Absence of a synergic nigral proapoptotic effect triggered by REM sleep deprivation in the rotenone model of Parkinson’s disease

Luana C Kmita 1  
Jessica L Ilkiw 1  
Lais S Rodrigues 1,2  
Adriano DS Targa 1,2  
Ana Carolina D Noseda 1,2  
Patrícia dos-Santos 1  
Juliane Fagotti 1  
Edvaldo S. Trindade 3  
Marcelo MS Lima 1,2

1 Federal University of Paraná, Department of Physiology - Curitiba - Paraná - Brazil.  
2 Federal University of Paraná, Department of Pharmacology - Curitiba - Paraná - Brazil.  
3 Federal University of Paraná, Department of Cell Biology - Curitiba - Paraná - Brazil.

ABSTRACT

Excitotoxicity has been related to play a crucial role in Parkinson's disease (PD) pathogenesis. Pedunculopontine tegmental nucleus (PPT) represents one of the major sources of glutamatergic afferences to nigrostriatal pathway and putative reciprocal connectivity between these structures may exert a potential influence on rapid eye movement (REM) sleep control. Also, PPT could be overactive in PD, it seems that dopaminergic neurons are under abnormally high levels of glutamate and consequently might be more vulnerable to neurodegeneration. We decided to investigate the neuroprotective effect of riluzole administration, a N-methyl-D-aspartate (NMDA) receptor antagonist, in rats submitted simultaneously to nigrostriatal rotenone and 24h of REM sleep deprivation (REMSD). Our findings showed that blocking NMDA glutamatergic receptors in the SNpc, after REMSD challenge, protected the dopaminergic neurons from rotenone lesion. Concerning rotenone-induced hypolocomotion, riluzole reversed this impairment in the control groups. Also, REMSD prevented the occurrence of rotenone-induced motor impairment as a result of dopaminergic supersensitivity. In addition, higher Fluoro Jade C (FJC) staining within the SNpc was associated with decreased cognitive performance observed in rotenone groups. Such effect was counteracted by riluzole suggesting the occurrence of an antiapoptotic effect. Moreover, riluzole did not rescue cognitive impairment impinged by rotenone, REMSD or their combination. These data indicated that reductions of excitotoxicity, by riluzole, partially protected dopamine neurons from neuronal death and appeared to be effective in relieve specific rotenone-induce motor disabilities.

Keywords: Excitotoxicity; Neuroprotection; Riluzole; REM sleep deprivation; Intranigral rotenone; Parkinson’s disease.

Corresponding author:  
Marcelo MS Lima  
E-mail: mmslima@ufpr.br  
Received: Month April 2, 2019;  
Accepted: Month August 20, 2019.

DOI: 10.5935/1984-0063.20190078
INTRODUCTION

Parkinson’s disease (PD) is the second most common progressive neurodegenerative disorder, especially prominent in aging societies1,2. PD is characterized by progressive dopaminergic neuronal loss within the substantia nigra pars compacta (SNpc) that tends to happen more broadly in the ventrolateral layer followed by medial ventral and dorsal layers3,4. PD pathogenesis is a complex and multifactorial process, both genetic features and environmental stressors converge and compromising neuronal activity5.

The precise intrinsic mechanism of the dopaminergic neuronal death remains unknown, however, several lines of evidence highlighted the importance of enhanced glutamatergic neurotransmission in basal ganglia and related structures in the PD development6. In PD, some glutamatergic systems are somewhat hyperactive, such as corticostriatal and subthalamic nigral pathways, potentially contributing to dopaminergic neuronal death, through the excitotoxic process5,7,8.

In this sense, pedunculopontine tegmental nucleus (PPT) is one of the major source of glutamatergic afferences to nigrostriatal pathway9. Remarkably, the basal ganglia, including SNpc, and PPT share numerous similarities in projections to cortex, thalamus amygdala and brainstem10. Accordingly, there is a hypothesis that PPT could play an excitotoxic role in dopaminergic neurons, suggesting some level of participation in PD pathogenesis, since PPT demonstrated to be highly active in the 6-hydroxydopamine (6-OHDA) animal model11,12.

Furthermore, mutual interactions between PPT and SNpc are associated with REM sleep generation. Thus, nigral degeneration has been related with significant disruption in REM sleep in both animal models13,14 and PD15. In this context, PPT could play a role in neurodegenerative process, triggering excitotoxicity within the SNpc, contributing to the occurrence of sleep disorders16.

Therefore, it is considered that reduced nigrostriatal activity would result in a compensatory PPT firing, resulting in increased glutamatergic neurotransmission to SNpc, with potential excitotoxic mechanisms. Based on these observations, we promoted nigrostriatal lesions with the neurotoxin rotenone17, associated with REM sleep deprivation (REMSD) and/or riluzole administration, a NMDA receptor antagonist with clinical neuroprotective applications, p.e., in amyotrophic lateral sclerosis18. Our hypothesis is also based on a 24 h REMSD-induced down-regulation of tyrosine hydroxylase (TH) expression, within the nigrostriatal pathway19,20, possibly promoting an increased PPT activity, negatively impacting SNpc neurons through excessive glutamatergic signaling.

MATERIAL AND METHODS

Animals and house conditions

All of the experiments performed in this study were approved by the ethics committee of Federal University of Paraná (approval ID#918) and conducted according to the guidelines of ethics and experimental care and use of laboratory animals (SBCAL). Male Wistar rats weighing 280-230 g at the beginning of the experiment were used. The animals were randomly housed in groups of 5 in polypropylene cages and maintained in a temperature controlled room (22°C ± 2°C) with a 12h light-dark cycle (lights on at 7:00 am). Bottles of water and pellets of food were available throughout the entire experiment.

Experimental design

Before the stereotoxic surgeries the animals were distributed randomly in two groups: sham (n=48) and rotenone (n=48). Afterwards, the animals were redistributed in eight groups (n=12/group): control sham vehicle, control sham riluzole, control rotenone vehicle, control rotenone riluzole, REMSD sham vehicle, REMSD sham riluzole, REMSD rotenone vehicle and REMSD rotenone riluzole. On the day 0, the animals underwent stereotoxic surgery for bilateral guide cannulas implantation within the SNpc. On day 7 the animals received a bilateral intranigral infusion of 1µl of rotenone (12 µg/µl) or equal volume of its vehicle dimethylsulfoxide (DMSO) and then the rats were subjected to 24 h of REM sleep deprivation (REMSD) or were kept in their home cages (control). Immediately after this period, the animals received a single bilateral intranigral infusion of 1µl of riluzole (10µg/µl) or equal volume of DMSO, subsequently (30 minutes after) they were tested in the open field (OFT) and in the object recognition test (ORT). At the end of these experiments, the animals were intracardially perfused (under deep anesthesia) for tissue fixation and the brains were removed for subsequent confocal microscopy analysis of apoptotic cells within the SNpc.

Stereotoxic surgery and intranigral infusions

The animals were sedated with intraperitoneal xylazine (10 mg/kg, Syntec do Brasil Ltda, Brazil) and anaesthetized with intraperitoneal ketamine (90 mg/kg, Syntec do Brasil Ltda, Brazil). The following coordinates were used for bilateral guide cannulas implantation, bregma as a reference: SNpc (AP) = - 5,0 mm, (ML) = ± 2,1 mm e (DV) = - 7,8 mm.21

Bilateral intranigral infusions of 1 µl of Rotenone (12µg/µl), 1 µl of riluzole (10µg/µl); Sigma-Aldrich®, St. Louis, MO, USA, or 1 µl of DMSO (Sigma-Aldrich®) were all made through the guide cannulas, at a rate of 0.33 ml/min for 3 min, with the assistance of an electronic infusion pump (Insight Instruments, Ribeirão Preto, SP, Brazil).

REMSD procedure

REMSD was attained by means of the single platform method. The animals were individually placed in a circular platform (6.5 cm in diameter) inside of a tank (23 x 23 x 35 cm) filled with water up 1 cm below the platform surface for 24 h. Once the animal experiences a REM sleep episode, it loses its muscular tonus and falls into the water, being awakened. This procedure has demonstrated effectiveness in ablation of REM sleep without affecting NREM sleep22. Throughout the study, the experimental room was maintained at controlled conditions.
Absence of nigral proapoptotic effect induced by REMSD

(22 ± 2 ºC, 12:12 h light/dark cycle, lights on 7:00 a.m.). The control group (non-sleep deprived) was maintained in the same room during the period, but isolated in their usual home cages, to mimic a possible effect of isolation caused by the procedure. Water and food were available during the entire experiment.

Open Field Test (OFT)

The apparatus consists of a circular arena (1 m of diameter) limited by a 40 cm high wall and illuminated by four 60 W lamps situated 48 cm above the arena floor, providing illumination around 300 lx. The animals were gently placed in the center of the arena and were allowed to freely explore the area for 5 min. During the experiments, the open field test was video recorded and the measures of locomotion of the groups were computed by an image analyzer system (Smart junior, PanLab, Harvard Apparatus, Spain).

Object Recognition Test (ORT)

The apparatus consists of an open box (width × length × height = 80 cm × 80 cm × 50 cm) made of wood and covered with a black opaque plastic film. The illumination on the floor of the box apparatus was around 186 lx. The objects to be discriminated were available in triplicate copies and were made of a biologically neutral material such as glass, plastic, or metal. The objects were weighted so that the animals could not move them around in the arena. They are not known to have any ethological significance for the rats and they had never been associated with any reinforcement 27. The object recognition test consists of two phases, a sample phase (3-min duration) and a choice phase (3-min duration). In the sample phase, two identical objects are exposed in the back corners of the open box, 10 cm away from the sidewall.

The rat is placed in the open box facing away from the objects for 3 times with a 15 min retention interval between the times. After 24 h, the rat is reintroduced to the open box and the choice phase is started. In the choice phase, two different objects are exposed in the same locations that were occupied by the previous sample objects. One of the objects is identical to the object seen in the sample phase and the other is a novel object, the total time spent in exploring the two objects was video recorded. The frequencies of approaches of each object are recorded. The exploration is recorded only when the rat touches the object with its nose or that the rat’s nose is directed toward an object at a distance ≤ 2 cm. As a measure of discrimination, “discrimination index (DI)” was calculated by dividing the difference in number of explorations between the two objects (object novel - object familiar) by the total amount of exploration for both objects (object novel + object familiar). DI was then multiplied by 100 to express as a percentage.

Fluoro Jade C (FJC) staining within SNpc

Animals were deeply anesthetized with ketamine (100 mg/kg) immediately after the behavioral tests and were intracardially perfused with saline first, then with 4% of the fixative solution formaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed from the skulls and were immersed for 48 hours in that fixative solution at 4°C. Subsequently, the brains were placed in 30% sucrose solution for 3 days and were freeze at -80°C before sectioning. Four 40 μm sections per animal were taken from the SNpc (~4.92 mm and ~5.28 mm). The coordinates were obtained from 28.

Prior to staining, sections were mounted from distilled water onto gelled slides. Gelatin coated slides were prepared by immersion in a 60 °C solution of 1% pig skin gelatin (Sigma-Aldrich; type A, 300 Bloom) and then oven dried overnight at the same temperature. The sections were mounted onto the slides from distilled water and then air dried for at least 30 min on a slide warmer at 50 °C. Slides bearing frozen cut tissue sections were first immersed in a solution containing 1% sodium hydroxide in 80% ethanol for 5 min. They were then rinsed for 2 min in 70% ethanol, for 2 min in distilled water, and then incubated in 0.06% potassium permanganate solution for 10 min. Slides were then transferred for 10 min to a 0.001% solution of Fluoro Jade C as indicated by the fabricant (Thermo Fischer Scientific). The proper dilution was accomplished by first making a 0.01% stock solution of the dye in distilled water and then adding 1 ml of the stock solution to 99 ml of 0.1% acetic acid vehicle. The working solution was used within 2 h of preparation. The slides were then rinsed through three changes of distilled water for 1 min per change. Excess water was drained onto a paper towel, and the slides were then air dried on a slide warmer at 50 °C for at least 5 min. The air dried slides were then cleared in xylene for 5 min and then coverslipped with Fluoromount-G (SouthernBiotech, Birmingham, Alabama, USA) non-fluorescent mounting media.

The images were analyzed in Nikon Confocal Microscope AIRSi+MP (Nikon Instruments Inc.; Tokyo, Japan), using 20x lenses. For FJC, 488 nm laser was used for excitation and the imagens was obtained using a 500-550nm band pass filter. The Imaging Software Nis Elements 4.20 (Nikon) was used for z-stack visualization and to generate maximum projectin image.

Statistical analysis

Homogeneity of variance was assessed by the Bartlett test and normal distribution of the data was assessed by the Kolmogorov-Smirnov test. Differences between groups in the OF, ORT and discrimination index were analyzed by two-way analysis of variance (ANOVA) followed by the Tukey’s post hoc test. Fluorescence intensity was analyzed by one-way analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test. Pearson’s correlation coefficients (r) were calculated to establish relationships between fluorescence intensity and behavioral parameters. Values were expressed as mean ± standard error of mean (SEM). The level of significance was set at p ≤ 0.05.

RESULTS

Open Field Test (OFT)

As can be seen in Figure 1, the control rotenone vehicle group presented an impaired locomotion in comparison to the control sham vehicle group (p ≤ 0.05), as indicated by the group factor [F(3,54) =2.21; p ≤ 0.09]. Interestingly, the same effect
was not observed in the control rotenone riluzole group when compared to the control sham vehicle \( (p \geq 0.96) \) and control sham riluzole \( (p \geq 0.99) \) groups. Complementarily, the rotenone REMSD groups did not exhibit reductions in locomotion when compared to the control sham \( (p \geq 0.95) \) and REMSD sham \( (p \geq 0.97) \) groups, according to the treatment \( [F(1.54) = 0.063; p = 0.8] \) and interaction \( [F(3.54) = 1.59; p = 0.20] \) factors.

Object Recognition Test (ORT)

Regarding the cognitive analysis, we found that the control sham vehicle group showed an increment in the time exploring the novel object in comparison to the familiar \( (p \leq 0.01) \) during the choice phase, as well as the control sham riluzole group \( (p \leq 0.01) \), by means of the treatment \( [F(7.10)=3.17; p \leq 0.01] \) and interaction \( [F(7.10)=3.63; p \leq 0.001] \) factors (Fig. 2A). Conversely, the control rotenone groups demonstrated a similar exploration time for both objects, indicating memory impairment as showed by the object \( [F(1.10)=2.57; p = 0.1] \) factor. Besides, it was detected that REMSD, itself, produced a remarkable impairment in the object recognition, since all the sleep deprived groups explored both objects equally.

Figure 2B shows the discrimination index (DI) obtained from the time of objects exploration recorded for each control and REMSD groups. Accordingly, the control rotenone vehicle exhibited a significant reduction in this parameter when compared to the control sham vehicle \( (p \leq 0.05) \). As observed in the previous parameter, REMSD, per se, promoted a noteworthy decrease in this index for all the groups that were sleep deprived. Therefore, we observed a significant decrease in the DI for the REMSD sham vehicle \( (p \leq 0.01) \) and REMSD sham riluzole \( (p \leq 0.05) \) groups when compared to their respective controls \( [F(3.57)=10.84; p < 0.0001] \).

Fluoro Jade C (FJC) staining within SNpc

FJC labeling can sensitively and selectively stain degenerating neurons. In this sense, Figure 3 depicts the differences on fluorescence intensity among the groups. In fact, the groups control sham vehicle (Fig. 3A), REMSD sham vehicle (Fig. 4A), control sham riluzole (Fig. 3B) and REMSD sham riluzole (Fig. 4B) did not exhibit FJC labeling. In opposite, the control rotenone vehicle (Fig. 3C) and REMSD rotenone vehicle (Fig. 4C) groups have shown remarkable fluorescence labeling compared to the control sham vehicle and REMSD sham vehicle groups. Conspicuously, we detected that riluzole treatment produced a prominent reduction in the FJC fluorescence intensity as can be seen in the control rotenone riluzole (Fig. 3D) and REMSD rotenone riluzole (Fig. 4D) groups.

DISCUSSION

In the present study we observed that blocking NMDA glutamatergic receptors, within the SNpc, immediately after an acute REMSD challenge, partially protected dopaminergic neurons from the rotenone lesion and the excitotoxicity purportedly inflicted by PPT increased activity. Such limited protection was also manifested as an absence of memory preservation observed in the rotenone-treated groups. Besides, sleep deprivation generated massive memory impairment in all the groups subjected to this condition, hampering the identification of any possible protective effect of the drug in this context. Nevertheless, we detected an interesting effect of riluzole preserving the locomotor activity of the control vehicle (Fig. 4A), control sham riluzole (Fig. 3B) and REMSD sham riluzole (Fig. 4B) did not exhibit FJC labeling. In opposite, the control rotenone vehicle (Fig. 3C) and REMSD rotenone vehicle (Fig. 4C) groups have shown remarkable fluorescence labeling compared to the control sham vehicle and REMSD sham vehicle groups. Conspicuously, we detected that riluzole treatment produced a prominent reduction in the FJC fluorescence intensity as can be seen in the control rotenone riluzole (Fig. 3D) and REMSD rotenone riluzole (Fig. 4D) groups.
Figure 3. Comparison of Fluoro-Jade C (FJC) staining in the SNpc between: (A) Control sham vehicle, (B) Control sham riluzole, (C) Control rotenone vehicle, (D) Control rotenone riluzole. Arrows indicate FJC-positive neurons.

Figure 4. Comparison of Fluoro-Jade C (FJC) staining in the SNpc between: (A) REMSD sham vehicle, (B) REMSD sham riluzole, (C) REMSD rotenone vehicle, (D) REMSD rotenone riluzole. Arrows indicate FJC-positive neurons.

Absence of nigral proapoptotic effect induced by REMSD

Riluzole has been consistently demonstrated to provide a multitude of neuroprotective effects in neurodegenerative disorders, such as amyotrophic lateral sclerosis. Moreover, analogous effects were reported in studies from animal models of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 6-hydroxydopamine, showing reduction in the dopaminergic neuronal loss, covered by a variety of administration protocols. It is described that the pharmacological mechanism of riluzole is based on the noncompetitive blockade of NMDA receptors, reduction of glutamate release from presynaptic terminals, inhibition of voltage-gated sodium channels and inhibition of protein kinase C activity, hence counteracting excitotoxic processes.

Rotenone is a neurotoxin that produces massive inhibition of mitochondrial complex I, increase of reactive oxygen species and selective degeneration of the dopaminergic neurons, reproducing key pathological features of PD. Evidence suggest that rotenone might induce neuronal damage via excitotoxic mechanisms potentiating NMDA-mediated currents in the dopaminergic neurons. Similar results were found in human postmortem tissues, indicating that mitochondrial alterations are important in the pathogenesis of sporadic PD.

The localization and activity profile of the ionotropic glutamate receptors in the SNpc suggest that these receptors may provide a positive feedback mechanism triggered by PPT activation. As a result, a putative reciprocal connectivity between those structures may exert a potential influence on the mechanism of REM sleep. This raises an intriguing possibility, for which there is currently no further evidence, that reducing the glutamatergic firing from PPT to SNpc, this could generate neuroprotective effects and influence the rate of the progression of PD. In view of that, our FJC-staining investigation showed some level of neuroprotection inflicted by riluzole particularly when fluorescence is compared to the rotenone groups. This result suggests the occurrence of and antiapoptotic effect. Besides, REMSD did not inflict increased or synergic neuronal death associated to rotenone lesions, as originally expected.

In fact, rotenone produces a considerable reduction on the percentage of SNpc tyrosine hydroxylase immunoreactive neurons. Furthermore, it seems to be more potent when compared to other neurotoxins requiring lower doses to produce the lesion. Thus, additional studies, with lower neurotoxin dosages, will be necessary to completely refute the current hypothesis; hence, we cannot entirely exclude the potential deleterious effects of REMSD as demonstrated by other studies.

Regarding the rotenone-induced hypolocomotion, riluzole rescued this impairment in the control groups. This is in accordance to previous reports that described a positive effect of riluzole in the motor performance in PD animal models. However, we did not found locomotion differences between the sham REMSD groups, neither in the rotenone REMSD groups. Probably due to REMSD is able to elicit a locomotion increase, per se, associated to the well-known supersensitivity of dopaminergic receptors.

Concerning the cognition, ORT is a familiarity-based memory task correlated with the human episodic-like memory which is compromised in early-stages PD patients. In light of these results, the possible effect of riluzole on the cognitive performance in PD needs to be further investigated.

rot enone-treated rats. In addition, REMSD counteracted the locomotion deficit induced by the lesion due to the occurrence of a vastly reported effect of dopaminergic supersensitivity.
of this, given the correlation between sleep disturbances and cognitive impairments, it is possible to consider that sleep disorders observed in PD patients, might be considered as an early marker for dementia processes. Accordingly, our results demonstrated that rivulose did not affect memory processes of control groups and also not rescued the impairment impinged by rotenone, REMSD or their combination. Furthermore, REMSD generated remarkable memory impairment, corroborating with previous reports. Such result is opposite to other studies that tested rivulose in different chronic and systemic protocols showing levels of improvement of cognitive performance. This discrepancy could be related to our experimental design that projected rivulose administration to a period of high neurotoxic condition, i.e., 24 h after rotenone and immediately after the end of REMSD (challenge to induce PPT activation). Complementarily, decreased FJC labeling within the SNpc could be associated with increased cognitive performance in controls, but not in REMSD groups, possibly due to massive memory disruption inflicted by sleep loss. These data indicate improvement of memory performance in the control animals with lower dopaminergic neuronal loss, as an outcome of the excitotoxicity blockade.

In summary, the intranigral administration of rivulose partially protected the dopaminergic neurons from the rotenone-induced lesion, particularly preventing the occurrence of locomotor, but not declarative-like memory deficits. The data also indicate absence of a synergic excitotoxic mechanism triggered by a supposed PPT overactivity towards SNpc, through reciprocal projections. Although, we cannot completely exclude this potential association because more studies will be necessary to identify the levels of glutamatergic PPT activation inflicted by different protocols of REMSD, perhaps in a more gradual and chronic situation, mimicking PD.

ACKNOWLEDGMENTS

This study was supported by CAPES, FINEP - Edital Pró-Infra 2009 (CT-INFRA), Fundação Araucária (Programa de Apoio a Núcleos de Excelência - PRONEX - Convênio 116/2018) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq. MMSL is the recipient of a CNPq fellowship, Research Grants 431279/2016-0, 305986/2016-3.

REFERENCES

1. Alam, M, A Mayerhofer and WJ Schmidt (2004). The neurobehavioral changes induced by bilateral rotenone lesion in medial forebrain bundle of rats are reversed by L-DOPA. Behavioural Brain Research 151: 117-124.

2. Ambrosi, G, S Cerri and F Blandini (2014). A further update on the role of excitotoxicity in the pathogenesis of Parkinson’s disease. Journal of Neurotransmission 121:849-859.

3. Araki, T, Y Muramatsu, K Tanaka, M Matsubara and Y Imai (2001a). Riluzole (2-amino-6-trifluoromethoxy benzothiazole) attenuates MPTP (1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine) neurotoxicity in mice. Neuroscience Letters 312:50-54.

4. Araki, T, T Kumagai, K Tanaka, M Matsubara, H Kato, Y Itoyama et al (2001b). Neuroprotective effect of riluzole in MPTP-treated mice. Brain Research 918:176-181.

5. Barnoud, P, M Mazadier, JM Miquet, S Parmentier, P Dubiada, A Doble et al (1996). Neuroprotective effects of riluzole on a model of Parkinson's disease in the rat. Neuroscience 43:971-983.

6. Barnaud, Q, V Lambregt, C Forni, S Meguire, M Hill, B Boulac et al (2000). Sleep disorders in Parkinson's disease: the contribution of the MPTP non-human primate model. Exp Neurol 219:574-582.

7. Benazzouz, A, T Boudiet, A Boireau, JM Stutzmann, C Gross (1995). Riluzole prevents MPTP-induced parkinsonism in the resus monkey: a pilot study. European Journal of Pharmacology 284:299-307.

8. Biswas, S, P Mishra and BN Mallick (2006). Increased apoptosis in rats brain after rapid eye movement sleep loss. Neuroscience 142:315-331.

9. Boireau, A, P Dubédat, B Bordier, C Peny, JM Miquet, G Durand, et al (1994). Riluzole and experimental parkinsonism: antagonism of MPTP-induced decrease in central dopamine levels in mice. Neuroreport 5:257-260.

10. Bredt, S, R Boulai-Benazzouz, AL Benabid, A Benazzouz (2001). Unilateral lesion of the nigrostriatal pathway induces an increase of neuronal activity of the pedunculopontine nucleus, with is reversed by the lesion of the subthalamic nucleus in rats. European Journal of Neuroscience 14:1833-1842.

11. Carbone, M, S Duty and M Rattray (2012). Riluzole neuroprotection in a Parkinson’s disease model involves suppression of reactive astrocytosis but not GLT-1 regulation. BMC Neuroscience 13:1-8.

12. Chen, L, S Tian and J Ke (2014). Rapid eye movement sleep deprivation disrupts consolidation but not reconsolidation of novel object recognition memory in rats. Neurosci Lett 20:12-16.

13. Charrara, A, Smith and A Parent (1996). Glutamatergic inputs from the pedunculopontine nucleus to midbrain dopaminergic neurons in primates: Phaseolus vulgaris-leucoagglutinin anterograde labeling combined with postembedding glutamate and GABA immunohistochemistry. The journal of Comparative Neurology 364:254-266.

14. Dawson, TM and VL Dawson (2002). Neuroprotective and neurorestorative strategies for Parkinson’s disease. Nature Neuroscience supplement 5:1058-1061.

15. Dere E, MA Silva and JP Huston (2004). Higher order memories for objects encountered in different spatio-temporal contexts in mice: evidence for episodic memory. Rev Neurosci 15:231–240.

16. Doble, A (1999). The role of excitotoxicity in neurodegenerative disease: Implications for therapy. Pharmacol. Ther 81:163-221.

17. Dos Santos, ACD, MAV Castro, EAK Jose, AM Delattre, PA Dombrowski, C Da Cunha, et al (2013). REM sleep deprivation generates cognitive and neurochemical disruptions in the intranigral rotenone model of Parkinson’s disease. Journal of Neuroscience Research 91:1508-1516.

18. Ennaceur, A and J Delacour (1988). A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. Behav. Brain Res 31:47-59.

19. Erró, R, G Sanchez, M Picillo, C Vitale, M Amboni, K Longo et al (2012). Link between non-motor symptoms and cognitive dysfunctions in de novo, drug naïve patients. J Neurolog 259:1808-1813.

20. Fagotti, T, Tanga ADS, Rodrigues, LK Noseda, AC Dorcier, FWG, Scarabossio, F; Ikile, JI; Louzada, FM, Chowdhury, NR, Van der Ven, DR, Middleton, B, Pennings, J LA, Swann, JR, Skene, DJ, Lima, MMS (2019). Chronic sleep restriction in the rotenone Parkinson’s disease model in rats reveals peripheral early-phase biomarkers. Sci. Reports 9:1898.

21. Furnagall, E, M Fuscinello, T Rauen, M Gobbi, T Mennini (2008). Riluzole enhances the activity of glutamate transporters GLAST, GLT-1 and EAAC1. European Journal of Pharmacology 578:171-176.

22. Khardarw, YA, NA Nour and HSA Ezz (2011). Effect of oxidative stress induced by parkinson like sleep deprivation on the activities of Na+, K+ and Acetylcholinesterase in the cortex and hippocampus of rat. Translational Research 157:100-107.

23. Jatwal, NK (2017). Riluzole but not melatonin ameliorates acute motor neuron degeneration and moderately inhibits SOD1-mediated excitotoxicity induced disrupted mitochondrial Ca2+ signaling in amyotrophic lateral sclerosis. Front. Cell. Neurosci 14:1833-1842.

24. Lang, AE and AM Lozano (1998). Parkinson’s disease. The New England Journal of Medicine 339:1044-1053.

25. Lavoz, B and A Parent (1994). Pedunculopontine nucleus in the squirrel monkey: Projections to the basal ganglia as revealed by anterograde tract-tracing methods. The Journal of Comparative Neurology 364:210-231.

26. Lima, MMS, AL Delattre, EAK Jose, AM Delattre, PA Dombrowski, C Da Cunha, et al (2013). REM sleep deprivation generates cognitive and neurochemical disruptions in the intranigral rotenone model of Parkinson’s disease. Journal of Neuroscience Research 91:1508-1516.

27. Lima, MMS, AB Reksidler and MABF Vital (2008). The dopaminergic dilemma: Sleep or wake? Implications in Parkinson’s disease. Bioscience Hypotheses 1:9-13.

Sleep Sci. 2019;12(3):196-202
Absence of nigral proapoptotic effect induced by REMSD

28. Lima, MMS, ML Andersen, AB Reksidler, AC Ferraz, MABF Vital, S Tufik, S (2012). Paradoxical sleep deprivation modulates tyrosine hydroxylase expression in the nigrostriatal pathway and attenuates motor deficits induced by dopaminergic depletion. CNS & Neurological Disorders - Drug Targets 11:359-368.

29. Lima, MMS (2013). Sleep disturbances in Parkinson’s disease: The contribution of dopamine in REM sleep regulation. Sleep Medicine Reviews 17:367-375.

30. Luquin, MR, I Saldicé, J Guillén, S Belzungeui, W San Sebastián, A Izal, et al (2006). Does increase excitatory drive from the subthalamic nucleus contribute to dopaminergic neuronal death in Parkinson’s disease? Experimental Neurology 201:407-415.

31. Meta-Segovia, J, JP Bolam and PJ Magil (2004). Pedunculopontine nucleous and basal ganglia: distant relatives or part of the same Family? TRENDS in Neuroscience 27:585-588.

32. Mohktari, Z, T Balouchnejadmojarad, F Nikbakht, M Mansouri, M Roghani (2017). Riluzole ameliorates learning and memory deficits in Aβ25-35-induced rat model of Alzheimer’s disease and is independent of cholinoreceptor activation. Biomedec & Pharmacotherapy 87:135-144.

33. Moreira, CG, D Ariza, PA Dombrowski, P Sabioni, M Bortolanza, C da Cunha, et al (2012). Behavioral, neurochemical and histological alterations promoted by bilateral intranigral rotenone administration. Neurotox Res 21: 291-301.

34. Morris RG (2001). Episodic-like memory in animals: psychological criteria, neural mechanisms and the value of episodic-like tasks to investigate animal models of neurodegenerative disease. Philos Trans R Soc Lond B Biol Sci 356:1453–1465.

35. Munhall, AC, YN Wu, JK Belknap, CK Meshul, SW Johnson (2012). NMDA alters rotenone toxicity in rat substantia nigra zona compacta and ventral tegmental area dopaminergic neurons. NeuroToxicology 33:429-435.

36. Nunes, GP, S Tufik and JN Nobrega (1994). Autoradiographic analysis of D1 and D2 dopaminergic receptors in rat brain after paradoxical sleep deprivation. Brain Res. Bull 34:453-456.

37. Olszewski, JW (1969). Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 164:719-721.

38. Oles, JW (1989). Excitatory amino acids and neuropsychiatric disorders. Biol Psychiatry 26:505-525.

39. Palchykova, S, R Winsky-Sommerer, P Meerlo, P Merticar, R Smit, HCHM Philippens (2012). Neuroprotective effects of riluzole in early phase Parkinson’s disease on clinically relevant parameters in the marmoset MPTP model. Neuropharmacology 62:1700-1707.

40. Piacentini, M, F Placidi, C Liquori, M Albanese, P Imbriani, MG Marcelli, et al (2016). Rotigotine may improve sleep architecture in Parkinson’s disease: A double-blind, randomized, placebo-controlled polysomnographic study. Sleep Med 21:140-144.

41. Prousa, MB, PA Dombrowski, C Cunha, L Fischer, AC Ferraz and MMS Lima (2014). Dopaminergic D2 receptor is a key player in the substantia nigra par compacta neuronal activation mediated by REM sleep deprivation. Neuropsychopharmacology 76:118-126.

42. Rodrigues, LS, ADS Tanga, ACD Noseda, MF Aurich, LC Cunha and MMS Lima (2014). Offactory impairment in the rotenone model of Parkinson’s disease is associated with bulbar dopaminergic D2 activity after REM sleep deprivation. Frontiers in Cellular Neuroscience 8:1-11.

43. Rouach, C, M Jiang, and R Gal (2010). Receptor monitoring and Parkinson’s disease. J Clin Exp Neuropsychol 28:618–630.

44. Souza, I, SS Smaili, R Ureshino, R Sinagaila-Coimbra, ML Andersen, GS Lopes, et al (2012). Effect of chronic sleep restriction and aging on calcium signaling and apoptosis in the hippocampus of young and aged animals. Progress in Neuro-Psychopharmacology & Biological Psychiatry 39:23–30.

45. Sugiyama, A, A Saitoh, M Inagaki, O Jun-Ichiro, M Yamada (2015). Systemic administration of riluzole enhances recognition memory and facilitates extinction of fear memory in rats. Neuropharmacology 97:322-328.

46. Schmaed, LC, CC Stowers, AC Seallet, L Xu (2005). Fluoro-Jade C results in ultra high resolution and contrast labeling of degenerating neurons. Brain Research 1: 24-34.

47. Tanga, ADS, LS Rodrigues, ACD Noseda, M Aurich, ML Andersen, S Tufik, et al (2016) Unraveling a new circuitry for sleep regulation in Parkinson’s disease. Neuropharmacology 108:161-171.

48. Targa, ADS, ACD Noseda, LS Rodrigues, M Aurich, Lima MMS (2018). REM sleep deprivation and dopaminergic D2 receptors modulation increase recognition memory in an animal model of Parkinson’s disease Behav Brain Res 339:239-248.

49. Testa, CM, TB Sherer and JT Greenamyre (2005). Rotenone induces oxidative stress and dopaminergic neuron damage in organotypic substantia nigra cultures. Molecular Brain Research 134:109-118.

50. Tufik S, CJ Lindsey and EA Carlini (1978). Does REM sleep deprivation induce a supersensitivity of dopaminergic receptors in the rat brain? Pharmacology 16:98-105.

51. Van Der Berg, RM Vanwersch, AB Smit, HCHM Philippens (2012). Neuroprotective effects of riluzole in early phase Parkinson’s disease on clinically relevant parameters in the marmoset MPTP model. Neuropharmacology 62:1700-1707.

52. Wu, YN and SW Johnson (2007). Rotenone potentiates NMDA currents in substantia nigra dopamine neurons. Neuroscience Letters 1: 24-34.

53. Wu, YN and SW Johnson (2011). Dopamine oxidation facilitates dopamine in substantia nigra zona compacta and ventral tegmental area dopaminergic neurons. Neuropharmacology 58:1-11.

54. Verhave, PS, MJ Jongsma, RM Van Der Berg, RAP Vanwersch, AB Smit, HCHM Philippens (2012). Neuroprotective effects of riluzole in early phase Parkinson’s disease on clinically relevant parameters in the marmoset MPTP model. Neuropharmacology 62:1700-1707.

55. Wu, YN and SW Johnson (2011). Dopamine oxidation facilitates rotenone-dependent potentiation of N-methyl-D-aspartate currents in rat substantia nigra dopamine neurons. Neuroscience 195:138-144.

56. Zhang, ZN, JS Zhang, J Xiang, ZH Yu, W Zhang, M Cai, et al (2017). Subcutaneous rotenone rat model of Parkinson’s disease: Dose exploration study. Brain Research 1655:104-113.

57. Zhang, LY, WY Liu, WY Kang, Q Yang, XY Wang, JQ Ding, et al (2016). Association of rapid eye movement sleep behavioral disorder with sleep-disordered breathing in Parkinson’s disease. Sleep Med 20:110-115.