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Physical exercise during pregnancy minimizes PTZ-induced behavioral manifestations in prenatally stressed offspring

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Abstract

Stress during gestation has been shown to affect susceptibility and intensity of seizures in offspring. Environmental stimuli, such as maternal physical exercise, have shown to be beneficial for brain development. Although studies have demonstrated the deleterious influence of stress during pregnancy on seizure manifestation in offspring, very little is known on how to minimize these effects. This study verified whether physical exercise during the pregnancy associated with prenatal stress minimizes seizure susceptibility in offspring at the beginning of postnatal development. Pregnant rats and male pups were divided into the following groups: control, stress, stress/forced exercise, and stress/voluntary exercise. Behavioral manifestations were analyzed after injection of pentylenetetrazol (PTZ; 45 and 60 mg/kg) at ages P15 and P25. Increased behavioral manifestations and seizure severity was observed in the stress group compared with the control group at both ages. At the dose of 45 mg/kg, offspring of stressed mothers who performed both physical exercise models showed an increase in latency for the first manifestation and decrease in the seizures severity at both ages compared with the mothers groups who were only stressed. Prenatal restraint stress potentiated PTZ-induced seizure behavior, and both forced and voluntary exercise during gestation attenuates the negative effects of PTZ-induced offspring.

KEYWORDS

epilepsy, pentylenetetrazol, physical exercise, prenatal stress

1 | INTRODUCTION

Epilepsy is a chronic neurological disorder in which different underlying pathologies can cause abnormal excessive synchronous neuronal activity in the brain, resulting in epileptic seizures (Fisher et al., 2014, 2005). It affects people of all ages and has social, behavioral, health, and economic consequences for the patients and their families (WHO, 2005). Studies have indicated that, in general, 91% of patients with epilepsy can identify at least one precipitating factor of seizure (Nakken et al., 2005; Tan, Wilder-Smith, Lim, Ong, & K., 2005). The precipitating factor of seizure that is most frequently reported by patients with epilepsy is caused by several endogenous and exogenous factors, such as sleep deprivation, fever, intermittent light exposure, hyperventilation, and stress (Ferlisi & Shorvon, 2014; Lunardi, Sukys-Claudino, Guarnieri, Walz, & Lin, 2011; Nakken et al., 2005; Sperling, Schilling, Glosser, Tracy, & Asadi-Pooya, 2008; Tan et al., 2005). Stress has been considered the most important factor in seizure susceptibility (McKee & Privitera, 2017), especially when the stressor is severe, prolonged, or experienced in early life (van Campen, Jansen, Graan, Braun, & Joels, 2014). In this regard,

prenatal environments exert a profound influence on the development of the fetus and may predispose it to adaptive disorders throughout life (Heinrichs, 2010).

Prenatal stress (PS) signifies the exposure of a pregnant mother to distress and can lead to neurological disorders in offspring (Berger, Barros, Sarchi, Tarazi, & Antonelli, 2002; Patin, Lordi, & Caston, 2004). In humans, fetal exposure to chronically high levels of endogenous maternal corticosteroids (Takahashi, Turner, & Kalin, 1998) has been associated with adverse birth outcomes, including preterm birth (Stott, 1973), fetal growth retardation (Cliver et al., 1992), delays in early motor development (Sandman, Wadhwa, Chicz-DeMet, Dunkel-Schetter, & Porto, 1997), behavioral abnormalities (Trautman, Meyer-Bahlburg, Postelnek, & New, 1995), sleep disturbances (Weinstock, 1997), the development of psychiatric disorders, such schizophrenia and depression, in later life (Huttenen & Niskanem, 1978; Meier, 1985), and epileptic seizures (Gholipoor et al., 2017; Thébault-Dagher et al., 2017).

Studies conducted in rats have demonstrated that PS leads to an impaired feedback regulation of the hypothalamic–pituitary–adrenal (HPA) axis due to decreased hippocampal corticosteroid receptor expression (Sadaghiani & Saboory, 2010). Repetitive activation of the HPA axis during frequent bouts of stress results in elevated concentrations of glucocorticoids (GCs) in both the peripheral and central nervous system (Kofman, 2002; Mabandla, Kellaway, Daniels, & Russell, 2009). High levels of GCs have been shown to be toxic to regions of the nervous system that are easily excitable, such as the pyramidal cells of the hippocampus. These regions may therefore be involved in the development of seizure activity (McEwen & Magarinos, 2001; Weinstock, 2007).

Different PS protocols have been used to verify behavioral manifestations following brain insult in offspring. For instance, restraint stress (RS) under bright light in pregnancy increased the rate of hippocampal kindling in pups (Edwards, Dortok, Tam, Won, & Burnham, 2002; Edwards Vimal, & Burnham, 2002, 2002b). Hashemi et al. (2013) observed a decrease in latency for the first behavioral manifestation in offspring injected with PTZ from stressed mothers during the last week of gestation. The same research group also demonstrated that pilocarpine-induced seizure in offspring from stressed mothers increased the severity of the seizures and decreased the latency for the first behavioral manifestation (Ahmadzadeh, Saboory, Roshan-Milani, & Pelehvarian, 2011; Sadaghiani & Saboory, 2010).

Although studies have demonstrated the deleterious influence of stress during pregnancy on behavioral manifestation and seizure susceptibility in offspring, very little is known about how to minimize these negative effects. Although complementary therapies, such as yoga (Jiang, Wu, Zhou, Dunlop, & Chen, 2015), massage (Filed, Diego, Hernandez-Reif, Deeds, & Figueiredo, 2009), acupuncture (Ormsby, Smith, Dahlen, Hay, & Lind, 2016), and physical exercise (Haakstad, Torset, & Bø, 2016), have been used to mitigate the negative effects caused by stress during the gestational period, no studies have been conducted to understand the impact of a physical exercise program during the period of gestational PS and on seizure susceptibility in offspring. This study, therefore, aimed to investigate whether two types of exercise programs (forced and voluntary) during the pregnancy of mothers submitted to prenatal RS minimizes seizure susceptibility and intensity in offspring in the beginning of postnatal development.

Highlights

- Stress during pregnancy increased seizure susceptibility in offspring;
- Intensity of seizures was increased in offspring in the early periods of postnatal development;
- Offspring of stressed exercised mothers presented decreased seizure susceptibility.

2 | METHODS

2.1 | Animals

In total, 46 female and 22 male Wistar rats, aged 8 weeks (230–250 g), were initially used for this study. The rats were obtained from CEDEME/Universidade Federal de São Paulo, São Paulo, Brazil, and were housed in a group of four rats of same sex in plastic home cages. The colony room was maintained at 21 ± 2°C with a 12-hr light/dark schedule (lights on at 7 a.m.) and ad libitum food and water throughout the experiments. All procedures involving animals were approved by the ethics committee (#2441171115). Two female rats were randomly paired with one male. Vaginal smears were taken daily in the morning (8:00–10:00) using a Pasteur's pipette filled with saline buffer (20 µl) which was introduced in the rats' vagina to look for the presence of sperm. The presence of sperm marked day 1 (G1) of pregnancy. According to the voluntary exercise procedure used in our protocol, two rats were excluded from the stress/voluntary group (see description in the section, "Voluntary exercise procedure2.3"). Therefore, pregnant dams were randomly divided into one of four experimental groups: (a) control (CTL, n = 11); (b) stress (STR, n = 10); (c) stress/forced exercise (STR/FOR, n = 12; adn (d) stress/soluntary exercise (STR/VOL, n = 11). After delivery, male offspring were divided into the following groups: CTL (n = 66); STR (n = 62); STR/FOR (n = 70); and STR/VOL (n = 67). The experimental design is shown in Figure 1.

2.2 | PS procedure

The stressed groups were exposed to the stressor throughout the whole gestational period. For restraint-stressed rats, stress involved the transportation of the home cage to the experimental room and placement of the pregnant female in a restraint chamber (transparent, plastic, cylindrical chamber, 6 cm diameter, 16 cm length). Rats were restrained for 120 min once per day (between 08:00 and 11:00). The non-stressed rats were transported to the experimental

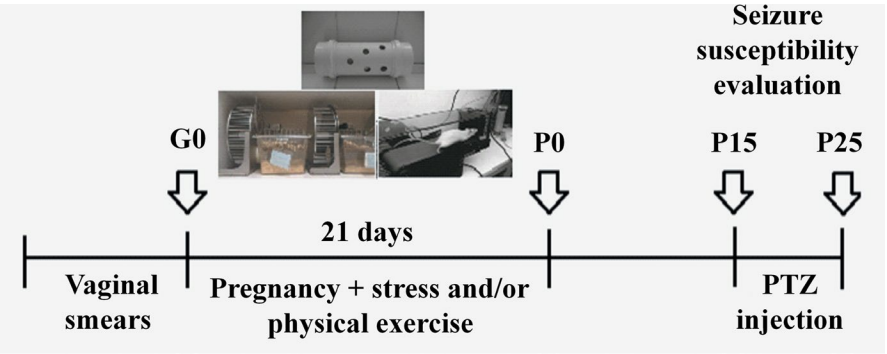

FIGURE 1 Experimental design of study. G0, gestational day 0; P0, postnatal day 0; P15, postnatal day 15; P25, postnatal day 25

room throughout the whole gestation period and were handled similarly to other groups but not stressed.

2.3 | Forced exercise procedure

Rats from the stress/forced groups were familiarized with the apparatus (treadmill—Columbus instruments) for 3 days before the match. The rats were placed on the treadmill, which was switched off during the familiarization period. Following day G1, pregnant rats were subjected to 20 sessions of an aerobic exercise program at a 0%-degree incline for 30 min/day, reaching 18 m/min in the last week. Electric shocks were not used to motivate rats to run. Therefore, a measure of trainability was used according to their performance on a scale between 1 and 5 (Dishman, Armstrong, Delp, Graham, & Dunn, 1988). The parameters of the scale were as follows: 1—refused to run; 2—below average runner (sporadic, stop, and go, wrong direction); 3—average runner; 4—above average runner (consistent runner occasionally fell back on the treadmill); and 5—good runner (consistently stayed at the front of the treadmill). The protocol states that rats that score below 3 are excluded from the experiment. However, no pregnant rats were excluded. The training protocol was performed between 11:00 and 13:00, after the stress procedure. Daily running distance for this group was quantified by multiplying the speed programmed in the treadmill with the time that the rats were kept running during every session. Subsequently, the daily values that were obtained were added together and divided by 20 (total number of sessions).

2.4 | Voluntary exercise procedure

After pregnancy was confirmed, rats submitted to the stress/voluntary exercise group were placed in a cage with voluntary wheel running (Panlab Harvard Apparatus) throughout the whole gestational period, with free access to water and food. Daily running distance was quantified using an odometer, which registered the number of wheel revolutions. The daily odometer value which was obtained was multiplied by $2\pi R$, considering R as the radius measured from the center of the wheel to its inner surface. Daily values were added together and divided by 20 (total number of days that rats had free access to the wheel). To establish a pattern and to compare voluntary exercise with forced exercise, we used the rats that covered more than 7,000 revolutions during the gestational period.

2.5 | Distribution of the offspring groups

At postnatal day 1, offspring were weighed. Female offspring were removed from the litter to maintain an equal number of eight pups per mother. This procedure was performed to avoid possible nutritional changes in the litter as well as to achieve our objective of analyzing only male pups. After this procedure, the offspring groups were divided as presented in the Table 1.

2.6 | Pentylenetetrazol injection

PTZ (Sigma) 1% was given to pups at two different postnatal ages (P15 and P25) and in two different doses (45 and 60 mg/kg). Studies have shown that different doses of PTZ present subconvulsive (45 mg/kg), convulsive (60 mg/kg), and lethal (100 mg/kg) characteristics (Ebrahimi, Saboory, Roshan-Milani, & Hashemi, 2014; Jandová, Riljak, Pokorný, & Langmeier, 2007). Immediately after the PTZ injection, the rats were transferred to a transparent cage and monitored for 120 min to observe the occurrence of the first behavioral manifestations and seizure intensity. Record analyzes were performed by the study's lead investigator and by another co-author independently using Intelbras Media Player software, version 3.36.12.

2.7 | Behavioral assessment

Seizure rating was assessed using a previously defined scale (Gholami, Saboory, & Roshan-Milani, 2014; Gholami, Saboory, Zare, Roshan-Milani, & Hajizadeh-Moghaddam, 2012): 0 = normal; 1 = immobilization, sniffing; 2 = head nodding, facial, and forelimb clonus (short myoclonic jerk); 3 = continuous myoclonic jerk, tail rigidity; 4 = generalized limbic seizure with kangaroo posture or violent convulsion; and 5 = continuous generalized seizures (tonic or tonic-clonic convulsions). Seizure intensity was evaluated by the total score of the seizure as follows: TSS = SBS + (1/LTCS × 100) + NTCS + DTCS, where TSS stands for the total score of seizure, SBS stands for the sum of behavioral stages, LTCS is the latency for tonic-clonic seizure

(sec), NTCS refers to the number of tonic-clonic seizures, and DTCS indicates the duration of tonic-clonic seziures (sec; Gholipoor, Saboory, Roshan-Milani, & Fereidoni, 2013).

2.8 | Removal of the adrenal gland

On the last day of gestation, pregnant rats were anesthetized intraperitoneally with ketamine and xilazine (66.6 and 13.3 mg/kg, respectively) and the adrenal glands from six rats per group were quickly removed and weighed.

2.9 | Statistical analysis

Data distribution was controlled using the Shapiro–Wilk test. The data that were normally distributed were analyzed using parametric techniques, one-way ANOVA followed by Tukey's post–hoc test when required. Results with non-normal distribution were compared using Kruskall–Wallis and Mann–Whitney nonparametric tests. Significant differences were indicated when the *p*-value was lower than .05. All values are expressed as mean and standard error of mean ($M \pm SEM$). Analyses were performed using IBM SPSS version 20.0 software (SPSS Inc., IBM Company).

3 | RESULTS

3.1 | Effect of RS on offspring weight

On the day of birth, offspring from all groups were weighed. The Shapiro–Wilk test indicated a normal distribution ($p > .05$; $n = 30$ for each group). ANOVA showed a difference between the groups [$F(3.116) = 101.257$; $p < .001$]. The Tukey's post-hoc test presented a difference in weight (grams) between the groups (Table 2). No difference in offspring weight was found at the time of the PTZ injection at ages P15 and P25.

TABLE 1 Division of the offspring number into groups based in the doses and ages

	CTL	STR	STR/FOR	STR/VOL
45 mg/kg				
P15	17	16	18	16
P25	17	14	17	17
60 mg/kg				
P15	18	17	19	17
P25	14	15	16	14

Abbreviations: CTL, control; P15, postnatal age 15 days; P25, postnatal age 25 days; STR, stress; STR/FOR, stress/forced; STR/VOL, stress/voluntary.

3.2 | Behavior analysis

3.2.1 | Latency for the first manifestation in seconds (P15 and P25—45 mg/kg)

For 45 mg/kg of PTZ, all rats (P15 and P25) presented behavioral manifestations. The latency to onset of first manifestation was significantly different between each age group. At age P15, the Shapiro–Wilk test indicated a normal distribution ($p > .05$) and ANOVA showed a significant difference between the groups [$F(3.63) = 8.478$; $p < .001$]. Tukey's post-hoc analysis showed a significant difference between the groups as shown in Figure 2. At age P25, the Shapiro–Wilk test indicated a normal distribution ($p > .05$) and ANOVA showed a significant difference between the groups [$F(3.61) = 18.584$; $p < .001$]. Tukey's post-hoc analysis showed a significant difference between the groups as shown in Figure 3.

3.2.2 | Latency for the first behavioral manifestation in seconds (P15 and P25—60 mg/kg)

For 60 mg/kg of PTZ at age P15, the Shapiro–Wilk test indicated a normal distribution ($p > .05$) and ANOVA test showed no difference between the groups [$F(3.67) = 2.586$; $p = .060$]. The values of latency for this group were: CTL (74.8 ± 19.2); STR (61.5 ± 9.9); STR/FOR (62.8 ± 18.9); and STR/VOL (63.4 ± 14.4). The same occurred for age P25: the Shapiro–Wilk test indicated a normal distribution ($p > .05$) and ANOVA showed no difference between the groups [$F(3.55) = 2.587$; $p = .062$].

3.2.3 | Score of severity of motor manifestations (P15 and P25—45 mg/kg)

We used the formula proposed by Gholipoor et al. (2013) and the scale of manifestations proposed by Gholami et al. (2012) and Gholami et al. (2014) to analyze the general severity of the behavioral manifestations. Therefore, for ages P15 and P25, the statistical analysis indicated a non-normal distribution (Shapiro–Wilk: $p < .05$ for both ages).

TABLE 2 Difference between weights in offspring at postnatal day 1

Difference between the groups	*p* value
CTL (6.49 g ± 0.32 g) versus STR (5.24 g ± 0.24 g)	<0
CTL (6.49 g ± 0.32 g) versus STR/FOR (5.35 g ± 0.24 g)	<0
CTL (6.49 g ± 0.32 g) versus STR/VOL (5.53 g ± 0.33 g)	<0
STR (5.24 g ± 0.24 g) versus STR/FOR (5.53 g ± 0.33 g)	<.004
STR (5.24 g ± 0.24 g) versus EST/VOL (5.53 g ± 0.33 g)	<.002

Abbreviations: CTL, control; STR, stress; STR/FOR, stress/forced; STR/VOL, stress/voluntary.

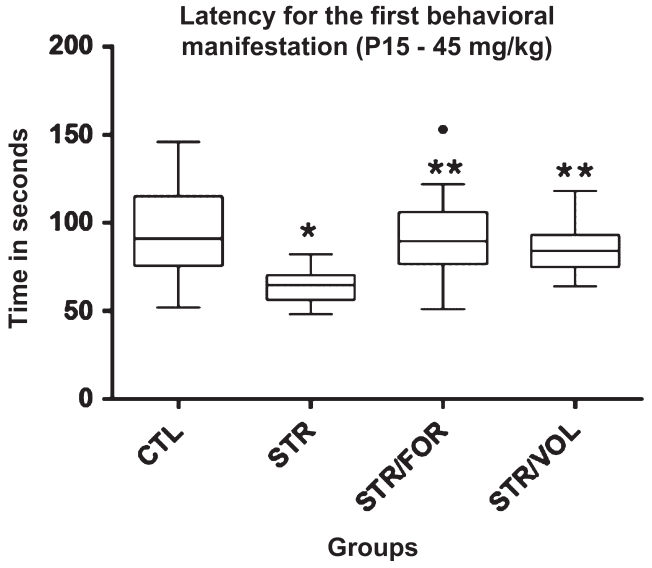

FIGURE 2 Latency for the first behavioral manifestation in seconds (P15, 45 mg/kg). *different from the control group; and **different from the stress group. CTL × STR (74.8 ± 19.2 vs. 63.9 ± 9.8; $p < .001$); STR × STR/FOR (63.9 ± 9.8 vs. 92.4 ± 23; $p < .001$); STR × STR/VOL (63.9 ± 9.8 vs. 87.1 ± 16.4; $p < .001$). CTL, control; STR, stress; STR/FOR, stress/forced; STR/VOL, stress/voluntary

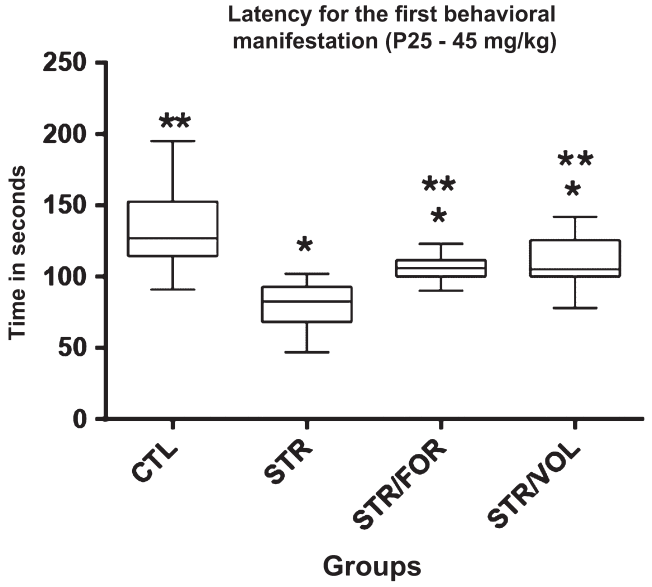

FIGURE 3 Latency for the first behavioral manifestation in seconds (P25, 45 mg/kg). *different from the control group; and **different from the stress group. CTL × STR (132.6 ± 27.4 vs. 80.1 ± 15.5; $p < .001$); CTL × STR/FOR (132.6 ± 27.4 vs. 105.2 ± 9.4; $p < .001$); CTR × STR/VOL (132.6 ± 27.4 vs. 109.6 ± 17; $p < .007$); STR × STR/FOR (80.1 ± 15.5 vs. 105.2 ± 9.4; $p < .005$); STR × STR/VOL (80.1 ± 15.5 vs. 109.6 ± 17; $p < .002$). CTL, control; STR, stress; STR/FOR, stress/forced; STR/VOL, stress/voluntary

First, for age P15, the Kruskal–Wallis nonparametric test showed a significant difference between the groups (H (3) = 13.056; $p < .05$).

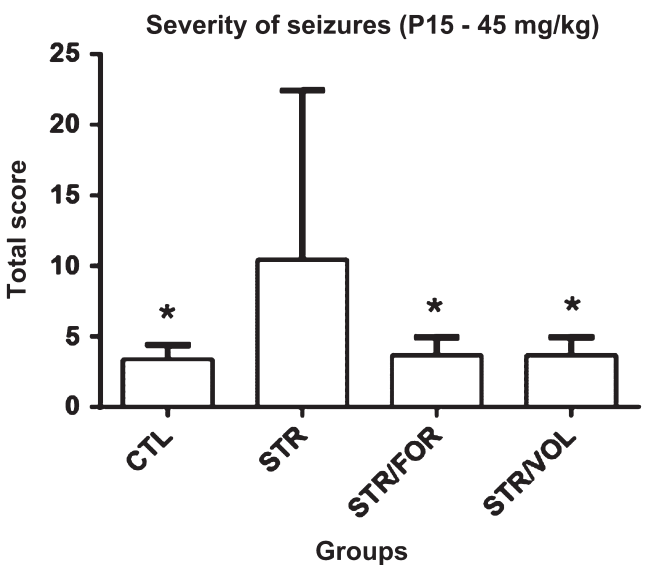

FIGURE 4 Total score seizure severity (P15 – 45 mg/kg). *different from the stress group. CTL vs. STR (U = 64.000; $p < .004$); STR vs. STR/FOR (U = 79.000; $p < .01$); STR vs. STR/VOL (U = 79.000; $p < .01$). CTL, control; STR, stress; STR/FOR, stress/forced; STR/VOL, stress/voluntary

The Mann–Whitney test showed a significant difference between the following groups as shown in the Figure 4.

For age P25, the Kruskal–Wallis nonparametric test showed a significant difference between the groups (H (3) = 16.229; $p < .05$). The Mann–Whitney test showed a significant difference between the following groups as shown in the Figure 5.

3.2.4 | Score of severity of motor manifestations (P15 and P25—60 mg/kg)

The Shapiro–Wilk test revealed a non-normal distribution for both ages ($p < .05$). In the Kruskal–Wallis nonparametric test, a significant difference between the groups at P15 was not observed (H (3) = 5.171; $p = .160$) and at P25 (H (3) = 2.610; $p = .456$).

3.3 | Weight of the adrenal gland

Statistical analysis of the weight of the adrenal gland presented a normal distribution (Shapiro–Wilk; $p = .455$). ANOVA indicated a significant difference between the groups [F(3.44) = 48.142; $p < .05$]: CTL versus STR ($p < .001$); CTL × STR/FOR ($p < .008$); CTL × STR/VOL ($p < .003$); STR versus STR/FOR ($p < .001$); and STR × STR/VOL ($p < .001$) (Figure 6).

4 | DISCUSSION

A growing number of studies have shown the benefits of physical exercise during the gestational period for mothers and for fetal neurodevelopment (Haakstad et al., 2016; Mudd et al., 2015). However,

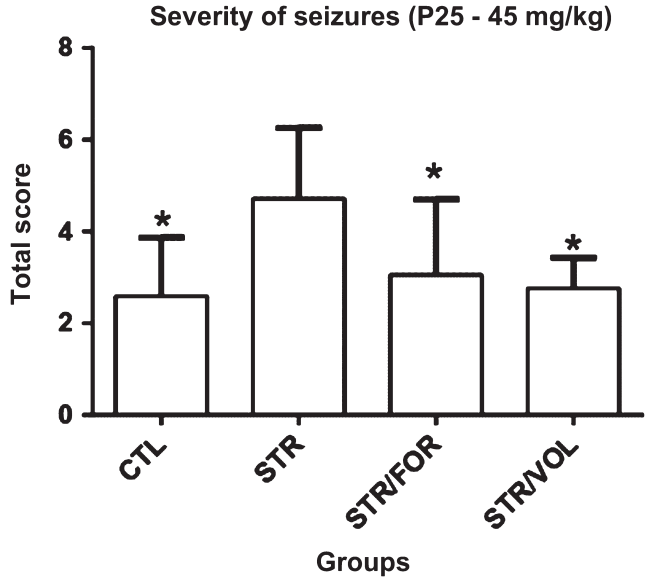

FIGURE 5 Total score seizure severity (P25 – 45 mg/kg). *different from stress the group. CTL vs. STR (U = 43.000; p < .001); STR vs. STR/FOR (U = 60.000; p < .01); STR vs. STR/VOL (U = 45.000; p < .01). CTL, control; STR, stress; STR/FOR, stress/forced; STR/VOL, stress/voluntary

it is not clear whether physical exercise in stressed pregnant subjects affects offspring submitted to brain insult early in life. Our findings show that stress during the gestational period reduced the latency for behavioral manifestations and increased seizure severity. In contrast, both physical exercise programs attenuated the negative effects of PTZ-induced in offspring from mothers submitted to PS.

On postnatal days 1, 15, and 25, the offspring were weighed to assess the effect of stress and/or physical exercise during gestation.

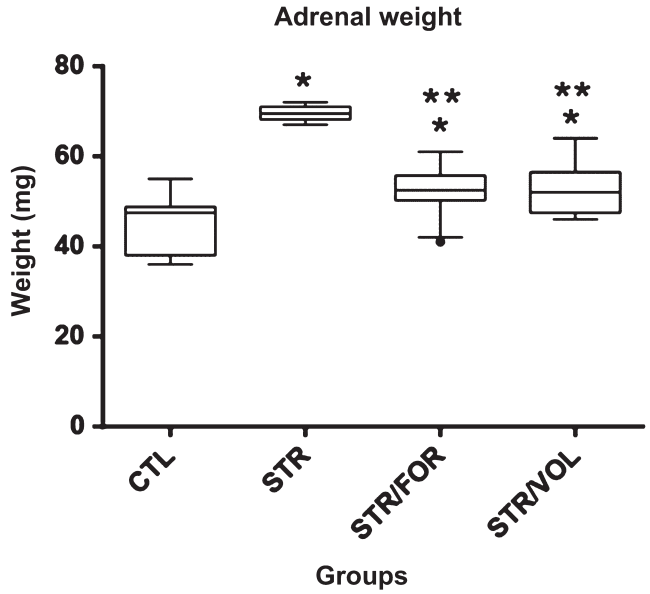

FIGURE 6 Weight of adrenal gland of pregnant rats (in mg). *different from hte control group; and **different from the stress group. CTL, control; STR, stress; STR/FOR, stress/forced; STR/VOL, stress/voluntary

It has been suggested that deregulation of the 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme increases the exposure of the GCs from the placenta to the fetus and reduces the activity and expression of 11β-HSD2 in the placenta, conferring less protection to the fetus (Avishai-Eliner, Brunson, Sandman, & Baram, 2002; Mairesse et al., 2007). Previous studies have demonstrated that inhibition of 11β-HSD2 contributes to weight loss at birth, intrauterine growth restriction, preterm birth, and preeclampsia (Causevic & Mohaupt, 2007; Michael & Papageorghiou, 2008). Our findings corroborated other studies (Ebrahimi et al., 2014; Hashemi et al., 2013) showing that stress during pregnancy, whether or not associated with a physical exercise program, reduced the weight of offspring at birth. However, at P15 and P25, discrepant outcomes were noted between our study and those by Hashemi et al. (2013) and Ebrahimi et al. (2014). While no significant difference was noted in pups' weight at P15 and P25 in our investigation, reduced weight was found in their studies at the same age. This difference may be related to the period of gestation during which the pregnant rats were exposed to the RS protocol. In our study, pregnant rats were stressed throughout gestation (G1–G20), unlike the other two studies where the stress protocol was performed only during the last week of gestation (between days 17 and 19).

For the investigation of behavioral manifestations, we used PTZ which is a noncompetitive antagonist of $GABA_A$ (Meilleur et al., 2003). According to Velisek et al. (1992), PTZ, when used in rodents, produces two different types of motor seizures—mild seizures, characterized by clonic manifestations, and intense seizures, characterized by generalized tonic-clonic manifestations. Our results showed that stress during gestation decreased the latency of motor manifestations in pups at both ages at a PTZ dose of 45 mg/kg. These findings accord with the study by Hashemi et al. (2013) which used similar doses of PTZ (40–50 mg/kg) at the same ages and found lower latency in pups of stressed mothers when compared with pups from control mothers. However, at 60 mg/kg, no significant difference was found in behavioral manifestations for all ages and groups; this may be attributable to the higher dose of PTZ.

Adrenal hypertrophy has been associated with various forms of chronic stress and states of anxiety (Kvetnansky & Mikulaj, 1970), including some forms of depression in humans (Gold, Licinio, Wong, & Chrousos, 1995). It has been used as a good measure of stress perception over periods of time and is thought to be caused by chronic overstimulation of the adrenal gland by elevated plasma GCs and ACTH. PS alters the regulation of the HPA axis, increasing the basal secretion of CRF and the production of GCs (Mairesse et al., 2007). CRF and GCs exert potent pro-convulsive effects on offspring by lowering the threshold for convulsive manifestations (Baram, 1993; Ebrahimi et al., 2014). Increased weight of the adrenal gland of stressed mothers was observed in our study, suggesting that our stress protocol was effective in inducing significant behavioral manifestations and seizure severity.

Regarding the severity of the behavioral manifestations, rats at an earlier postnatal period, that is, at P15, submitted to 45 mg/kg of PTZ, presented increased seizure severity compared with P25 rats.

One possible explanation for this difference may be related to the alteration in GABAergic maturation. According to Lubbers, Wolff, and Frotscher (1985), the maturation process of the GABAergic system occurs during the second and third postnatal weeks. The GABAergic synapse is responsible for providing most of the inhibitory drive in adult neural networks, but in the immature brain, the GABAergic system is excitatory due to different intracellular concentrations of Cl^- (Ben-Ari, 2002; Ben-Ari, Khazipov, Leinekugel, Caillard, & Gaiarsa, 1997). Thus, since GABAergic synapses are also formed prior to glutamatergic in the hippocampus, GABA provides most of the excitatory stimulus at an early stage of development (Ben-Ari, Khalilov, Represa, & Gozlan, 2004) and thus actively contributes to seizures (Dzhala & Staley, 2003). A study with similar characteristics to our investigation (Hashemi et al., 2013) reported that, at P15, generalized tonic-clonic seizures were more evident than at P25, where focal convulsions predominated. Velisek et al. (1992), comparing doses and ages of rats injected with PTZ, observed greater seizure severity at a postnatal age of less than 18 days than in rats at greater ages, where the manifestations occurred only with higher doses of PTZ.

In this study, both types of physical exercise intervention during pregnancy in stressed mothers were able to revert the negative manifestations caused by stress after 45 mg/kg of PTZ was injected into pups at both ages. Indeed, several human and animal investigations have reported a beneficial effect of exercise during brain development. In humans, a favorable effect of physical exercise has been demonstrated during pre- and postnatal neurodevelopment (Wolfe, Brenner, & Mottola, 1994). Clapp (1996) showed that children from exercised mothers 5 years after delivery presented better results in tests of oral language skills and in intelligence scores. Another investigation by the same group showed that 5 days after birth, the infants of the physically active mothers were able to orient the stimuli from the environment more efficiently and to self-regulate after the presentation of sound and luminous stimuli when compared with the infants from the group of inactive mothers (Clapp, Simonian, Lopez, Appleby-Wineberg, & Harcar-Sevcik, 1998). Better scores in academic performance in young people (6–18 years) whose mothers practiced physical exercise in pregnancy were also observed (Esteban-Cornejo, Martinez-Gomez, Tejero-González, Izquierdo-Gomez, & Carbonell-Baeza, 2016). In rats, physical exercise during the gestational period contributes to better brain function in offspring (Parnpiansil, Jutapakdeegul, Chentanez, & Kotchabhakdi, 2003). For example, pups from mothers exercised during gestation, and analyzed at P28, presented a significant increase in the expression of BDNF mRNA, enhanced hippocampal cell survival, and improved short-term memory when compared with those measured in the control group (Kim, Lee, Kim, Yoo, & Kim, 2007). Similarly, in a more recent work, Gomes da Silva et al. (2016) reported an increase in hippocampal BDNF levels, better performance in cognitive tests, and an increase in the number of hippocampal cells in adult offspring (P60) of mothers exercised throughout the gestational period.

It is important to mention that beneficial effects of exercise in reducing the deleterious effect of a brain insult during neurodevelopment have also been reported in adult rats. For instance, an aerobic exercise program reduced the frequency of seizures induced by pilocarpine (Arida, Scorza, Peres, & Cavalheiro, 1999); retarded the development of amygdala kindling (Arida, Jesus Vieira, & Cavalheiro, 1998); decreased CA1 hyper-responsiveness (Arida et al., 2004), and increased the staining of parvalbumin—a calcium protein binder present in GABAergic interneurons in rats with epilepsy (Arida et al., 2007). During the adolescent period, our group research showed that, when compared with rats that remained sedentary during adolescence, rats trained from P21 to P60, then subsequently left untrained for 90 days, presented an increase in the threshold for motor manifestation and reduction in the intensity of the motor symptoms when submitted to pilocarpine injection at P150. This suggests that early exercise interferes positively in the later ictogenesis process (Gomes da Silva et al., 2011). The above findings support the notion that physical exercise acts positively in protecting the brain against insults at different stages of life.

To our knowledge, this is the first study to evaluate the impact of physical exercise on the offspring of stressed mothers during pregnancy. In conclusion, our results reveal that PS creates deleterious effects for pups' brain development, and both forced and voluntary exercise during gestation attenuated these negative effects. Further investigations are needed to elucidate possible positive morphological, cellular and molecular alterations induced by physical exercise after PS.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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