim & Seo, 2014; Navarrete-Opazo, Gonzalez, & Nahuelhual, 2016; Sasaki & Ogiwara, 2016).

In the final part of this study, our results showed that interaction of mild forced treadmill exercise and Baclofen microinjection in the GPi could improve significantly spatial learning and memory and motor activity. Comparing the average distance and escape latency for finding hidden platform demonstrated a significant difference between Str (IA) and Str (IA) + GPi (Bac) + T groups. Moreover, results of open field test demonstrated that Str (IA) + GPi (Bac) + T group increased significantly total distance than Str (IA) group.

Our experiment concluded that interaction treatment of drug and exercise could improve spatial learning and memory by IA-induced striatum lesion in an animal model. Previous studies showed that physical exercise causes neurogenesis, cell proliferation, and dendritic branching (Berchtold, Castello, & Cotman, 2010; Cassilhas et al., 2016; Petzinger et al., 2013; Petzinger et al., 2010). It is also interesting to note that such benefits of exercise might be the result of increased endogenous neurotrophic factors (Wu et al., 2011). It has previously been reported that hippocampal BDNF and NGF protein levels are below control levels 6 weeks after exercise (8 weeks swimming, 5×/week) (Radak et al., 2006). Exercise-induced changes in the AMPA-R, as it is responsible for the majority of fast excitatory neurotransmission in the CNS and mediates activity-dependent processes that alter synaptic strength (Isaac, Ashby, & McBain, 2007). Slow synaptic inhibition is mediated by metabotropic GABA_B receptors and it can be activated directly by GABA binding, and facilitate postsynaptic action potential. Baclofen produces slow presynaptic inhibition by inhibiting Ca^{2+} channels, while postsynaptic GABA_B receptors induce a slow inhibitory postsynaptic current, activating G protein-coupled inwardly rectifying K^+ (GIRK) channels (Kim & Seo, 2014; Ladera et al., 2008). So, Baclofen microinjection in the GPi through modulating direct pathway and following final output of BG had a subsidiary role in our experiment for helping exercise program and improving spatial learning and motor activity (Kim & Seo, 2014; Nourjah et al., 2006).

The main hypothesis of our experiments was to determine whether GABA_B receptor agonist and exercise composition could affect motor activity and spatial learning and memory in an animal with striatum lesion. This result is a new approach in this filed and our results support it.

In summary, our findings suggest that the interaction of mild forced treadmill exercise and Baclofen microinjection in the GPi contribute to motor activity and spatial learning and memory improvement in striatum lesion rats. Although our results suggest new treatment for brain dysfunctions specifically BG disorders but it still needs more studies in this matter. Physical exercise protocols have needed widely to study raising the question of whether there is an optimal intensity, duration, and frequency that would produce maximal benefits in attenuating symptoms related to movement disorders.

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