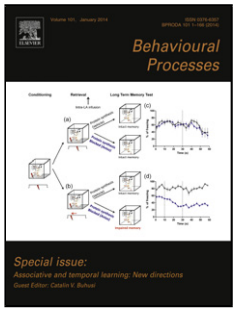

Title: EFFECTS OF PHYSICAL EXERCISE AND SOCIAL ISOLATION ON ANXIETY-RELATED BEHAVIORS IN TWO INBRED RAT STRAINS

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<AT>EFFECTS OF PHYSICAL EXERCISE AND SOCIAL ISOLATION ON ANXIETY-RELATED BEHAVIORS IN TWO INBRED RAT STRAINS

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<ABS-HEAD>Highlights► Physical exercise on a treadmill reduced anxiety-like behavior ► Spontaneous activity levels are genotype-dependent ► Benefits of physical exercise depend on the type of exercise performed

<ABS-HEAD>Abstract

<ABS-P>We investigated the effects of physical exercise (PE) on locomotor activity and anxiety-like behavior in Lewis (LEW) and Spontaneously Hypertensive Rats (SHR) male rats. Rats received either four weeks of forced training, 5 days/week, on a treadmill (experiment 1) or were given 21 days of free access to running wheels (experiment 2). We also tested the effects of social isolation (SI) (seven days of isolation - experiment 3) on behavior. In experiment 1, 20% of LEW rats and 63% of SHR rats completed the training protocol. PE significantly increased central and peripheral locomotion in the open field (OF) and entries into the open arms in the elevated plus-maze (EPM) in both strains. In experiment 2, the distance traveled by SHR rats on running wheels was significantly higher compared with LEW rats. PE on running wheels also increased the time spent in the center of the OF in SHR rats only. In experiment 3, SI decreased central and peripheral locomotion in the OF in both strains. In summary, forced PE on a treadmill reduced anxiety-like behavior and increased locomotion in male rats of both strains, whereas voluntary PE on running wheels decreased anxiety-like behavior in SHR rats only. SI decreased locomotion in both strains in the OF. This study suggests that spontaneous activity levels are genotype-dependent and the effects of PE depend on the type of exercise performed.
<KWD>Keywords: treadmill; running wheels; elevated plus-maze; open field.

<H1>1. Introduction

A growing body of evidence indicates the positive influence that lifestyle factors, including physical exercise (PE), social interaction and nutrition, can have on emotionality in humans (Kramer et al., 1985; Fox, 1999; Ratey & Loehr, 2011; Hötting & Röder, 2013). For example, periodic PE decreases anxiety, improves mood, physical well-being and mental disposition (McKercher et al., 2009; Samulski et al., 2009; UNESCO, 2013). It also may have a positive influence on the modulation of brain neurotransmitter systems (Hill et al., 2010), related to

improvements in cardiovascular function (Murlasits, 2015; Dunn et al., 2005), and increases brain plasticity and resilience to stress, which could prevent the development of mental disorders (Cotman & Berchtold, 2002). Although the biological mechanisms responsible for these improvements are not yet fully understood, PE is recommended as a complementary treatment for mental disorders, such as anxiety, depression and attention deficit hyperactivity disorder (ADHD) (Berwid & Halperin, 2012; Lees & Hopkins, 2013; Ströhle, 2009).
Studies using genetic models can be useful to investigate the benefits of PE in humans (White, 2016). However, most studies use socially isolated animals, at least in some periods of time (e.g., during training). This procedure allows one to monitor PE levels for each individual; however, social isolation (SI) per se may be associated with anxiety-like behaviors (Butler et al., 2014; Skelly et al., 2015), cognitive dysfunction (Li et al., 2016) and depressive behaviors (Zanier-Gomes et al., 2015) in rodents, which could confound data interpretation.
The Lewis (LEW) and Spontaneously Hypertensive Rats (SHR) inbred rat strains show contrasting levels of anxiety-related behavior, with the former exhibiting increased levels of anxiety-like behavior (Chiavegatto et al., 2009). These strain differences were reliably found in different substrains, testing conditions, laboratories and countries (Ramos et al., 2002; Izídio et al., 2005a;b; Vendruscolo et al., 2006). The effects of PE on behavior and neurochemical measures have been tested independently in these two rat strains. It is interesting to note that PE decreased hyperactivity and aggressive behavior, while it improved attention and spatial learning in SHR rats (Kim et al., 2011; Baek et al., 2014; Jeong et al., 2014; Hoffmann et al., 1990; Robinson et al., 2015). In LEW rats, PE has been shown to reduce plasma corticosterone levels and cocaine-induced conditioned place preference (Thanos et al., 2010; Calik et al., 2015). It has been suggested that SI can affect brain catecholamine levels and heart rate, depending on strain and experimental design (Varty & Geyer, 1998; Gavrilovic et al., 2005; Azar et al., 2011; Meyer & Bardo, 2015). However, to our knowledge, anxiety-related and locomotor behavior in LEW and SHR rats have not been directly compared following PE on treadmills or running wheels, neither following SI.
The aim of the present study was to investigate the impact of PE and SI on the behavior of LEW and SHR rats. To this end, LEW and SHR rats were tested in two behavioral models of anxiety, namely the open field (OF) and the elevated plus-maze (EPM), after forced (treadmill; experiment 1) or voluntary (running wheels; experiment 2) PE. These experiments were conducted in animals that were socially isolated, at least during the period of PE. Therefore, we performed a third experiment to evaluate the potential effects of SI on behavior of LEW and SHR rats (experiment 3). We hypothesized that PE would decrease anxiety levels at least in LEW rats (``high anxious rats''), whereas increases in locomotor activity would be observed for both LEW and SHR rats. Additionally, we expected SI to increase anxiety-like behavior at least in SHR rats (``low anxious rats'').
<H1>2. Animals

The inbred LEW (LEW/HsdUnibAnra) rats came originally from Harlan Sprague Dawley, IN, and were then bred at UNICAMP (Campinas, SP, Brazil). The inbred SHR (SHR/NCrlAnra) rats were originally from Harvard University, Boston MA, and were then bred at UNESP, Botucatu, SP, Brazil. Both strains were obtained from UNICAMP or UNESP and have been maintained at the Behavior Genetics Laboratory (Federal University of Santa Catarina, Florianópolis, Brazil) for more than 40 generations, under a brother-sister mating system (described at the Rat Genome Database).
Animals were kept in collective plastic cages (4-5 animals/cage), except during the isolation period, where they were kept in individual cages. They had food and water available *ad libitum* and were kept under a 12 h light/dark schedule (lights on at 7:00 a.m.) at 22±2 °C. All

procedures were performed according to the guidelines of the local committee for Animal Care in Research (CEUA/UFSC) and had the valid permissions PP00046 and PP00903.

<H1>3. Experiment 1

<H2>3.1 Material & Methods - Forced physical exercise on a treadmill

One hundred and four male rats (22-30 rats/strain/training), 56-day old, were used. LEW and SHR rats were randomly divided into sedentary (SED) or PE groups. SED rats were kept in their home-cage near the treadmill during the same period that the PE group was training. Training consisted of forced running sessions on a treadmill adapted to rodents, with six individual compartments (Insight, Brazil). The daily running sessions were performed during four weeks (5 days/week). The protocol was adapted from Real et al. (2010) and is presented in Table 1.

During each daily session, rats that remained for more than 1 minute without running were eliminated from the experiment. Only animals that successfully completed the four weeks of forced training were used in the behavioral experiments. One day after the end of the PE protocol, animals were submitted to the behavioral experiments starting at 2:00 p.m.

<H2>3.2 Behavioral experiments

<H3>3.2.1 Open field (OF)

The apparatus was made of white Formica surrounded by white walls (40 cm high). The floor of 100 cm × 100 cm was divided by black lines into 25 squares of 20 cm × 20 cm and was under 7 lx illumination. Each rat was placed in the center of the OF and the following behaviors were registered for 5 min: peripheral locomotion (adjacent to the walls), central locomotion (apart from the walls) and time spent in the center of the apparatus. The apparatus was cleaned with a 5% ethanol solution after each test. A camera positioned above the apparatus recorded the tests, and behaviors were quantified by a trained observer. Methods for cleaning and recording were identical for all tests.

<H3>3.2.2 Elevated-plus maze (EPM)

The apparatus was made of black Formica and had four elevated arms (52 cm from the floor) 50 cm long and 10 cm wide. The arms were arranged in a cross, with two opposite arms being enclosed (by 40 cm high walls) and two being open, having at their intersection a central platform (10 cm × 13.5 cm) that gives access to any of the four arms. A raised ledge (1 mm thick and 5 mm high) surrounded the open arms. The central platform was under 70 lx. Rats were placed in the central platform facing the open arm and the following behaviors were registered for 5 min: number of entries and time spent (with all four paws) inside each arm.

<H2>3.3 Statistical analysis

Data was expressed as mean + standard error of the mean (SEM) and analyzed using two-way analysis of variance (ANOVA). Results with $p \leq 0.05$ were considered significant. All analyses were performed using the Statistica 7.0 software package.

<H2>3.4 Results - Experiment 1

Figure 1 shows the percentage of rats from both strains that completed the forced PE training on the treadmill. Only 20% of LEW (6 from 30 rats) and 63% of SHR (14 from 22 rats) rats satisfactorily completed the training and, therefore, were used in the behavioral tests.

One SHR rat was excluded after training, and thus experimental groups were composed of: 13 SHR PE, 13 SHR SED, 6 LEW PE and 6 LEW SED.

Figure 2 shows (a) central and (b) peripheral squares crossed by LEW and SHR male rats in the OF. The two-way ANOVA showed significant effects of strain (SHR > LEW; $F(1,34) = 29.48$; $p \leq 0.0001$; partial $\eta 2 = 0.46$) and training (PE > SED; $F(1,34) = 11.04$; $p = 0.0022$; partial $\eta 2 = 0.25$) on central locomotion and a significant effect of training (PE > SED; $F(1,34) = 15.19$; $p = 0.0004$; partial $\eta 2 = 0.31$), but not strain ($F(1,34) = 3.46$; $p = 0.0712$; partial $\eta 2 = 0.09$), on peripheral locomotion. The two-way ANOVA also showed a significant effect of strain (SHR > LEW; $F(1,33) = 8.64$; $p = 0.0060$; partial $\eta 2 = 0.21$), but not training ($F(1,33) = 2.51$; $p = 0.1223$; partial $\eta 2 = 0.07$), on time spent in the center of the OF (data not shown).

Figure 3 shows (a) open and (b) closed arm entries and time in the (c) open and (d) closed arms by LEW and SHR male rats in the EPM. The two-way ANOVA revealed significant effects of strain (SHR > LEW; $F(1,34) = 4.31$; $p = 0.0453$; partial $\eta 2 = 0.11$) and training (PE > SED; $F(1,34) = 5.41$; $p = 0.0262$; partial $\eta 2 = 0.14$) for open arm entries (Figure 3a). No significant effects of strain ($F(1,34) < 1$; $p = 0.5880$; partial $\eta 2 = 0.01$) or training ($F(1,34) < 1$; $p = 0.5134$; partial $\eta 2 = 0.01$) were found for closed arm entries (Figure 3b). There was a significant effect of strain for time spent in the open (SHR > LEW; $F(1,34) = 23.28$; $p \leq 0.0001$; partial $\eta 2 = 0.41$) and closed (LEW > SHR; $F(1,34) = 53.75$; $p \leq 0.0001$; partial $\eta 2 = 0.61$) arms (Figure 3c-d). No significant effects of training were found for time spent in the open ($F(1,34) = 1.15$; $p = 0.2914$; partial $\eta 2 = 0.03$) or closed arms ($F(1,34) < 1$; $p = 0.8600$; partial $\eta 2 < 0.01$) (Figure 3c-d).

<H2>3.5. Discussion - Experiment 1

The results showed that four weeks of forced PE on a treadmill significantly increased central locomotion in the OF and open arm entries in the EPM for both strains compared with their respective SED controls, indicating an anxiolytic-like effect of forced PE. In humans, PE has been shown to decrease anxiety and depression levels and improve physical well-being

(McKercher et al., 2009; Samulski et al., 2009; Unesco, 2013). Some studies also suggest that PE could be used as a treatment for anxiety and depression (Ströhle, 2009). In rodents, PE was shown to attenuate the negative behavioral changes associated with exposure to predator scent (Hoffman et al., 2015), reduce responsiveness to stress (Lalanza et al., 2012) and decrease anxiety-related behaviors in the OF and EPM (Pietrelli et al., 2011; 2012). However, the mechanisms responsible for these improvements are not yet fully understood (Cotman & Berchtold, 2002; Hill et al., 2010).

Forced PE also caused an increase in peripheral locomotion in the OF in both strains. Although the meaning of this behavior is complex, peripheral locomotion is often interpreted as locomotor activity (Ramos et al., 2003; Izídio et al., 2011). Thus, it can be suggested that the PE-induced increase in cardiovascular or muscular capacity of these animals (Moraska et al., 2000) facilitated locomotion in the OF.

Alternatively, the increase in general locomotion may be a result of the selection of animals that completed the treadmill training, i.e., rats with baseline increased locomotion. Because the behavioral outcomes in the OF and EPM tests change with repetition, we did not perform these tests before the period of PE to properly address this question. The possibility that baseline locomotion affects the percentage of rats that conclude the treadmill training could be investigated in the future. Another possible explanation was suggested by Malisch et al. (2016) that showed that acute stress can increase the patterns of locomotor activity of mice. However, studies using Sprague Dawley or Long-Evans rats submitted to the treadmill or the running wheel, reported that PE has either no effects (Fulk et al., 2004; Hopkins et al., 2011; Patki et al., 2014; Sciolino et al., 2015) or reduces locomotor activity in rats (Grace et al., 2009). Therefore, some caution is needed to suggest that PE caused stimulant motor effects in LEW and SHR rats and this point deserves a further evaluation in future experiments.

<H1>4. Experiment 2

<H2>4.1 Material & Methods - Voluntary physical exercise on running wheels

Thirty-six male (9 rats/strain/training), 56-day old, rats were used. LEW and SHR rats were randomly divided into SED or PE groups. Rats were individually placed in a cage with free access (PE group) to a running wheel (31 cm in diameter) for 21 days (24 h/day). The SED group had no access to the running wheel. One day after the final exercise day, animals were submitted to the behavioral experiments starting at 2:00 p.m.

<H2>4.2 Behavioral experiments

The animals were submitted to the OF and EPM, as described in 3.2.1 and 3.2.2.

<H2>4.3 Statistical analysis

Data was expressed as mean + SEM and analyzed using two-way ANOVA or two-way ANOVA for repeated measures. When a significant interaction between factors was detected, we used the *post hoc* Tukey HSD. Results with $p \leq 0.05$ were considered significant. All analyses were performed using the Statistica 7.0 software package.

<H2>4.4 Results - Experiment 2

Figure 4 shows the average distance traveled during the voluntary PE on running wheels by rats from both strains. LEW animals travelled 0.05 km/day, whereas SHR travelled 3.63 km/day. The two-way ANOVA for repeated measures revealed a significant strain x day

interaction ($F(20,240) = 2.65$; $p = 0.0003$; partial $\eta 2 < 0.18$). The *post hoc* tests indicate that SHR rats showed higher traveled distance compared with LEW rats from the third day on.

Figure 5 shows (a) central and (b) peripheral squares crossed and (c) time spent in the center of the OF by LEW and SHR rats. The two-way ANOVA revealed a significant effect of strain (SHR > LEW; $F(1,28) = 9.37$; $p = 0.0048$; partial $\eta 2 = 0.25$), but not training ($F(1,28) < 1$; $p = 0.9124$; partial $\eta 2 < 0.01$), for central locomotion (Figure 5a). There was no significant effect of strain ($F(1,28) < 1$; $p = 0.8836$; partial $\eta 2 < 0.01$) or training ($F(1,28) < 1$; $p = 0.5517$; partial $\eta 2 = 0.01$), for peripheral locomotion (Figure 5b). There was a significant strain x training interaction ($F(1,28) = 4.73$; $p = 0.0383$; partial $\eta 2 = 0.14$) for the time spent in the center. The *post hoc* test showed that SHR spent more time in the center of the OF than LEW rats ($p = 0.0319$), but only after PE training (Figure 5c).

Figure 6 shows (a) open and (b) closed arm entries and time in the (c) open and (d) closed arms by LEW and SHR rats in the EPM. The two-way ANOVA showed a significant effect of strain (SHR > LEW; $F(1,26) = 7.34$; $p = 0.0118$; partial $\eta 2 = 0.22$), but not training ($F(1,26) < 1$; $p = 0.6055$; partial $\eta 2 = 0.01$), for open arms entries (Figure 6a). There was no significant effect of strain ($F(1,26) < 1$; $p = 0.5126$; partial $\eta 2 = 0.02$) or training ($F(1,26) = 1.62$; $p = 0.2144$; partial $\eta 2 = 0.06$) for closed arm entries (Figure 6b). There were significant strain effects for time spent in the open (SHR > LEW; $F(1,26) = 19.54$; $p = 0.0002$; partial $\eta 2 = 0.43$) and closed (LEW > SHR; $F(1,26) = 25.32$; $p \leq 0.0001$; partial $\eta 2 = 0.49$) arms (Figure 6c-d). No significant effect of training was found for the time spent in the open ($F(1,26) = 3.65$; $p = 0.0670$; partial $\eta 2 = 0.12$) or closed arms ($F(1,26) < 1$; $p = 0.7855$; partial $\eta 2 < 0.01$) (Figure 6c-d).

<H2>4.5. Discussion - Experiment 2

In the running wheel, 21 days of voluntary PE increased the time spent in center of the OF only in SHR rats (Figure 5c). The same trend was observed for central locomotion in SHR rats (Figure 5a). These results suggest an anxiolytic-like effect of voluntary PE in this strain, given that the central area of the OF is considered aversive to rats (Prut & Belzung, 2003).

Clark et al. (1995) showed that mice of 12 different strains that exercised in running wheels display increased neurogenesis in the hippocampus, a brain area involved in cognition and emotionality (Bannerman et al., 2004; Juruena et al., 2004; Kalisch et al., 2006). Moreover, 14 to 21 days of PE in running wheels have been shown to improve attention of male and female SHR rats (Robinson et al., 2011) and have been suggested to be effective in decreasing anxiety symptoms (Haydari et al., 2014). However, caution is needed because increased activity or locomotion could also be considered as a stress response to a new environment (Thorsell et al., 2006; Ago et al., 2007; Malisch et al., 2016). Increased locomotion may also lead to freezing behavior, increasing the time spent in the center in the OF (where the animal is place at the beginning of the test), thereby masking anxiolytic effects. Moreover, time spent in the center of the OF could be interpreted as an inappropriate behavioral response caused by previous stress (Raineki et al., 2016).

The anxiolytic-like effect of voluntary PE was only evident in SHR rats. This result can be explained by the fact that SHR rats ran voluntarily about 3.6 km/day, whereas LEW ran only 0.05 km/day. Surprisingly, LEW rats stopped running after 17 days, whereas SHR rats reached a total of 4.7 km on 21th day. Interestingly, studies have been demonstrating great variability in the distance traveled by LEW rats. For example, Makatsori et al. (2003) showed that individual running distance traveled by LEW rats varied considerably, ranging from 4 to 6 km, whereas Roebuck et al. (1990), similarly to the present results, reported that LEW rats ran voluntarily less than 1 km/day. The discrepancies in the distance traveled by LEW rats may be due to differences in experimental protocols, including type and size of the running wheel, sub-strain, acclimatization to the apparatus and other conditions in the maintenance of rats. Some studies show that intense PE can cause muscle pain and hyperalgesia (Borghi et al., 2014). Considering that LEW rats exhibit high secretion of cytokines (Elenkov et al., 2008), these animals could present higher levels of inflammation and oxidative stress in the muscle, thus impairing the maintenance of voluntary PE for long periods in this strain. Consistent with our results, SHR rats have been shown to run an average distance of 3-10 km/day in running wheels (Hoffmann et al., 1987; 1990; Jonsdottir et al., 1996; Jonsdottir & Hoffmann, 2000). These results suggest that genetic background largely influences the individual's willingness/capacity to engage in regular physical activity.

Some studies suggest that voluntary PE on running wheels is rewarding and sometimes addictive-like (Belke & Wagner, 2005; Rhodes et al., 2003; 2005). Several studies investigating the effects of PE have focused on the hippocampus. For example, voluntary wheel running has been shown to enhance learning and hippocampal neurogenesis in rodents (van Praag et al., 2005; Merkley et al., 2014). However, other brain regions must be also considered. For example, mice that were continuously selected for voluntary running exhibited, after 29 generations, differentiation of brain regions associated with motivation, such as lateral hypothalamus, sensory cortex, nucleus *accumbens* and putamen. In addition, altered dopaminergic and glutamatergic functions were also reported in rodent runners, which may contribute to behavioral outcomes observed after PE (Rhodes & Garland, 2003; Staples et al., 2015).

Using a technique for QTL (Quantitative Traits Loci) identification, Kelly et al. (1996) mapped regions on chromosomes 1, 2, 7, 11 and 14 of the mouse that influence PE in running wheels. Although very interesting, this study just associated PE in running wheels to genomic regions without finding the genes responsible for this behavior, which would be an important advance in understanding the genetic basis of voluntary PE. The results of the present study suggest that LEW and SHR rats constitute a good rat model for the search of the genetic bases underlying voluntary PE because of their contrasting voluntary running patterns. As already reported for anxiety-like behaviors (Ramos et al., 1997; Izídio et al., 2005a;b; Vendruscolo et

al., 2006; Chiavegatto et al., 2009), this genetic model can also be an important tool for the identification of genes influencing voluntary PE.

<H1>5. Experiment 3

<H2>5.1 Material & Methods - Social isolation

Thirty-two male rats (8 rats/strain/training), 56-day old, were used. LEW and SHR rats were randomly divided into SI (socially isolated) or GR (grouped, four animals per cage) group. After seven days of social isolation, the rats were submitted to the behavioral experiments starting at 2:00 p.m.

<H2>5.2 Behavioral experiments

The animals were submitted to the OF and EPM, as described in 3.2.1 and 3.2.2.

<H2>5.3 Statistical analysis

Data was expressed as mean + SEM and analyzed using multivariate analysis of variance (MANOVA) followed by *post hoc* Tukey HSD. Results with $p \leq 0.05$ were considered significant. All analyses were performed using the Statistica 7.0 software package.

<H2>5.4 Experiment 3

Table 2 shows the behaviors exhibited by rats of both strains in the OF and EPM while single (a week of social isolation) or group-housed. The MANOVA revealed a significant effect of strain (Wilks value = 0.486; $F(3, 26) = 9.17$; $p = 0.0002$) and social condition (Wilks value = 0.718; $F(3, 26) = 3.40$; $p = 0.0325$) in the OF. The *post hoc* tests showed that SHR rats presented higher central locomotion ($p = 0.0002$) and spent more time in the center ($p = 0.0012$) of the OF than LEW rats (Table 2). Moreover, SI animals presented lower central ($p = 0.0270$) and peripheral ($p = 0.0100$) locomotion than GR animals (Table 2).

The MANOVA also revealed a significant effect of strain (Wilks value = 0.388; $F(4, 25) = 9.85$; $p = 0.0001$), but not social condition (Wilks value = 0.908; $F(4, 25) = 0.63$; $p = 0.6436$) in the EPM. The *post hoc* tests showed that SHR presented fewer entries ($p = 0.0341$) and spent less time ($p = 0.0002$) in the closed arms of the EPM than LEW rats (Table 2). The MANOVA did not reveal significant interactions for the OF (Wilks value = 0.903; $F(3, 26) = 0.93$; $p = 0.4402$) or the EPM (Wilks value = 0.952; $F(4, 25) = 0.32$; $p = 0.8633$) (Table 2).

<H2>5.5. Discussion - Experiment 3

It is important to highlight that experiments 1 and 2 were performed with animals that were socially isolated, at least during the period of PE. Therefore, we designed this third experiment to evaluate the potential effects of SI on behavior of LEW and SHR rats. The results showed that seven days of SI caused a decrease in central and peripheral locomotion in the OF. These results suggest a hypolocomotor effect of SI in rats of both strains. No significant effects were observed in the EPM (Table 2).

Rats usually live in groups and have high contact with other rats to establish their social organization (Varlinskaya & Spear, 2008). The lack of physical contact generates a series of behavioral and physiological reactions that affect emotional reactivity of adult rats (Weiss et al., 2004). Results in the literature vary greatly, both in rats or mice, with SI increasing anxiety levels in the OF (Hall et al., 2000) and in the EPM (Maisonnette et al., 1993; Weiss et al., 2004) or showing anxiolytic-like effects in the EPM (Voikar et al., 2005; Thorsell et al., 2006). The LEW and SHR inbred rat strains have already been tested in OF and EPM showing contrasting anxiety-like behaviors, with the former presenting higher levels of

anxiety/emotionality than the latter (Chiavegatto et al., 2009). Such differences are robust and have been confirmed using different substrains, at different laboratories and countries. Then, we initially hypothesized that SI would increase anxiety-related behaviors, at least in the low anxious-like SHR rats. However, the effects of SI were found not to depend on strain and were more pronounced on locomotion (central and peripheral were decreased) than in anxiety-behaviors.

SI may increase locomotor activity in a new environment (Hall et al., 1998; Thorsell et al., 2006; Ago et al., 2007), trigger aggressive behaviors (Wongwitdecha et al., 1996), induce deficits in prepulse inhibition (Weiss et al., 1999), and cause changes in endocrine parameters (Pohorecky et al., 2008). SI is also capable of inducing morphological changes in length and dendritic density of neurons localized in the prefrontal cortex and *nucleus accumbens*, which are associated with increased locomotor activity in a novel and stressful environment (Alquicer et al., 2004; 2008). However, in the present study, SI rats exhibited hypo instead of hyperlocomotion in the OF, and SI did not cause changes in anxiety levels, as measured in the EPM. Thus, it is possible to suggest that, in our study, the SI period was not long enough (only seven days) to cause the expected effects (e.g., hyperlocomotion and anxiogenic-like effects). Still, this could be a specific effect of SI in the LEW and SHR strains or, alternatively, our results could indicate that the OF is more sensitive to the effects of SI than the EPM.

<H1>6. General discussion

We have investigated the effects of PE and SI on anxiety-like behavior using a genetic model that has been well validated for the study of anxiety. Our results confirm the behavioral profiles of LEW and SHR rats reported in previously published articles. SHR rats approach more and spent more time exploring potentially dangerous situations, such as the center of the OF and the open arms of the EPM, than LEW rats (Ramos et al., 1997; Izídio et al., 2005a;b; Vendruscolo et al., 2006; Chiavegatto et al., 2009).

We show that forced PE on a treadmill decreases anxiety-like behavior and increases locomotion in both LEW and SHR rats. Voluntary PE on the running wheel, on the other hand, decreases anxiety-like behavior in SHR rats only. Finally, SI decreased locomotion in rats from both strains.

Our study also suggests that the effects of PE depend on the type of exercise performed. PE in the treadmill has an inherent stress component, because the rats are forced to run. On the other hand, voluntary PE in the running wheel is a rewarding and voluntary physical activity. Some studies propose that forced and voluntary PE may produce different brain adaptations. For example, Ke et al. (2011) showed that voluntary exercise is more effective than forced exercise in upregulating hippocampal BDNF levels. Moreover, Yuede et al. (2009) suggested that voluntary exercise may be superior to forced exercise in reducing memory impairment in a mouse model of Alzheimer's disease. Furthermore, Arida et al. (2004) proposed that PE leads to changes in the hippocampal formation of rats, which were more evident following voluntary activity. However, this matter is not completely unraveled in the literature. Some researchers have found beneficial effects of the forced when compared to spontaneous PE. For example, Cheong et al. (2013) showed greater improvement in cognitive function in rats submitted to forced exercise than in the spontaneous exercise group.

<H1>7. Conclusions

In summary, forced PE on a treadmill decreased anxiety/emotionality in the EPM and OF, and increased locomotion in male rats of both strains. In contrast, PE on running wheels only decreased anxiety/emotionality in SHR rats tested in the OF. SI caused hypolocomotor effects in both strains in the OF. Finally, SHR displayed higher levels of spontaneous exercising in

the running wheel than LEW rats. This study suggests that PE affect spontaneous activity levels in a genotype-dependent manner and the effects of PE also depend on the type of exercise performed.

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Captions to illustrations

[Figure 1]. Percentage of Lewis (LEW) and SHR male rats that completed the training by week.

[Figure 2]. Means + standard error of the mean of (a) central and (b) peripheral squares crossed from Lewis (LEW) and SHR male rats in the open field. Two-way ANOVA was performed. **p<0.01 represents overall strain effects and ##p<0.01; #p<0.05 represents overall physical exercise effects.

[Figure 3]. Means + standard error of the mean of (a) open and (b) closed arm entries and time in the (c) open and (d) closed from Lewis (LEW) and SHR male rats in the elevated plus-maze. Two-way ANOVA was performed. **p<0.01 represents overall strain effects and #p<0.05 represents overall physical exercise effects.

[Figure 4]. Means + standard error of the mean of traveled distance from Lewis (LEW) and SHR male rats in the running wheels. Two-way ANOVA for repeated measures and the *post hoc* Tukey HSD were performed. **p<0.01; *p<0.05 represent differences between strains.

[Figure 5]. Means + standard error of the mean of (a) central and (b) peripheral squares crossed and (c) time spent in the center from Lewis (LEW) and SHR male rats in the open field. Two-way ANOVA and the *post hoc* Tukey HSD were performed. *p<0.05 represents overall strain effects and &p<0.05 represents differences between strains only in physical exercise group.

[Figure 6]. Means + standard error of the mean of (a) open and (b) closed arm entries and time in the (c) open and (d) closed from Lewis (LEW) and SHR male rats in the elevated plus-maze. Two-way ANOVA was performed. **p<0.01 represents overall strain effects.

<Table>Table 1- Protocol of four weeks of forced training with LEW and SHR male rats. The daily running sessions were conducted for four weeks (5 days/week).

Weeks	**Days**	**Velocity**	**Total time in running**
First	1th	8 m/min	15 min
	2th	8 m/min (first 10 min) and 10 m/min (last 5 min)	15 min
	3th	10 m/min (first 10 min) and 12 m/min (last 5 min)	15 min
	4th	10 m/min (first 10 min) and 12 m/min (last 15 min)	25 min
	5th	10 m/min (first 5 min), 12 m/min (for 20 min) and 15	30 min

		m/min (last 5 min)	
Second	Five days	15 m/min	40 min
Third	Five days	15 m/min	40 min
Fourth	Five days	15 m/min	40 min
Fifth	Five days	15 m/min	40 min

<Table>Table 2- Means ± SEM of behaviors from Lewis (LEW) and SHR male rats tested in the open field and elevated plus-maze. MANOVA followed by *post hoc* Tukey HSD were performed.

	LEW		**SHR**	
	GR	**SI**	**GR**	**SI**
Central squares crossed	6.4 ± 1.45 ** &	3.9 ± 1.17 **	17.1 ± 2.35 &	10.6 ± 2.42
Peripheral squares crossed	101.1 ± 2.64 &	81.0 ± 8.23	88.8 ± 2.88 &	78.3 ± 6.27
Time in the center (s)	<H2>8.4 ± 2.12 **	12.0 ± 5.17 **	<H2>2.5 ± 0.91	21.9 ± 4.06
Open arms entries	29.1 ± 4.74	<H2>1.3 ± 0.62	<H2>1.1 ± 0.61	<H2>0.9 ± 0.30
Closed arms entries	<H2>8.5 ± 1.22 *	7.8 ± 0.56 *	5.9 ± 0.91	16.0 ± 5.61
Time in the open arms (s)	6.4 ± 0.75	10.5 ± 4.54	7.3 ± 3.62	175.6 ± 5.12 **
Time in the closed arms (s)	12.1 ± 6.30	139.6 ± 9.27	169.9 ± 11.86 **	128.8 ± 7.37

<PA>GR = grouped and SI = social isolated animals. * or ** ($p<0.05$; $p<0.01$; respectively) strain (LEW vs. SHR); & or && ($p<0.05$; $p<0.01$; respectively) social condition (GR vs. SI) Tukey HSD effects.
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