Research report

Repeated treatment with a low dose of reserpine as a progressive model of Parkinson’s disease

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ABSTRACT

Animal models are widely used to study alterations caused by Parkinson’s disease (PD). However, in general, pharmacological models do not express the progressive nature of the disease, being characterized by immediate severe motor impairment after a single dose of the drug. Reserpine administration in rodents has been suggested as a pharmacological model of PD based on the effects of this monoamine-depleting agent on motor activity. Here, we describe that repeated administration with a low dose (0.1 mg/kg) of reserpine in rats induces a gradual appearance of motor signs, evaluated by catalepsy behavior. Furthermore, these motor signs are accompanied by increased levels of striatal lipid peroxidation. However, treatment with reserpine failed to induce memory impairments (evaluated by novel object recognition and discriminative avoidance tasks) and alterations in hippocampal lipid peroxidation. Thus, repeated treatment with low doses of reserpine progressively induces alterations in motor function and an increase in striatal oxidative stress, indicating a possible application of this model in the study of the neuroprogressive nature of the motor signs in PD.

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1. Introduction

Parkinson’s disease (PD) is a progressive neurodegenerative disorder, characterized by bradykinesia, tremor, rigidity and postural abnormalities [1]. However, cognitive impairments can also be observed in PD patients [2–4]. The pattern of cognitive disturbances associated with PD includes learning impairments [5], deficits of executive functions such as planning or working memory [6–8], and attentional deficits [9].

Animal models have been extensively used to study neuronal and behavioral alterations caused by PD [10]. However, in general, pharmacological models do not express the progressive nature of the disease. Indeed, these models are achieved by a single administration of the drug, which causes immediate severe motor impairment [11–13].

The administration of reserpine to rodents has been suggested as a pharmacological model of PD based on the effects of this monoamine-depleting agent on motor activity. Reserpine interferes with the storage of monoamines in intracellular vesicles, causing monoamine depletion in nerve terminals and transient hypolocomotion and muscular rigidity, depending on the dose [14,15]. The dose range that usually induces such motor alterations in rodents is 1–5 mg/kg [16–20]. The severe motor impairment after reserpine administration prevents other kinds of behavioral evaluations, such as memory tests and other cognitive/ emotional assessments. However, previous results from our group [21,22] have shown that a single administration of reserpine in low doses (0.1–0.5 mg/kg) can induce deficits in emotional memory without causing motor alterations. These findings corroborate studies with PD patients showing deficits in emotional processing previously to the appearance of motor deficits [23–25]. Therefore, the previous studies suggest that depending on the dose, reserpine is able to induce changes in rodents similar to the cognitive or motor symptoms found in humans with PD.

Reserpine is an irreversible inhibitor of the vesicular monoamine transporter 2 (VMAT-2). The blockade of dopamine vesicular uptake results in the accumulation of neurotoxic dopamine oxidation byproducts [26]. Dopamine (DA) reacts with molecular oxygen to form dopamine-quinones which can deplete the antioxidant glutathione, generating reactive oxygen species...
(ROS) during this process [27]. In addition, enzymatic metabolic breakdown of dopamine (via monoamine oxidase) increases the formation of ROS [28]. When the production of ROS exceeds the ability of the antioxidant system to eliminate them, oxidative damage occurs [29].

Neuronal damages caused by oxidative stress can induce alterations in both motor [18,30] and cognitive skills [31]. Therefore, several studies have been performed to investigate the role of oxidative injury in neurodegenerative diseases, including PD [32,33]. Evidences of damage induced by oxidative stress are found in both brain tissue from PD patients [34] and in pharmacological models such as the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [35], 6-hydroxydopamine (6-OHDA) [36] and reserpine [37,38] models.

The present study evaluated the repeated administration of reserpine as a possible pharmacological model with progressive features, similar to those in patients with PD. We submitted Wistar rats to a repeated treatment with a sub-effective dose of reser- pine and evaluated motor behavior (catalepsy, motor activity and oral movements) and memory performance (novel object recognition and plus-maze discriminative avoidance tasks). Furthermore, we assessed oxidative stress in the striatum and hippocampus by measuring lipid peroxidation.

2. Materials and methods

2.1. Animals

Five-month-old male Wistar rats were used. All animals were housed in groups of 4–5 per cage (30 cm × 37 cm × 16 cm) in a room with isolation and air flow as well as controlled temperature (25 ± 1 °C), humidity and luminosity (12 h light: 12 h dark, light on 6:30 a.m.), Food and water were available ad libitum. The rats were handled accordingly to Brazilian law for the use of animals in scientific research (Law Number 11.794) and all procedures were approved by the local ethics committee. All efforts were made to minimize animal pain, suffering or discomfort as well as the number of rats used.

2.2. Drug treatment, general procedures and experimental design

Reserpine (Sigma Chemical Co. St. Louis, MO) was dissolved in glacial acetic acid and diluted to the correct concentration in distilled water. Vehicle consisted of the same amount of acetic acid and water as in the reserpine solution. These solutions were injected subcutaneously (s.c.).

Before the beginning of the experimental procedures, the animals were gently handled for 10 min in a daily schedule for 5 days. Afterwards, the rats received subcutaneous injections of vehicle (VEH) or 0.1 mg/kg of reserpine (RES), at a volume of 1 ml/kg body weight, every other day. During treatment, rats were submitted to the following procedures (from 8:00 to 10:00 a.m.): (1) catalepsy test every day throughout the treatment, i.e., 24 and 48 h after each injection (VEH: n = 13 and RES: n = 12); (2) evaluation of open field behavior 24 h after the 4th injection (n = 17 per group); (3) assessment of oral movements before starting the treatment, 24 h after the 5th and the 10th injections and 48 h after the 10th injection (n = 8 per group); (4) training and test sessions of novel object recognition task, 24h and 48h after the 5th injection, respectively (n = 7 per group); (5) training and test sessions of plus-maze discriminative avoidance task, 24h and 48h after the 7th injection, respectively (n = 23 per group); (6) weight measurement was performed on days 2, 6, 10, 14, and 20 before injections (n = 13 per group), (7) quantification of striatal and hippocampal lipid peroxidation 48h after the 7th (n = 10 per group) and the 10th (n = 12 per group) injections. Experimental design is shown in Fig. 1.

The behavioral quantification of the catalepsy test and oral movements was performed by direct observation with the use of stopwatches and counters. All other behavioral sessions were recorded by a camera placed above the apparatus and the behavioral parameters were registered by the animal video-tracking software Anymaze (Stoelting, USA). During behavioral sessions, all apparatuses were washed with a water/alcohol (5%) solution before behavioral testing to eliminate possible bias due to odors left by previous subjects.

2.3. Behavioral testing

2.3.1. Catalepsy test

The catalepsy behavior was assessed by placing the animal’s forepaws on a horizontal platform positioned at 90 cm above the floor. The latency to start catalepsy, which was defined as an immobile posture, keeping both forepaws on the bar, was measured up to a maximum of 180 s. Three trials were carried out for each animal in each observation day and the results were analyzed considering the mean value of the three trials.

2.3.2. Open field

The apparatus was a circular open field arena (84 cm in diameter) with 32 cm high walls, made of wood and painted in black. We quantified the distance traveled (in meters), the frequency of rearing (partial or total rising onto hind limbs), immobility duration (time of complete absence of paw movements), the latency to start movement from the initial position, and the time in center (time spent in the center of the open field).

2.3.3. Oral movements

Rats were placed individually in wired cages (40 cm × 40.5 cm × 20 cm) with mirrors positioned under the floor and behind the back wall of the cage to allow behavioral quantification when the animal faced away from the observer. The number of tongue protrusions (projection of the tongue out of the oral cavity), vacuous chewing movement frequency (mouth openings in the vertical plane not directed toward physical material), and duration of twitching of the facial muscle were measured continuously for 15 min.

2.3.4. Novel object recognition task

The task was carried out in a circular open field arena (84 cm in diameter) with 32 cm high walls, made of wood and painted in black. The objects used were a sugar bowl and a plastic stem glass, which were alternately assigned to the familiar or new condition to avoid the effect of a possible preference. In the training session, rats were exposed to two copies of an object in the open field arena for 10 min. The same procedure was carried out 24 h later (test session), except that one of the objects was replaced for a new one. The time rats spent exploring each object was measured in both sessions. Exploration behavior included touching with forepaws or nose, sniffing and biting the objects. The percent time exploring each object (time exploring old or new object/time exploring both objects) was calculated.

2.3.5. Plus-maze discriminative avoidance task

The apparatus employed was a modified elevated plus-maze, made of wood, containing two enclosed arms (50 cm × 15 cm × 40 cm) opposite to two open arms (50 cm × 15 cm). In the training session, each rat was placed in the center of the apparatus and, over a period of 10 min, every time the animal entered the aversive enclosed arm, the rat encountered an aversive situation that lasted until the animal left the arm. The aversive stimuli were the 100 W light and an 80 dB noise applied through a speaker placed over the aversive enclosed arm. In the test session held 24 h later, the rats were again placed in the apparatus for 10 min, without receiving the aversive stimulation. The lamp and the speaker were still present over the aversive arm, but turned off. Distance traveled in the apparatus (used for motor activity evaluation) and time spent in each arm (aversive, non-aversive and open arms) were registered. Percent time in aversive arm (time spent in aversive enclosed arm/time spent in both enclosed arms) and percent time spent in open arms (time spent in open arms/time spent in both open and enclosed arms) considering the whole duration of behavioral sessions were used to evaluate memory and anxiety, respectively [39]. Percent time spent in the aversive enclosed arm assessed minute by minute.
across the training and test sessions were used to evaluate learning and extinction of the task [40].

2.4. Tissue preparation and oxidative stress parameters

Rats were euthanized by decapitation 48 h after the 7th or the 10th injections. The brains were quickly removed, and the striatum and hippocampus were dissected. Tissue samples were homogenized in ice-cold 0.1 M phosphate buffer (1:50, w/v). A duplicate of each sample was used to determine malondialdehyde (MDA) by measurement of a fluorescent product formed from the reaction of this aldehyde with thiobarbituric acid, as described by Tanizawa et al. [41]. The results are expressed as nmol MDA/g tissue, calculated by plotting the obtained fluorescence (Fluorescence Spectrophotometer F-2000, Hitachi, Japan) against an MDA concentration standard curve.

2.5. Statistical analysis

Catalepsy behavior, oral movements, and body weight were compared across the treatment using ANOVA with repeated measures. This repeated measures procedure were also applied to analyze the percentage of time in aversive enclosed arm measured minute by minute across the behavioral sessions of the discriminative avoidance task. When necessary, pairwise comparisons were held with multiple t tests. The independent samples t test was used to analyze differences between groups RES and VEH in all parameters of the open field behavior and the discriminative avoidance task (when considering the total duration of the behavioral sessions). In the novel object recognition task, within-subject comparisons for percentage of time to explore old × new objects were conducted with paired-samples t tests. Results are expressed as mean ± S.E.M. and p < 0.05 was considered to reflect significant differences, except for multiple comparisons by paired-samples t tests, when sequential Bonferroni corrections were applied.

3. Results

3.1. Effects of repeated administration of reserpine on catalepsy behavior

ANOVA with repeated measures revealed time (days of treatment) [F(21, 483) = 18.16, p < 0.001], treatment (reserpine or vehicle) [F(1, 23) = 12.19, p = 0.002] and time × treatment interaction effects [F(21, 483) = 9.29, p < 0.001]. Rats repeatedly treated with reserpine showed progressive increases in the catalepsy behavior, which were significantly different from VEH on days 16 (48 h after the 7th injection) [t(23) = 3.43, p = 0.002], and from day 18 onwards: 48 h after the 8th injection [t(23) = 4.42, p < 0.001], 24 h after the 9th injection [t(23) = 5.52, p < 0.001], 48 h after the 9th injection [t(23) = 4.42, p < 0.001], 24 h after the 10th injection [t(23) = 7.40, p < 0.001] (Fig. 2).

Fig. 2. Effects of repeated administration of reserpine (RES – 0.1 mg/kg, n = 13) or vehicle (VEH, n = 12) on catalepsy behavior. Data are expressed as mean ± S.E.M. ANOVA with repeated measures revealed time, treatment and time × treatment interaction effects. * p < 0.05 compared to VEH group (independent samples t test).

Fig. 3. Effects of repeated administration of reserpine (RES – 0.1 mg/kg, n = 17) or vehicle (VEH, n = 17) on motor behavior in an open field evaluated 24 h after the 4th injection. Data are expressed as the mean ± S.E.M. of distance traveled (A), total rearing counts (B), immobility duration (C), latency to initiate the movement (D) and center zone time of the open field (E) (independent samples t test).
3.2. Effects of repeated administration of reserpine on motor activity in an open field

No effects of repeated administration of reserpine were found on motor behavior in the open field. The distance traveled \( [t(32) = 0.73, p = 0.46] \), total rearing counts \( [t(32) = 0.91, p = 0.36] \), the immobility duration \( [t(32) = 0.23, p = 0.81] \), the latency to initiate the movement \( [t(32) = 0.68, p = 0.49] \) and the time spent in the center of the open field \( [t(32) = 0.53, p = 0.59] \) of the reserpine group were not different from those presented by the vehicle group (Fig. 3A, B, C, D and E, respectively).

3.3. Effects of repeated administration of reserpine on oral movements

ANOVA with repeated measures revealed time (days of treatment) \( [F(3,42) = 16.34, p < 0.001] \), treatment (reserpine or vehicle) \( [F(1,14) = 7.65, p = 0.015] \) and time × treatment interaction effects \( [F(3,42) = 7.58, p = 0.001] \) for the number of vacuous chewing movements. Significant increases due to reserpine treatment compared to VEH were detected on days 21 (24h after the 10th injection) \( [t(14) = 3.36, p = 0.005] \) and 22 (48h after the 10th injection) \( [t(14) = 2.89, p = 0.012] \) (Fig. 4A).

Regarding duration of facial twitching, ANOVA with repeated measures revealed a treatment (reserpine or vehicle) effect \( [F(1,14) = 7.52, p = 0.016] \). No effect of time (days or treatment) \( [F(3,42) = 0.83, p = 0.47] \) or time × treatment interaction \( [F(3,42) = 1.75, p = 0.17] \) were found. Significant increases due to reserpine treatment compared to VEH were detected on day 21 (24h after the 10th injection) \( [t(14) = 3.72, p = 0.002] \) (Fig. 4B).

For the number of tongue protrusions, ANOVA with repeated measures revealed a treatment (reserpine or vehicle) effect \( [F(1,14) = 6.44, p = 0.024] \). No effect of time (days of treatment) \( [F(3,42) = 2.57, p = 0.09] \) or time × treatment interaction effects were found. Significant increases due to reserpine treatment compared to VEH were detected on day 22 (48h after the 10th injection) \( [t(14) = 2.91, p = 0.01] \) (Fig. 4C).

3.4. Effects of repeated administration of reserpine on novel object recognition task

Both groups showed an increased percentage of novel object exploration compared to the old object \( [VEH: t(6) = 3.73, p = 0.01 \) and RES: \( t(6) = 3.36, p = 0.01 \), respectively], indicating adequate performance in the task independently of the drug treatment (Fig. 5).

![Fig. 4. Effects of repeated administration of reserpine (RES = 0.1 mg/kg, n = 8) or vehicle (VEH, n = 8) on orofacial movements. Data are expressed as the mean ± S.E.M. of the frequency of vacuous chewing movements (A), the duration of facial twitching (B) and the number of tongue protrusions (C). ANOVA with repeated measures revealed treatment effects for all parameters, as well as time and time × treatment interaction effects for vacuous chewing. *p < 0.05 compared to VEH group (independent samples t test with Bonferroni’s correction).](image)

![Fig. 5. Effects of repeated administration of reserpine (RES = 0.1 mg/kg, n = 7) or vehicle (VEH, n = 7) on novel object recognition task performed 48 h after the 5th injection. Data are expressed as the mean ± S.E.M. *p < 0.05 compared to percent of old object exploration.](image)
3.5. Effects of repeated administration of reserpine on plus-maze discriminative avoidance task

Repeated treatment with reserpine decreased the distance traveled in the maze 24 h after the 7th injection [t(44) = 3.31, p = 0.002 in the training session, Fig. 6A], and 48 h after the 7th injection [t(44) = 2.39, p = 0.02 in the test session, Fig. 6C], when compared to the vehicle group. However, no effects of repeated administration of reserpine were found on the latency to initiate the movement in the training or test sessions (Fig. 6B and D, respectively).

Regarding anxiety-like behavior, the percentage of time in the open arms (%TO) in the training [t(44) = 0.43, p = 0.66] and in the test [t(44) = 1.07, p = 0.28] sessions presented by the reserpine group were not different from those presented by the vehicle group (Fig. 7A and B, respectively).

No effects of repeated administration of reserpine were found on the percent time in the aversive enclosed arm (%TAV), in the training [t(44) = 1.76, p = 0.08] and test [t(44) = 0.62, p = 0.53] sessions (Fig. 8A and B, respectively) when the whole sessions were considered for analysis.

In the training session, a significant effect of time (minutes) [F(9,396) = 8.18, p < 0.001] was found when the percentage of time in the aversive enclosed arm (%TAV) was evaluated across the session. No effect of the treatment (reserpine or vehicle) [F(1,44) = 1.24, p = 0.27] and the time x treatment interaction [F(9,396) = 1.02, p = 0.40] were found (Fig. 8D).

3.6. Effects of repeated administration of reserpine in the body weight

Regarding the body weight, ANOVA with repeated measures revealed no effect of time (number of injections) [F(4,96) = 0.72, p = 0.53], treatment (reserpine or vehicle) [F(1,24) = 0.82, p = 0.37] or time x treatment interaction [F(4,96) = 0.66, p = 0.57] (Table 1).

3.7. Effects of repeated administration of reserpine on striatal and hippocampal lipid peroxidation

Fig. 9A and B shows striatal and hippocampal levels of lipid peroxidation, respectively, 48 h after 7th and 10th injection of rats repeatedly treated with reserpine. No effects of repeated administration of reserpine were found after the 7th injection for the striatum [t(18) = 1.34, p = 0.19] (Fig. 9A) or the hippocampus [t(18) = 1.14, p = 0.26], and after the 10th injection for the hippocampus [t(22) = 2.08, p = 0.05] (Fig. 9B). However, reserpine-treated rats showed increased levels of lipid peroxidation in the striatum 48 h after the 10th injection [t(22) = 3.00, p = 0.01] (Fig. 9A).

4. Discussion

In this study, we investigated the effects of repeated administration with a low dose of reserpine on motor and cognitive parameters. We observed that this treatment induced a progressive motor impairment. In fact, these results can be seen in the evaluation of catalepsy behavior performed before, 24 and 48 h after each injection (Fig. 2). In addition, the motor parameters evaluated in the open field were not altered in the RES group 24 h after 4th injection (Fig. 3) but hypolocomotion was detected in the distance traveled in the discriminative avoidance apparatus 24 and 48 h after the 7th injection (Fig. 6A and C). However, none of the memory tests performed were affected by the treatment with reserpine (Figs. 5 and 8). The present study demonstrates that repeated reserpine treatment can induce motor abnormalities and concomitant
increases in striatal levels of lipid peroxidation, an indicative of oxidative stress-induced neuronal damage (Fig. 9).

PD is a neurodegenerative disorder of the basal ganglia characterized by a complex condition of behavioral disorders, including tremor, rigidity and bradykinesia [42–44]. These motor symptoms have been highlighted as those that characterize the clinical status of an affected person, so they are considered the most important signs associated with PD [1,45]. In rodents, dopamine hypofunction leads to Parkinsonian signs such as akinesia and rigidity (catalepsy). The evaluation of catalepsy has been used as an important parameter for the detection of motor impairment in animal models of PD [46,47]. These alterations can be induced not only by drugs that block dopamine receptors such as haloperidol [17,48] but also by substances that are potential inhibitors of mitochondrial complex I as MPTP [17] and rotenone [49], or the neurotoxin 6-OHDA [47]. Additionally, these effects are also present after monoamine vesicle depletion induced by reserpine [17]. Reserpine interferes with the storage of catecholamines by blocking the presynaptic vesicular carriers, resulting in depletion of monoamines in nerve terminals [26] and induction of hypolocomotion and muscular rigidity [14,16]. This study revealed that rats exposed to repeated administration of reserpine at 0.1 mg/kg showed a gradual increase of cataleptic immobility when compared to the control group (Fig. 2). Previous studies have demonstrated that a short-term treatment with high doses of reserpine (1.0 mg/kg every other day for 4 days) [16] or an acute injection of an even higher dose (5 mg/kg) [17] produce catalepsy and hypolocomotion. However, in the current study, it is unlikely that the increase in catalepsy behavior is due to the acute effect of reserpine since it is still present even 48 h after the last injection of this drug from the 7th injection onwards. Thus, a progressive neuronal effect of the repeated treatment leading to the motor impairment could be hypothesized. As discussed below, this progressive neuronal effect could be related to oxidative damage. Alternatively, the possibility that context-dependent sensitization

<table>
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<th>10th</th>
<th>14th</th>
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ANOVA did not reveal significant effects of the treatment.

![Fig. 8](image8.png)

**Fig. 8.** Effects of repeated administration of reserpine (RES – 0.1 mg/kg, n = 23) or vehicle (VEH, n = 23) on the percent time in the aversive enclosed arm (% TAV) of the plus-maze discriminative avoidance apparatus during the whole sessions (A and B) or minute by minute throughout the sessions (C and D), for training (A and C) and test (B and D), performed 24 and 48 h after the 7th injection, respectively. Data are expressed as the mean ± S.E.M. ANOVA with repeated measures revealed time (minutes) effects in C and D.

![Fig. 9](image9.png)

**Fig. 9.** Effects of repeated administration of reserpine (RES – 0.1 mg/kg) or vehicle (VEH) on striatal (A) and hippocampal (B) levels of lipid peroxidation 48 h after 7th (n = 10 per group) and 10th (n = 12 per group) injection. Data are expressed as the mean ± S.E.M. of MDA levels per gram of tissue. *p < 0.05 compared to VEH group (independent samples t test).
is related to the progressive behavioral effects observed cannot be ruled out. Indeed, studies have shown that context-dependent learning plays a role in sensitization of catalepsy behavior induced by dopamine blockers [46,50,51].

Data from catalepsy evaluation are corroborated by the fact that the repeated treatment with a low dose of reserpine was not able to impair motor parameters evaluated after the 4th injection in the open field (distance traveled, rearing frequency, immobility duration and latency to start movement) (Fig. 3). Previous research has demonstrated that acute administration of higher doses of reserpine induced locomotor alterations [18–20]. In this respect, hypokinesia is an important feature of animal models of PD and is often related to a significant loss of dopaminergic neurons [12,52–54]. The present results suggest that there was no acute effect of the dose used on motor behavior, and the continuation of the repeated treatment was necessary to produce motor abnormalities that were observed in the catalepsy test only after the 7th injection (Fig. 2). In this respect, another motor parameter used in this study was the distance traveled and the latency to start the movement in the elevated plus-maze discriminative avoidance task, which was performed 24 and 48 h after the 7th injection (Fig. 6). Corroborating the data from the catalepsy evaluation, animals treated with reserpine showed a significant decrease in distance traveled in the maze in both sessions. Previous studies have suggested reserpine-induced oral dyskinesia in as an animal model of tardive dyskinesia [55–58]. Tardive dyskinesia is a side effect of long-term treatment with typical antipsychotics characterized by severe motor symptoms affecting the face, mouth and tongue (oral dyskinesia) [59,60]. Conversely, some authors advocate the induction of oral movements as a model of the tremor-related symptoms found in patients with PD [61,62]. These movement alterations in rodents can be induced by a series of conditions related to the neurochemical and pathophysiology of parkinsonism such as depletion of dopamine levels caused by reserpine [61,62], dopamine antagonists such as haloperidol [63] and neurotoxins such as 6-OHDA [64]. Here, we found that the treatment with repeated administration with a low dose of reserpine was able to induce an increase in oral movements 24 h after the 10th injection (Fig. 4). It should be noted that neither oral movements nor catalepsy behavior were altered 24 h after the 5th injection (Figs. 2 and 4). However, 24 h after the 10th injection, concomitant motor alterations were observed in the catalepsy test and the oral movements evaluation. Indeed, previous research has shown that acute reserpine administration (at higher doses) induced decreased locomotion and increased duration of immobility concomitantly to increased oral movements [18,19]. In addition, there are descriptions of cases of PD patients who have concomitant usual motor symptoms (bradykinesia, disorders in walking, among others) and impaired oromotor control [65]. However, Sussman et al. [66] showed that reserpine-induced oral movements persisted despite repletion of dopamine in the caudate-putamen, suggesting that the persistent neuropathological change underlying this behavior occurs in a neural pathway other than the dopaminergic nigrostriatal pathway. Thus, the pathophysiological characteristics of oral movements are still controversial. Notwithstanding, the data presented here indicate a progressive increase in oral movements simultaneously to catalepsy behavior. Additionally, this result is corroborated by recent data from our laboratory showing the same pattern of concomitant appearance of both kinds of symptoms across a repeated treatment with 6-OHDA (unpublished results).

As mentioned before, besides motor symptoms, PD patients also display other symptoms such as cognitive, mood and sensory system alterations [67–70]. We have recently verified that single administrations of reserpine – at doses that do not modify motor function – impair memory in the discriminative avoidance task (a rodent model of aversive discrimination – [21]), while no effects of the same acute doses were detected in the novel object recognition task [22]. In the present study, we investigated the effects of a repeated treatment with 0.1 mg/kg reserpine on the performance of rats in these two tasks. Due to evidence that cognitive deficits can precede the appearance of the motor symptoms in the progress of the disease, we attempt to evaluate cognitive deficits before an expressive motor impairment was instated.

The novel object recognition task (performed after the 5th injection) is based on the fact that rats recognize a previously presented object, and therefore would spend more time exploring the new object presented in the test session. The preference for exploring new objects was shown by both groups (Fig. 5), indicating that the repeated administration of reserpine did not affect this kind of memory. Similar results were found with acute administration of reserpine (0.1, 0.25 or 0.5 mg/kg – [22]). In this respect, the lack of alteration in this task is in accordance with some clinical studies showing intact recognition memory in PD patients [71,72].

Besides evaluating memory, we used the plus-maze discriminative avoidance task (performed after the 7th injection) to assess learning, anxiety and motor behavior, since it has been shown that these evaluations can be performed concomitantly by different parameters in this paradigm [38,73]. The results have shown that there were no significant differences in the percentage of time in the aversive arm between the VEH and RES groups, both in the training and test sessions, indicating that repeated treatment with reserpine was not able to promote changes in learning or retrieval of the aversive task (Fig. 8). Moreover, by evaluating the distance traveled in the maze, we observed that animals treated with reserpine showed motor deficits during the acquisition and retrieval of the task (Fig. 6), corroborating the increase in catalepsy duration also shown at this point of the treatment (Fig. 2). This motor activity decrement, however, did not interfere with the analysis of the data related to the cognitive aspect of the task, since rats’ performances were evaluated by the time spent in the aversive enclosed arm. Indeed, previous studies conducted with this paradigm have shown the viability of separate and reliable analysis and interpretation of the two parameters [21,39,73–76]. Furthermore, the evaluation of behavior minute by minute throughout the training session indicated that even in the presence of motor deficits, animals treated with reserpine learned the task, as shown by a decrement of aversive arm exploration by the end of the session (Fig. 8C). Similarly, the analysis of aversive arm exploration throughout the test session indicated that animals treated with reserpine or vehicle showed retrieval of the task (low aversive arm exploration in the first minutes) followed by extinction of the task (increase in exploration towards the end of the session) (Fig. 8D).

In summary, the repeated administration with a low dose of reserpine did not produce changes in the memory task involving an emotional context, as opposed to what has been previously observed after single injections in this same paradigm [21,73] or in the contextual fear conditioning task [22]. In this respect, research has shown that excessive or insufficient levels of dopamine may have a negative effect on emotional memory [6,77]. Thus, the previous studies performed with acute treatments (with doses from 0.1 to 1 mg/kg) could reflect the effects of acute dopamine depletion on emotional memory. Although the dose used in this study is within this range (0.1 mg/kg), it was given several times, and it is possible that the decrease in the levels of dopamine depletion was outweighed by up-regulation of D1 and D2 receptors in the caudate-putamen [54] or by other compensatory mechanisms of plasticity [78–81]. Also, the repeated treatment, which was efficient in inducing progressive motor impairment, did not induce cognitive impairments in any of the paradigms used here. Thus, the data suggest dissociation between the cognitive deficits induced by reserpine treatment (observed in previous studies) and a
possible degenerative process induced by the repeated treatment. It is important to note, however, that cognitive impairments can be observed in rats injured with MPTP [12] and PD patients [23,77]. Thus, it would be interesting to verify the effects of the repeated treatment used here in other animal models of memory, or even in other aspects of cognitive function.

Another parameter evaluated in the plus-maze discriminative avoidance task was the time of exploration of the open arms of the maze, indicative of an anxiety-like behavior [39]. Results showed that treatment with reserpine did not induce alterations in anxiety-like behavior (Fig. 7), corroborating the previous studies that investigated the effects of reserpine in this task [21,73].

Increased oxidative stress with cumulative free radical damage is present in brain aging and neurodegenerative diseases such as PD [32,33]. In this respect, treatment with reserpine can result in the accumulation of neurotoxic dopamine oxidants that can induce the production of ROS exceeding the ability of the antioxidant system to eliminate them, thus resulting in oxidative damage [26,33]. Recent studies showed that acute administration of high doses of reserpine increases lipid peroxidation on striatum and antioxidant agents are able to reverse the behavioral effects induced by reserpine [18,55,56]. Herein, the repeated treatment with a low dose of reserpine induced an increased striatal level of lipid peroxidation 48 h after the 10th injection (Fig. 9A), when an important motor impairment was also present (Fig. 2). On the other hand, hippocampal levels of lipid peroxidation were not modified by the treatment. Interestingly, the absence of memory impairment may be related to lack of neuronal damage caused by oxidative stress in the hippocampus (Fig. 9B). These findings suggest that the treatment used here may induce a progressive neuronal damage similar to what is found in patients with PD, at least considering motor aspects of the pathology.

Although a quantitative assessment was not conducted, we did not observe significant peripheral autonomic changes in reserpine-treated animals throughout the treatment. Additionally, no change was found in body weight of rats during the repeated treatment with reserpine (Table 1), and all animals survived to the treatment. In this respect, Ferro et al. [53] found a significant change in weight in pharmacological models of MPTP and 6-OHDA when compared to control groups, with death of 20% of treated animals. In light of these findings, we suggest that reserpine may be a more favorable drug to the development of a pharmacological progressive model, which requires repeated treatment over time, compared to MPTP or 6-OHDA models. On the other hand, it is important to mention that reserpine, as a pharmacological model of PD, is considered to be unspecified, because this drug acts on the depletion of all monoamines. However, there is evidence in the literature that the physiopathology of PD itself is not exclusively related to dopamine, since other neurotransmitter systems have shown to be involved in the PD symptoms, such as the serotonergic and the GABAergic systems, among others [82,83].

Reserpine is an irreversible inhibitor of the vesicular monoamine transporter (VMAT). As mentioned above, the action of reserpine prevents the storage of monoamines in synaptic vesicles [26]. Interestingly, animals that express only 5% of VMAT have been suggested as a promising model for the study of PD. The VMAT deficient animals have increased oxidative stress, progressive loss of dopamine terminals and accumulation of α-synuclein [84]. In addition, in these animals, levels of dopamine, norepinephrine and serotonin are severely diminished [84,85]. As can be seen, most of the alterations present in the VMAT2-deficient animals are similar to those found in the animals treated with reserpine. In addition, studies using post mortem Western blot analysis showed reduced VMAT2 immunoreactivity in the putamen, caudate and nucleus accumbens of PD patients compared to control cases [86]. These findings show that alterations in VMAT can be one of the factors related to the development of PD, which would favor the use of reserpine treatment to induce PD animal models.

In conclusion, we found that repeated administration with a low dose of reserpine in rats induces a gradual appearance of motor signs. These motor alterations are accompanied by increased levels of oxidative stress in the striatum, corroborating studies showing an increase in oxidative stress as a possible pathophysiological mechanism in PD [32,33]. Nevertheless, the treatment protocol applied was not able to induce cognitive deficits, at least in the behavioral models used, which was corroborated by the absence of oxidative damage in the hippocampus. More studies are required to verify the possible progressive changes of the dopaminergic and other neurotransmitter systems in the PD model proposed here. Further investigation with other behavioral tasks could also clarify if the cognitive deficits related to PD can be observed in this new progressive pharmacological model.

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