

Exercise and Pharmacological Treatment: Influence on locomotor activity in SHR rats

NÁDIA E. SANTOS¹| CAMILA A. S. COELHO¹| MILCA A. MORAIS¹| BEATRIZ S. FRANCO¹| ANDREA M. ESTEVES¹

Faculdade de Ciências Aplicadas da Universidade Estadual de Campinas. Rua Pedro Zaccaria, 1300, Limeira – SP, Brazil, 13484-350.

Correspondence to: Andrea M. Esteves, Faculdade de Ciências Aplicadas da Universidade Estadual de Campinas. Rua Pedro Zaccaria, 1300, Limeira – SP, Brazil, 13484-350.

email: andrea.esteves@fca.unicamp.br

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HIGHLIGHTS

- Treatment with PPX reduced locomotor activity on specific days.
- Exercise has been shown to cause changes in locomotor activity, similarly to PPX.
- Treatment with PPX for 48 days did not engender the augmentation effect in SHR rats.

ABBREVIATIONS

OF	open field
log	transformation log
PE	physical exercise
PLM	Periodic Leg Movement
PPX	pramipexole
PPX1	PPX (0.1 mg/kg) at 75 days of age
PPX2	PPX (0.05 mg/kg) at 91 days of age
PPX3	PPX (0.05 mg/kg) at 125 days of age
RLS	Restless legs syndrome
SHR	spontaneously hypertensive rats

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BACKGROUND: Restless legs syndrome (RLS) is characterized by abnormal sensations in the lower limbs and negatively impacts on sleep. Dopaminergic agonists (pramipexole, PPX) may be used in the treatment. However, chronic use of these drugs may cause worsening of symptoms (augmentation). Physical exercise (PE) can also be effective in reducing RLS symptoms and improving sleep patterns.

AIM: The main purpose of this study was to evaluate the effect of PPX and acute PE on locomotor activity (RLS symptoms) of spontaneously hypertensive rats at different ages.

METHOD: Animals were distributed into 4 groups: Saline, PPX1 (0.1 mg/kg) started at 75 days of age, PPX2 (0.05 mg/kg) started at 91 days of age, and PPX3 (0.05 mg/kg) started at 125 days of age. The animals received a daily injection of PPX or saline (07:00 h) for 48 days. The PE (treadmill) consisted of a single session (30 min) at 20–22 m/min, performed on day 49. The evaluation of locomotor activity (open field) was performed at baseline and weekly, after administration of the drug.

RESULTS: Both PPX and PE caused changes in locomotor activity (↓ Total Ambulation, ↓ Rearing and ↑ Freezing), indicating reduction in RLS symptoms on specific days.

CONCLUSION: The groups receiving PPX did not present augmentation.

KEYWORDS: aerobic exercise | augmentation | Pramipexole | Restless Legs Syndrome

INTRODUCTION

Restless legs syndrome (RLS), classified as a sleep-related movement disorder, is a sensorimotor disorder, characterized by discomfort (lower limbs), with nocturnal worsening and relief with moving, generating a negative impact on sleep architecture and quality of life.¹ Its prevalence, as well as the severity of symptoms may increase with age², suggesting that the neurodegenerative process may play an important role in this syndrome. The life style of older adults as well as the senile changes, including cardiovascular and metabolism alterations, are also related to RLS.³ Moreover, patients with RLS may present Periodic Leg Movement (PLM), which is characterized by the rhythmic extension of the lower limbs, followed by ankle dorsiflexion, causing knee flexion and generalized motor activation in the lower limbs during sleep. PLM differs from RLS as it occurs involuntarily while the patient is sleeping.¹

Treatment for RLS may be pharmacological, including dopaminergic agents, such

as pramipexole (PPX)⁴, or non-pharmacological, such as physical exercise (PE)⁵. Moreover, scientific evidence demonstrates that the use of PPX may reduce locomotor activity in animal models, which is related to the reduction in RLS symptoms.^{6,7} A study performed with C57BL / 6 mice with iron deprivation plus bilateral 6-hydroxydopamine lesions in the A11 nuclei (animal model of RLS) showed that acute and chronic treatment with PPX attenuated locomotor activity.⁷ Oliveira de Almeida et al.⁶ also observed that animals (Wistar) that received PPX presented a significant reduction in locomotor activity.

Although pharmacological treatment is effective in reducing symptoms, it may cause some adverse effects, such as augmentation, characterized by the anticipation of symptoms relative to the typical time of onset; or even, worsening of symptoms. Augmentation may occur when treatment is performed in the long-term, or with an increased dose.⁴ Augmentation has been reported in 60–85% of patients on levodopa treatment for 6 months or more⁸ and in 30% of patients taking a dopamine agonist, such as PPX, for at least 3 years.⁹ Studies suggest that this continues to occur for up to 9 years after treatment onset.¹⁰ On the other hand, in a more recent study¹¹ conducted in Japanese patients with RLS, it was observed that short-term treatment with PPX (< 46 months) was a factor significantly associated with the presence of augmentation. It is not yet known if the incidence decreases after a longer duration of treatment, as well as which, it is not clear how rapidly augmentation develops or if it progresses over time to a more severe state.¹²

In previous studies that showed high rates of augmentation during PPX treatment, the mean daily dose ranged from 0.63 to 1.28 mg/day.^{9,13–15} Another study¹¹ showed that the daily dose of PPX of 0.375 mg/day was the optimal cutoff value to predict the occurrence of the augmentation symptom. Therefore, the daily dose of PPX should be kept low to prevent augmentation. However, it is not yet known whether decreasing the daily dose of PPX can prevent the presence of augmentation.¹¹

In this sense, studies in animal models^{5,16}, as well as in humans¹⁷, have shown the benefits of acute or chronic PE in reducing RLS symptoms. In addition, PE can improve sleep patterns, and represents a safe and low-cost treatment method.^{5,16,17} Frank et al.⁵ observed that chronic treatment with PE (8 weeks) reduced the locomotor activity of spontaneously hypertensive rats (SHR), suggesting a reduction in RLS symptoms. Esteves et al.¹⁶ verified, in an animal model of RLS, a reduction in the number of limb movements after chronic exercise. In addition, Esteves et al.¹⁷ also demonstrated that PE may improve sleep patterns and reduce PLM levels in humans.

In the present study, the lineage of (SHR), composed of rats that developed attention deficit and hyperactivity disorder symptoms along with arterial hypertension, was used. This lineage has been shown to present characteristics related to sleep and behavior suggestive of sleep-related movement disorders, such as RLS.¹⁸

We believed that PPX treatment, as well as a PE session, could reduce locomotor activity and, consequently, RLS symptoms over time. In addition, it was expected that chronic use of PPX, as well as an increased dose, may cause augmentation of symptoms in SHR rats.

Thus, this study aimed to evaluate the effect of PPX treatment, as well as of one PE session, on the symptoms of RLS, by monitoring the locomotor activity, at different ages. In addition, we verified if there is worsening of symptoms (augmentation) with chronic treatment, or an increased dose of PPX.

METHODS

The project was approved by the Universidade Estadual de Campinas (Unicamp) Ethics Committee (4559-1/17). The animals were distributed into 4 groups:

- 1) Saline (n = 4);
- 2) PPX (0.1 mg/kg) started at 75 days of age (PPX1) (n = 6);
- 3) PPX (0.05 mg/kg) started at 91 days of age (PPX2) (n = 4);
- 4) PPX (0.05 mg/kg) started at 125 days of age (PPX3) (n = 4).

As this study will present preliminary data (initial results), we used a small number of animals, and the sample size was not calculated.

Experimental Design

The animals were adapted to the open field (OF) test. After the adaptation period and baseline, treatment was started. Baseline assessments were performed according to the age at which the experiment was started in each group. Thus, for the PPX1 group, the baseline measurement was performed at 75 days, for the PPX2 group at 91 days, and for the PPX3 group at 125 days. Regarding the saline group, the baseline measurement was performed at 75 days. The animals were treated daily with PPX or saline, for 48 days, always at the same time (between 07:00 and 08:00 h). On day 37, the PPX dose was increased, and on day 49, one PE session was performed. The evaluation of locomotor activity was performed at baseline and weekly (1, 6, 14, 22, 29, 36, 37, 43, and 49 days), shortly after (15 min) the PPX administration, or at the end of the PE session. Following the last OF test, the animals were euthanized (Figure 1).

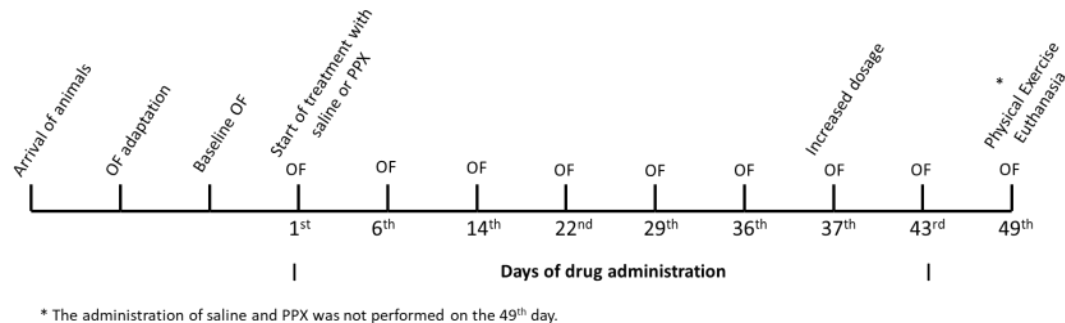


Figure 1. Experimental Design. OF: Open Field

Experimental Procedure

• Open field (OF)

Locomotor activity was evaluated in the OF, which consists of a circular arena of 81 cm (diameter), and 41-cm-high walls (open top). The lower part of the arena is divided into 19 quadrants, where the animals are placed individually for evaluation. Each animal remained in the test for 10 min, and only the final five were evaluated.¹⁹ This type of assessment serves as an indirect parameter to measure the "desire to move," one of the most common symptoms in RLS.²⁰

The analyzed parameters included total ambulation (the total number of quadrants that the animal stepped on with four paws); rearing frequency (the number of times that the animal supported itself with both hind legs); and freezing (total time the animal remained

perfectly still, moving only the vibrissae).²¹ An increase in locomotor activity is related to an increase in RLS symptoms. Therefore, the higher the Total Ambulation and Rearing parameters, the greater the locomotor activity, and, consequently, the greater the symptoms of RLS. The reverse is also true. On the other hand, the lower the freezing time, the greater the locomotor activity, and, consequently, the greater the symptoms of RLS. The opposite is also true.

- **Pharmacological Treatment**

Pharmacological treatment with PPX lasted 48 days. For the PPX1 group, the dose was 0.1 (first 4 weeks) and 0.25 mg/kg (weeks 5 and 6). For the PPX2 and PPX3 groups, the dose was 0.05 (first 4 weeks) and 0.25 mg/kg (weeks 5 and 6). Each 0.1 mg/kg was dissolved in 0.9% saline with the aid of a stirrer (Vortex-Genie 2®, Daigger®, USA). Animals from the saline group received a saline vehicle at 0.9% (0.1 mL/100 g). The drug/saline was administered intraperitoneally between 07:00 and 08:00 h, daily.⁷ The animals were weighed weekly on a Precision Electronic Digital Scale for administration of the correct amount of the drug.

Physical Exercise (PE)

The PE session (treadmill) occurred on the final day of the experiment (day 49), at a speed of 21 m/min (moderate intensity), which corresponds to approximately 60% of the maximal oxygen consumption.²² Exercise intensity was the same for all animals. The PE session started with a 5 min warm-up at 12–15 m/min, followed by 30 min at 20–22 m/min (5), and took place between 07:00 and 08:00 h.

Statistical analysis

Statistical analyses were performed using Statistica® for Windows® (version 7.0, StatSoft Inc., Tulsa, OK). To analyze the behavioral parameters, the Kolmogorov - Smirnov test was applied to verify the normality of the data. The distribution of the data was normalized by the transformation log (log). The open field and body mass results were analyzed by ANOVA for repeated measures (time - dependent variable & group-independent variable), followed by post hoc Tukey's test. The value of $\alpha = 5\%$ was considered to be statistically significant. The data are expressed as mean \pm standard error.

RESULTS

Groups that received PPX presented alterations in locomotor activity after chronic use of the drug and the PE session. These changes were demonstrated through the total ambulation, rearing, and freezing parameters.

The PPX1 group (on days 1, 37, and 49), the PPX2 group (on day 37), and the PPX3 group (on day 37) presented significantly reduced total ambulation compared to the PPX1 group (at baseline and day 22), the PPX2 group (at baseline and days 22, 36, and 43), and the PPX3 group (at baseline and day 22, Figure 2A). All groups receiving PPX reduced rearing on days 1 and 37 compared to the baseline test (Figure 2B). Finally, the PPX1 group (on days 1, 37, and 49), the PPX2 group (on day 37) and the PPX3 group (on days 37 and 49) presented increased freezing compared to the baseline test and days 22, 29, 36, and 43 (Figure 2C).

The PPX1 and PPX3 groups presented alterations in body mass from week 2 of treatment in comparison to the baseline data of all groups (Figure 3). The PPX3 group demonstrated a significant increase in body mass (weeks 5 and 6) relative to the saline group at baseline, 1, 2, and 3 weeks. However, although not significant, the PPX3 group presented higher values of body mass than the saline group, at all studied moments after starting the drug treatment.

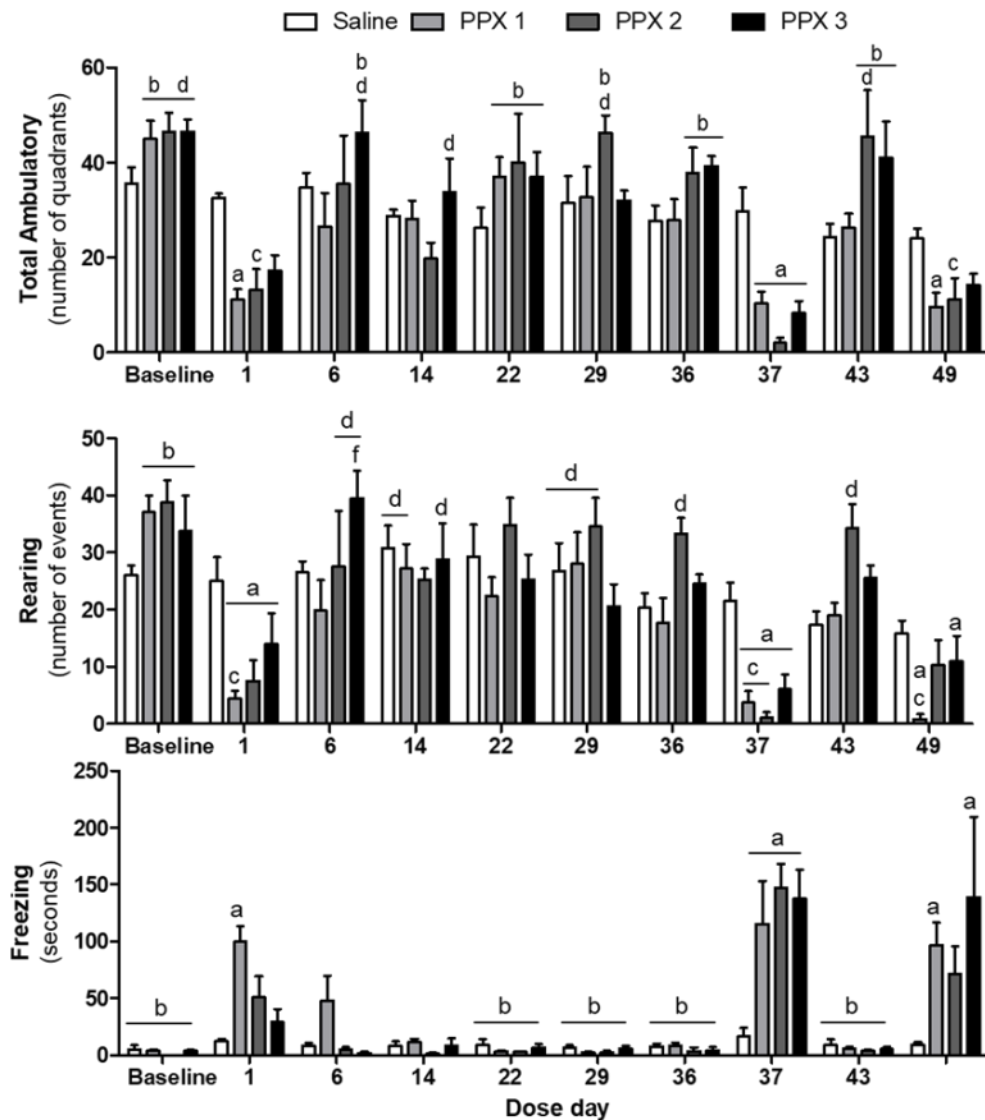


Figure 2. Parameters analyzed by the open field test of the animals in the saline group (control) and PPX groups at the ten assessment moments (baseline, 1, 6, 14, 22, 29, 36, 37, 43, and 49 days). ANOVA repeated measures, post hoc Tukey's test. (A) Total ambulatory: time [$F(9,126) = 19.7772$, $p < 0.00001$]; group [$F(3,126) = 2.6838$, $p < 0.001$]; interaction [$F(27,126) = 735.7397$, $p < 0.00001$]; "a" differs from "b"; "c" differs from "d". (B) Rearing: time [$F(9,126) = 26.4401$, $p < 0.00001$]; group [$F(3,126) = 3.4252$, $p < 0.00001$]; interaction [$F(27,126) = 335.9515$, $p < 0.00001$]; "a" differs from "b"; "c" differs from "d". (C) Freezing: time [$F(9,126) = 21.9069$, $p < 0.00001$]; group [$F(3,126) = 2.6523$, $p < 0.00001$]; interaction [$F(27,126) = 355.7966$, $p < 0.00001$]; "a" differs from "b".

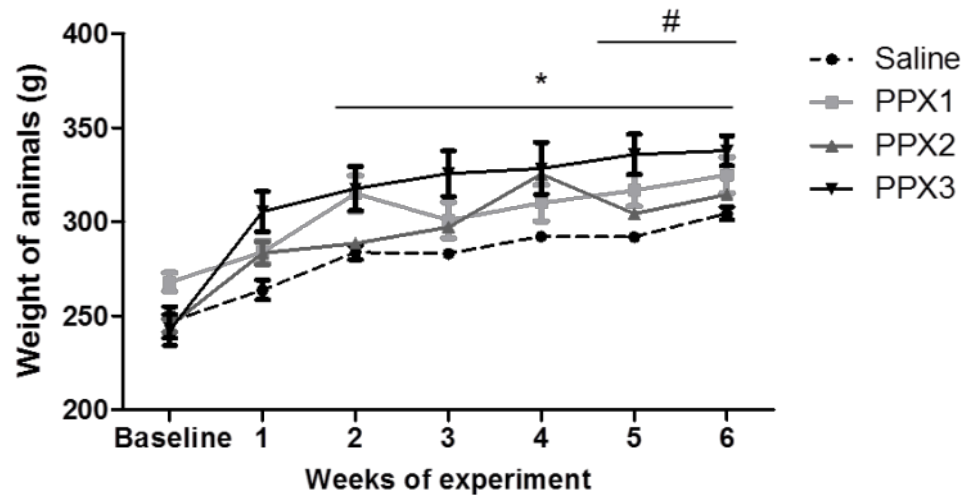


Figure 3. Body mass of the animals of the saline group (control) and PPX groups at baseline and during 6 weeks of treatment. ANOVA repeated measures, post hoc Tukey's test. Time [$F(6,84) = 5.990$, $p < 0.00001$]; group [$F(3,84) = 3.456$, $p < 0.0457$]; interaction [$F(18,84) = 5.990$; $p < 0.00001$]. * PPX1 and PPX3 (on the 2nd, 3rd, 4th, 5th and 6th weeks) differs from baseline data of the saline, PPX1, PPX2, and PPX3 groups; # PPX3 (5th and 6th weeks) differs from the saline group at baseline, 1, 2, and 3 weeks of experiment.

DISCUSSION

The present study aimed to evaluate the effect of chronic treatment with PPX, as well as of one PE session, on the symptoms of RLS, through evaluation of the locomotor activity, at different ages. Moreover, we aimed to check if there is worsening of symptoms (augmentation) with chronic treatment, or increased doses of PPX. The hypothesis of the study was that treatment with PPX, and a PE session, would reduce locomotor activity and, consequently, RLS symptoms, as age increased. In addition, it was expected that the chronic use of PPX, as well as increased doses, would cause the augmentation of symptoms in SHR rats.

Regarding the effect of PPX on locomotor activity, the total ambulation results were similar to the Rearing data, and all PPX groups presented lower values on days 1 (beginning of drug treatment) and 37 (dose increase) of treatment compared with the saline group. These findings reveal a reduction in locomotor activity and a possible decrease in RLS symptoms (days 1 and 37). Oliveira de Almeida et al.⁶ investigated the locomotor activity in Wistar rats after treatment with PPX (71 days), and observed a reduction in total ambulation (drug group) relative to both the basal period (days 1–57 of treatment) and the control group (days 8 – 43 of treatment). Conversely, similar to observations in the present study, there was a reduction in rearing (drug group) compared with the control group, only on day 1 of treatment. Overall, these outcomes also suggest a decrease in RLS symptoms, but for a longer period than in the present study, possibly because the treatment with PPX was longer.

In addition, in our study, the freezing values increased in all PPX groups on days 1 and 37 of treatment, inferring less need for locomotion by the animals and, consequently, a reduction in the symptoms of RLS. Oliveira de Almeida et al.⁶ documented an increase in

freezing (drug group) relative to both the basal period (days 1–64 of treatment) and the control group (days 1–43 and 57), thereby indicating reduced locomotor activity. However, it is worth mentioning that the PPX dose was constant and administered for a longer period (71 days) than in our study. In agreement with the present work, Oliveira de Almeida et al.⁶ did not observe augmentation of symptoms throughout the treatment.

A study⁷ that evaluated the effect of PPX treatment in another animal model of RLS noticed that the drug reduced locomotor activity and, similarly to our study, augmentation did not occur.

In the present study, different doses of PPX were used to verify if the effect of augmentation occurred. The dose choice of 0.1mg / kg was based on the studies of Oliveira de Almeida et al.⁶ and Luo et al.⁷ In both, changes in locomotor activity were observed in response to PPX treatment, but no manifestation of augmentation of symptoms. Thus, we decided to evaluate whether the treatment initiated with a lower dose of PPX (0.05mg / kg) and with a subsequent increase to 0.25mg / kg would cause alterations in locomotor activity, as well as augmentation of symptoms, since it is known that the effect of augmentation can be caused by the increased dose of dopaminergic agonists, over treatment.

In our study, the animals presented rapid habituation to the administration of pramipexole, where behavioral changes only occurred at the first dose and at the dose increase. This is the first study to evaluate whether augmentation occurs with the use of pramipexole in SHR rats. Therefore, more studies are needed to understand adaptation of the animals to different dosages and treatment periods.

Regarding the acute PE session, performed on the final day of the experiment (day 49), on which the animals did not receive PPX, results similar to the treatment with the drug were seen, with a reduction in total ambulation and rearing, and an increase in freezing. Thus, a single PE session promoted similar results to PPX treatment, reducing locomotor activity, indicating a decrease in RLS symptoms. Corroborating with our study, Esteves et al.¹⁷ noted a positive effect of acute aerobic PE in patients with PLM, concluding that PE may be a plausible alternative to pharmacological treatment, or as a complement to drug therapy in extreme cases.

Other research with animal models of RLS also demonstrated the beneficial effect of chronic PE on RLS/PLM symptoms^{5,16}. Frank et al.⁵ analyzed the impact of chronic PE (treadmill) on locomotor activity (SHR rats). While those authors did not note any difference in total ambulation between the PE groups and the control, the PE groups presented lower rearing values.

One of the mechanisms that suggest improvement in sleep-related movement disorder symptoms through PE is related to the participation of the opioidergic and dopaminergic systems, which are highly stimulated during exercise. Esteves et al.¹⁷ observed a negative correlation between beta-endorphin levels released in plasma and PLM after an acute PE session¹⁷.

Regarding treatment with PPX, we illustrated an increase in the body mass of the animals of the PPX1 and PPX3 groups from week 2 when compared with the baseline data of all groups. Although not significant, the animals in the PPX3 group were heavier than the saline group, corroborating the findings of Oliveira de Almeida et al.⁶ in rats (Wistar) after chronic treatment with PPX.

CONCLUSION

The groups that received PPX presented decreased locomotor activity compared with the saline group at the first dose (day 1), increased dose (day 37), and after the PE session (day 49). The PE session caused changes in locomotor activity similar to PPX, proving to be effective in reducing the symptoms of RLS. Furthermore, no augmentation of symptoms occurred with chronic treatment, or even with increasing doses of PPX. Further studies are needed to understand the augmentation of symptoms in SHR rats.

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