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ORIGINAL PAPER

# The role of calcium channel blockers and resveratrol in the prevention of paraquat-induced parkinsonism in *Drosophila melanogaster*: a locomotor analysis

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**Abstract** Studies have suggested that neuronal loss in Parkinson's disease (PD) could be related to the pacemaker activity of the substantia *nigra pars* compacta generated by L-type $Ca_v$ 1.3 calcium channels, which progressively substitute voltage-dependent sodium channels in this region during aging. Besides this mechanism, which leads to increases in intracellular calcium, other factors are also known to play a role in dopaminergic cell death due to overproduction of reactive oxygen species. Thus, dihydropyridines, a class of calcium channel blockers, and resveratrol, a polyphenol that presents antioxidant properties, may represent therapeutic alternatives for the prevention of PD. In the present study, we tested the effects of the dihydropyridines, isradipine, nifedipine, and nimodipine and of resveratrol upon locomotor behavior in *Drosophila melanogaster*. As previously described, paraquat induced parkinsonian-like motor deficits. Moreover, none of the drugs tested were able to prevent the motor deficits produced by paraquat. Additionally, isradipine, nifedipine, resveratrol, and ethanol (vehicle), when used in isolation, induced motor deficits in flies. This study is the first demonstration that dyhidropyridines and resveratrol are unable to reverse the locomotor impairments induced by paraquat in *Drosophila melanogaster*.

**Keywords** Dihydropyridines · Resveratrol · *Drosophila melanogaster* · Paraquat · Parkinson's disease · Voltage-dependent calcium channels

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## Introduction

Parkinson's disease (PD) is primarily characterized by motor symptoms including bradikinesia, akinesia, and tremor. PD patients present neuronal loss in different brain areas, mainly in the substantia *nigra pars* compacta (SNpc) and, to a lesser degree, in the ventral tegmental area (VTA) and retrorubral field (Halliday et al. 1996; Dauer and Przedborski 2003).

Recently, it has been suggested that the neuronal loss related to PD could be associated with SNpc neurons' pacemaker activity generated mainly by L-type voltage-dependent calcium channels (VDCC), which substitute voltage-dependent sodium channels (VDSC), in this region, during aging (Chan et al. 2007). In the VTA, a dopaminergic brain region damaged to a lesser extent in PD (Damier et al. 1999), the pacemaker activity is continuously generated by sodium channels, suggesting VDCC

activity may have a causal role in the neuronal loss seen in PD (Chan et al. 2007; Sulzer and Schmitz 2007).

Increases in intracellular calcium are known to play an important role in neuronal death, mainly by inducing dysfunctions in mitochondrial function and reactive oxygen species (ROS) production; protein synthesis, folding, and proteasomal degradation and transcriptional regulation in the nucleus (Mattson 2007; Chan et al. 2009).

High levels of VDCC $Ca_v$ 1.3 subunit mRNA are present in the dopaminergic neurons of the SNpc, suggesting that this subunit may be related to neuronal death in PD (Chan et al. 2007). Importantly, patients treated for hypertension with dihydropyridines exhibited a lower than expected incidence of PD when compared with patients not treated with calcium channel blockers (Rodnitzky 1999). Thus, it is possible that calcium channel blockers, particularly the dihydropyridines that have the capacity to cross the blood brain barrier, could be used as preventive and neuroprotective agents against PD (Farkas et al. 2001; Surmeier 2007).

Similarly, drugs that reduce ROS generation, such as resveratrol, can provide some degree of neuroprotection in PD, probably due to their ability to reduce oxidative damage to macromolecules, including proteins, nucleic acids, and polyunsaturated fatty acids, which are responsible for plasma membrane integrity (Sun et al. 2008; Marambaud et al. 2009). Factors such as gene mutations and exposure to herbicides are related to free-radical-mediated damage in the SNpc, contributing to the development of PD (Schapira 2009).

Resveratrol is a polyphenol found in red grapes, among other plants, to which the benefits from wine consumption have been attributed. Resveratrol presents antioxidant properties that are related to the activation of Sir2 enzymes, a family of protein deacetylases that are $NAD^+$-dependent, also called sirtuins, whose homolog in mammals is known as SIRT. These enzymes are important for many cellular processes including gene silencing, regulation of p53, fatty acid metabolism, cell cycle regulation and extension of longevity (Yang et al. 2007; Sun et al. 2010).

Furthermore, a recent study has shown that resveratrol promotes neuronal survival in a rat model of PD induced by 6-hydroxydopamine (6-OHDA), with enhancement in the content of dopamine and its metabolite DOPAC, tyrosine hydroxylase (TH) expression, and improvement in motor behavior (Khan et al., 2010). Thus, resveratrol could also be considered as a therapeutic candidate for PD treatment or prevention.

A number of animal models have been employed in order to better understand the mechanisms involved in PD, including primates (Decamp and Schneider 2009), rodents (Lindgren et al., 2010), and *Drosophila melanogaster* (Bayersdorfer et al. 2010). These models are mainly based on the neurotoxic induction of PD-like symptoms using different agents, such as 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP; Xu et al. 2010), 6-OHDA (Chuang et al. 2010), rotenone (Hu et al. 2010), and paraquat (Dinis-Oliveira et al. 2006; Jimenez-Del-Rio et al. 2008).

*Drosophila melanogaster* is considered one of the most suitable organisms to evaluate PD and other neurological dysfunctions for several reasons, such as (1) rapid generation time (Botella et al. 2009); (2) a well-described nervous system (Ito and Awasaki 2008); (3) similarities between the cellular and molecular mechanisms of *Drosophila melanogaster* neurons and those of vertebrates (Nichols 2006; Whitworth et al. 2006); (4) similarities, in terms of motor behavior, between the parkinsonism induced in flies and PD in humans (when flies with parkinsonism induced by neurotoxic agents, as paraquat, are compared with PD patients, for example, flies also present bradikinesia that can be easily estimated using very simple locomotor assays; Chaudhuri et al. 2007); (5) homologies in genes related to PD in humans (Whitworth et al. 2006; Botella et al. 2009); and (6) significant homologies between the *Drosophila melanogaster* and human calcium channels, for example, in *Drosophila melanogaster*, the $Ca_v$ 1 subunit, designated as Dmca1D, has a percentage identity of 66% with the $Ca_v$ 1.3 subunit of humans (King 2007).

The aim of the present study was evaluate the effects of chronic exposure to three different dihydropyridines—isradipine, nifedipine, and nimodipine—and resveratrol upon the motor behavior of *Drosophila melanogaster* with paraquat-induced parkinsonism.

## Materials and methods

### *Drosophila* strain and culture maintenance

A total of 1,440 mated wild-type *Canton-S Drosophila melanogaster* flies (from Bloomington *Drosophila* Stock Center, Indiana University, USA) were used, including 480 in the pilot study to determine the paraquat dose to be used and 960 in the behavioral screenings. They were maintained at 25°C on a 12 h light/dark cycle, under 60–70% humidity, in bottles containing standard medium prepared with 10 g agar, 10 g methylparaben (to prevent fungal growth), 100 g of rye flour, 50 g of brown sugar, 1 L of water, and dried yeast.

### Experimental groups

An initial study was performed in order to establish a sublethal dose of paraquat capable of significantly reducing the flies' locomotion to be used in the following analyzes.

Doses of 2.0, 7.5, 10, and 15 mM of paraquat (1,1′-dimethyl-4,4′-bipyridinium ion (Syngenta)) were tested in animals from the 14th–16th days of life. Control group animals received normal medium for the equivalent period. This experiment was performed using 14-day-old male flies, day one being considered the day of pupae eclosion, and the survival rate and locomotion were evaluated 48 h after.

Experimental groups for the main experiment are depicted in Table 1. Each experimental group consisted of 80 flies from both genders (40 males and 40 females).

## Experimental procedures

The dihydropyridines, isradipine (Novartis), nifedipine (Bayer), and nimodipine (Bayer), were purchased from commercial sources. Resveratrol (*trans*-3,4′,5-trihydroxystilbene, extracted from *Polygonum cuspidatum*) was purchased from Chengdu Hawk Bio-Engineering (Beijing, China) and presented >99% of purity (confirmed by high performance liquid chromatography), as previously described (Souto et al. 2001).

Treatments were performed by adding drugs (including ethanol, the vehicle of resveratrol) to the cooked medium of the corresponding group, at a final concentration of 1 mM. The choice of this dose was based on similar studies (Pendleton et al. 2002; Bass et al. 2007; Parashar and Rogina 2009).

The flies treated with paraquat received a dose of 10 mM of the herbicide during a 48-h period between the 14th and 16th days of life. The groups paraquat + dihydropyridines, paraquat + resveratrol, and paraquat + ethanol were treated with the corresponding drug or vehicle during the period of paraquat exposure (Chaudhuri et al. 2007; Table 1).

## Locomotor assay

Adult flies were separated by sex under cryoanesthesia for 5 min. Locomotor tests were conducted in the morning (between 9 and 12 p.m.), in a room with controlled temperature (25°C) and humidity (60–70%).

On the 22nd day of life, the flies were submitted to a locomotor evaluation according to Neckameyer and Matsuo (2008), with slight modifications as follows. A single fly was placed into an empty 60-mm Petri dish marked with a grid of 1-cm squares, allowed to recover for 30 s, after which the locomotor activity was observed for the first 2 min and last 2 min of a 10-min period. The locomotion observed in the first period was considered exploratory, while that in the second was considered basal locomotion, since 6–8 min was considered sufficient time for the flies to become accustomed to the environment (Neckameyer and Matsuo 2008). The number of grid lines crossed during each observation period was recorded by an observer. In this environment, the flies are unable to fly, so the number

**Table 1** Table showing the different treatments that flies were submitted to in the main experiment

| Experimental groups | Dihydropyridine treatments (1 mM—7th–22nd days) | | | Ethanol treatment (1 mM—7th–22nd days) | Resveratrol treatment (1 mM—7th–22nd days) | Paraquat treatment (10 mM—14th–16th days) |
|---|---|---|---|---|---|---|
| | Isradipine | Nifedipine | Nimodipine | | | |
| Control | | | | | | |
| Paraquat (PQ) | | | | | | X |
| Isradipine (Isr) | X | | | | | |
| Nifedipine (Nif) | | X | | | | |
| Nimodipine (Nim) | | | X | | | |
| Ethanol (vehicle of resveratrol) | | | | X | | |
| Resveratrol | | | | | X | |
| Isradipine + paraquat (Isr + PQ) | X | | | | | X |
| Nifedipine + paraquat (Nif + PQ) | | X | | | | X |
| Nimodipine + paraquat (Nim + PQ) | | | X | | | X |
| Ethanol + paraquat (PQ) | | | | X | | X |
| Resveratrol + paraquat (PQ) | | | | | X | X |

The concentrations presented (1 mM for dihydropyridines, ethanol and resveratrol, and 10 mM for paraquat) were the final concentrations of drugs in the medium. The first day of life of flies was considered the day of eclosion from pupae. Forty males and 40 females were used in each experimental group (960 flies in total)

of grid lines crossed represents a measure of locomotor activity. The chambers were wiped clean and exposed to air after use in order to minimize any pheromonal or waste products that might affect subsequently tested flies behavior.

### Statistical analysis

Statistical analyses were performed using two-way ANOVA followed by the Bonferroni post hoc test, using SPSS 11.0 software.

## Results

Results from the initial study performed to determine the paraquat dose to be used in the subsequent analyses. Fig. 1 confirmed paraquat's impact on the animals' survival and locomotion during both exploratory and basal activity periods (Girardot et al. 2004; Neckameyer and Matsuo 2008).

When given between the 14th and 16th days of life, paraquat significantly affected survival in a dose-dependent manner (Fig. 1a). While paraquat at 15 mM caused 100% lethality, 10, 7.5, and 2 mM caused 95.7, 70, and 19.6%, respectively. In the locomotor assay, the performance of flies from these three groups (2, 7.5, and 10 mM) revealed a significant reduction in the basal locomotor activity when compared with the control group (Fig. 1c). However, only flies exposed to 10 mM of paraquat presented a significant decrease in exploratory locomotion (Fig. 1b). Thus, the final concentration of paraquat selected in our study was 10 mM, because it was the most toxic sublethal dose, capable of inducing deficits in basal and exploratory locomotion in our flies.

Interestingly, when control males and females were compared in the locomotor assays, a significant decrease was revealed in the exploratory and basal locomotion in females ($P < 0.01$; Fig. 2a, b).

When analyzing the locomotor performance of the dihydropyridine groups composed of male flies, a decrease was observed in exploratory activity in the groups PQ ($P < 0.001$), Isr ($P < 0.01$), Isr + PQ ($P < 0.001$), Nif + PQ ($P < 0.01$), and Nim + PQ ($P < 0.01$) when compared with the control group (Fig. 3a). Observing basal locomotion in males, there was a significant decrease in the following groups: PQ ($P < 0.01$), Isr ($P < 0.001$), and Nif ($P < 0.05$; Fig. 3b). In females, the exploratory and basal locomotion were unaffected when compared with control (Fig. 3c, d).

The locomotor performance of male groups related to resveratrol, and ethanol treatments showed a significant decrease in exploratory locomotion of the groups PQ 10 mM ($P < 0.001$), resveratrol ($P < 0.05$), ethanol ($P < 0.001$), resveratrol + PQ ($P < 0.001$), and ethanol + PQ ($P < 0.001$) when compared to the control group (Fig. 3a). In relation to the basal locomotion of males, only the groups PQ 10 mM ($P < 0.01$) and ethanol + PQ ($P < 0.001$) revealed a reduced locomotor index when compared with the control group (Fig. 3b). In females, the exploratory and basal locomotion were unaffected when compared with control (Fig. 3c, d).

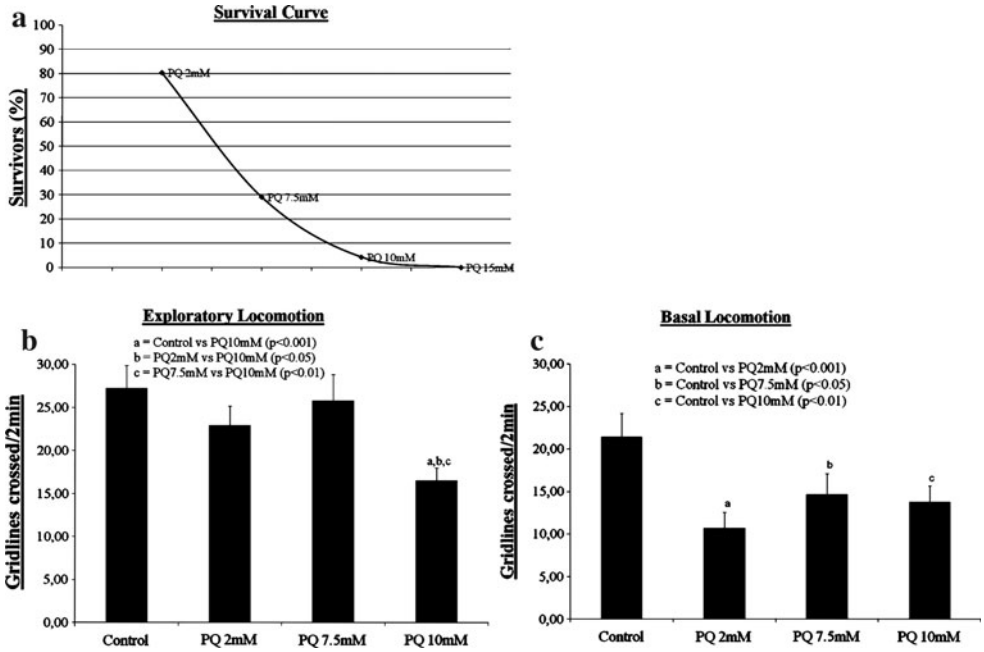


**Fig. 1** **a** Percentage of surviving flies exposed to four different concentrations of paraquat (2, 7.5, 10, and 15 mM) during 48 h. **b** Exploratory locomotion index of the dose-assessment study with control and surviving male flies exposed to three different concentrations of paraquat (2, 7.5, and 10 mM) during 48 h. **c** Basal locomotion index of the dose-assessment study with control and surviving male flies exposed to three different concentrations of paraquat (2, 7.5, and 10 mM) during 48 h (mean ± SE)

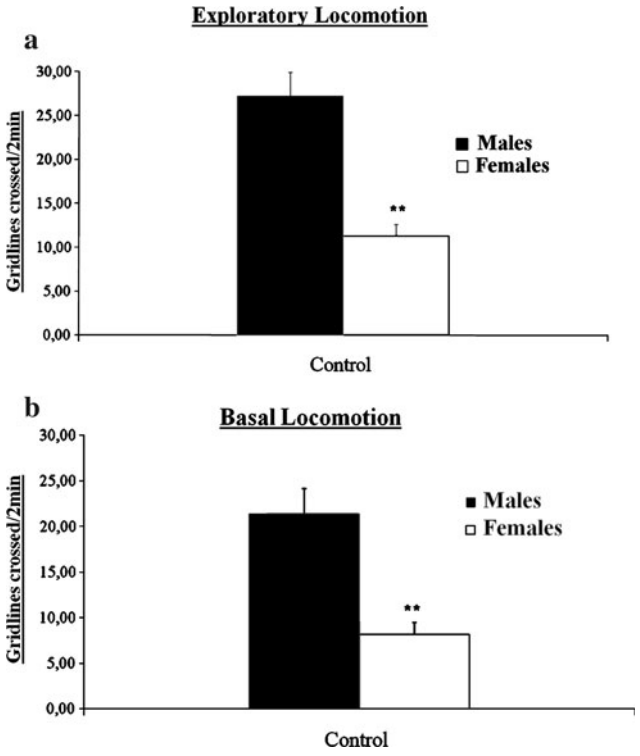


**Fig. 2** **a** Comparison of exploratory locomotion index between control males and control females. **b** Comparison of basal locomotion index between control males and control females (mean ± SE). ** $P < 0.01$

## Discussion

In our study, paraquat doses of 2, 7.5, 10, and 15 mM were found to be significantly lethal (Fig. 1a). This finding is similar to the results described by Girardot et al. (2004) and Chaudhuri et al. (2007). However, the 2 and 7.5 mM doses were insufficient to induce the impairments in exploratory and basal locomotion, which characterize parkinsonism. Therefore, our choice of the length of time of exposure (48 h) and the dose of paraquat used (10 mM) were mainly based on the induction of a significant deficit in both exploratory and basal locomotion (Fig. 1b, c), similar to that of other studies (Girardot et al. 2004; Chaudhuri et al. 2007). Moreover, Chaudhuri et al. (2007) also demonstrated a significant dopaminergic cell death in flies exposed to paraquat and concluded that temporal progression of neural degeneration coincided quite closely with the timing of symptom onset and progression of movement deficits.

In relation to the toxicity of paraquat, we found 100% lethality at the 15 mM paraquat dose, while previous studies performed using higher concentrations of paraquat, such as 20 mM, in the same *Drosophila melanogaster* strain, *Canton-S*, failed to observe such significant mortality (Jimenez-Del-Rio et al. 2008, 2010). We believe that the discrepancy between the lethal dose of paraquat found

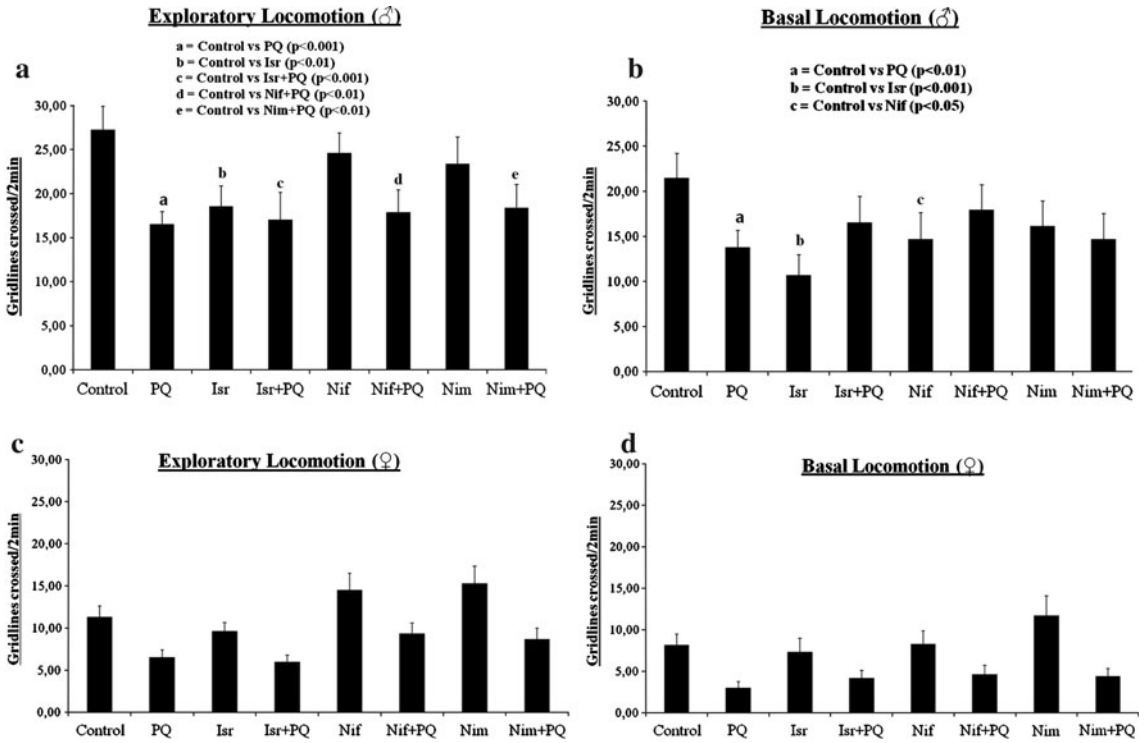


**Fig. 3** Treatment with dihydropyridines. **a** Exploratory locomotion index of experimental groups of males. **b** Basal locomotion index of experimental groups of males. **c** Exploratory locomotion index of experimental groups of females. **d** Basal locomotion index of experimental groups of females (mean ± SE)

in our study and those used in other related studies probably originates in the different administration methods used. In the other studies, paraquat was administrated using a filter paper saturated in paraquat; in our experiment, paraquat was dissolved in the medium (Jimenez-Del-Rio et al. 2008, 2010).

Our choice of drug administration was based on previous studies in which the drugs were dissolved in the medium, so increasing the exposure area of the drug and, consequently, the possibility of the flies coming into contact with the paraquat and other drugs are more homogeneous for all the animals maintained in the bottle and is not altered by the variations in motor behavior inherent to each fly (Pesah et al. 2004; Chaudhuri et al. 2007; Lee et al. 2008). In our dose-assessment study, we only used male flies. The rate of food ingestion is higher in female flies when compared with males, this is probably related to the higher metabolism of females required for ovipositing and large body mass when compared with males (Jimenez-Del-Rio et al. 2008). Thus, females are more susceptible to drugs mixed in the medium; for this reason, we chose to use males in the dose-assessment study because the first dose capable of reducing locomotion in females would probably be ineffective in males.

Analyzing different patterns of locomotion between the genders in control flies, male flies were found to have higher levels of exploratory and basal locomotion (Fig. 2a, b). This difference is well documented in previous studies that demonstrate that the average walking velocity is higher in males (Martin 2004), while females present more periods of locomotor inactivity than males (Belgacem and Martin 2002).

Analyzing the effects of dihydropyridines in the exploratory locomotion of males, we noticed that none of the drugs used (isradipine, nifedipine, and nimodipine) were able to prevent the locomotor impairment produced by paraquat exposure (Fig. 3a). Moreover, isradipine was found to reduce exploratory locomotion. This inhibitory effect of isradipine on spontaneous locomotor activity was previously described in mice with hyper-excitability induced by alcohol withdrawal (Watson and Little 2002). Our results are also in accordance with a previous study in which the rotarod test was used to demonstrate that isradipine was unable to inhibit akinesia generated by L-DOPA in rats (Rylander et al. 2009).

However, these results differ from data previously published showing the neuroprotective role of dihydropyridines in PD. Epidemiological data demonstrated a reduction of PD incidence in patients chronically treated for hypertension with this class of calcium channel blockers (McCann et al. 1998; Rodnitzky 1999; Paganini-Hill 2001). Furthermore, a neuroprotective effect was also observed in animal models of PD treated with dihydropyridines (Kupsch et al. 1995; Kupsch et al. 1996). The key event associated with this treatment is the "rejuvenation" of the pacemaker activity in adult dopaminergic neurons of SNpc. This mechanism is based on the VDCC blockade, leading to a switch of the pacemaker mechanism in these cells, which become VDSC-dependent and less susceptible to the calcium-induced oxidative stress (Surmeier 2007; Chan et al. 2007, 2009).

In mice with parkinsonism, produced by MPTP injection or a combination of MPTP and probenecid, isradipine was able to reduce the MPTP-induced motor deficits (Chan et al. 2007; Meredith et al. 2008). Isradipine also prevents the diskinesia induced by L-DOPA in rats with parkinsonism generated by 6-OHDA injection (Schuster et al. 2009). Regarding these studies and locomotor activity, it should be taken into account that none of them included a group treated only with isradipine (Chan et al. 2007; Meredith et al. 2008; Schuster et al. 2009). Thus, the role of isradipine alone in locomotor activity in rodents cannot be evaluated in these studies.

Like isradipine, nifedipine was unable to prevent the deficit in the exploratory locomotion of males after paraquat administration (Fig. 3a). Additionally, a decrease in basal locomotion could be observed in flies treated only with nifedipine (Fig. 3b). In mice, this drug did not change locomotor activity (Sansone et al. 1995). Nifedipine was previously tested in *Drosophila melanogaster* larvae and was shown to be unable to block the calcium inflow mediated by VDCC (Worrell and Levine 2008), but was able to reduce the contractility mediated by *DPKQDFMRFamide*, so generating inhibition in the larval muscular tonus (Clark et al. 2008).

Nimodipine was also unable to revert the paraquat-induced deficits in exploratory locomotion of males and used alone this drug does not present any effect on either exploratory or basal locomotion (Fig. 3a, b). Studies based on alcohol and nicotine withdrawal protocols show that nimodipine is able to reduce the resultant locomotor hyperactivity (Hart et al. 1996; Watson and Little 2002). However, it is difficult to compare the results from previous studies with those of the present study because studies showing the effects of nimodipine in non-treated animals are very scarce.

Analyzing the effects of resveratrol and ethanol in the exploratory locomotion of males, we notice that none of the drugs used were able to prevent the locomotor impairment produced by paraquat exposure (Fig. 4a). Furthermore, resveratrol and ethanol were found to reduce exploratory locomotion in this group. Similar findings were reported in a previous study that shows that doses of more than 200 μM of resveratrol in *Canton-S Drosophila melanogaster* inhibit spontaneous locomotor activity when they are submitted to caloric restriction (Parashar and Rogina 2009).

Likewise, another study showed that resveratrol is able to prevent diet-induced obesity generated by a high-fat diet in mice and consequently reduce body weight. This decrease is probably generated by enhancement of the basal metabolism, because resveratrol in this case was unable to increase locomotion, but instead promoted a significant decrease in ambulatory locomotor activity as well as a tendency to decrease the number of rears in these mice (Lagouge et al. 2006).

On the other hand, it was demonstrated that resveratrol can provide a neuroprotective effect in 6-OHDA-induced PD in rats. In these animals, resveratrol also led to a significant improvement in motor coordination (Khan et al. 2010). Other evidence also suggests that resveratrol improves neuromuscular function, generating increases in muscle force and improving motor performance in the rotarod test (Baur et al. 2006; Lagouge et al. 2006).

Our findings are also in contrast with the results found in flies that are used as a genetic model of PD (a transgenic synuclein *Drosophila melanogaster*). In these flies, treatment with Regrapex-R—a botanical formulation comprised of extract of whole grape (*Vitis vinifera*) and *Polygonum cuspidatum*—shows increases in antioxidant activity and improvements in the locomotor functions of these animals (Long et al. 2009). Some technical considerations should be noted when comparing our study with that of Long. The first is that, while both studies used *Drosophila melanogaster*, Long and coworkers used a transgenic type of fly; the second is, they also employed a climbing assay to estimate the locomotor activity in flies. We tested this assay exhaustively in pilot studies in our laboratory, but unfortunately, the results obtained using this protocol always presented high variability, a lower degree of reproducibility, and unreliable results, at least in our experiments. Thus, we decided to use a new protocol, the simple locomotor assay (Neckameyer and Matsuo 2008), which is currently one of the most widely used protocols for the evaluation of locomotion and dopaminergic function in flies, mainly because it is very similar to the open field protocol used in rodents and presents less variability when compared with the climbing assay. Hence, this kind of test could facilitate comparisons between parkinsonism and its treatments in flies and vertebrates.

We agree that in future studies, other doses of resveratrol should be tested to evaluate the effects of this substance in *Drosophila melanogaster* locomotor activity. Recent studies have shown that the effects of resveratrol are dose-dependent, a study using dopaminergic culture cells exposed to paraquat and higher doses of resveratrol showed that this association presents a strong neurotoxic effect, increasing endoplasmic reticulum stress, and caspase-mediated programmed cell death, while low doses of resveratrol do not present the same effects (Chinta et al. 2009). The choice of dose of resveratrol used in our study

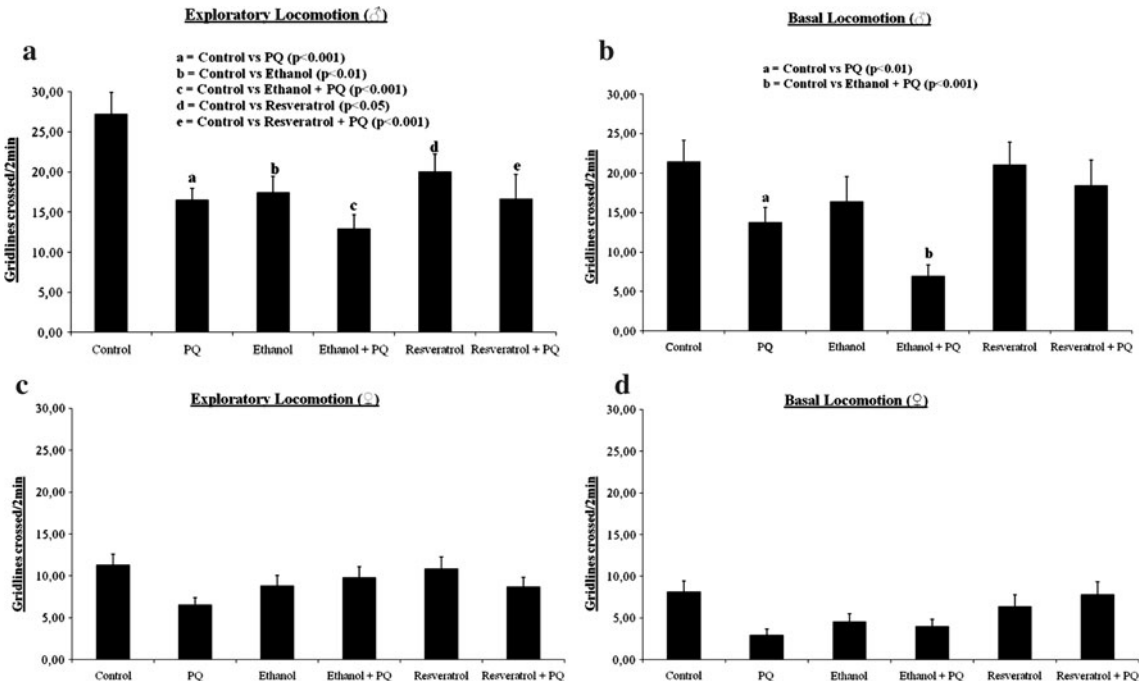


**Fig. 4** Resveratrol and ethanol treatments. **a** Exploratory locomotion index of experimental groups of males. **b** Basal locomotion index of experimental groups of males. **c** Exploratory locomotion index of experimental groups of females. **d** Basal locomotion index of experimental groups of females. *Note*: Control and paraquat groups are the same as those presented in Fig. 3 (mean ± SE)

was based on a previous study that showed that it to be the highest safe dose that does not alter the lifespan in *Drosophila melanogaster* (Bass et al. 2007).

Besides the locomotor analysis, our study attempted to demonstrate the dopaminergic neuronal alterations induced by paraquat in our experimental groups and the consequences of the drug treatments—dihydropyridines and resveratrol—in these cells, using TH immunohistochemistry. However, flies are a very different animal model to evaluate this kind of information, because it is very difficult to perform histological sections and immunohistochemistry. These difficulties are based on two facts: the reduced body size and the different densities found in the body and exoskeleton. We exhaustively tried different sectioning protocols, using cryostat, ultramicrotome, and microtome.

In relation to the methodology using cryostat and *tissue-tek* inclusion, the sections collected directly on gelatinized slips or coverslips were unsatisfactory due to the significant tissue disruption caused by freezing. Using the *free-floating* method, it was not possible to collect the sections because of their small size.

Using an ultramicrotome, different post-fixation and inclusion protocols were performed. None of which proved satisfactory, because only the soft tissue and not the exoskeleton was included. Finally, using the microtome, although several different fixative and fixation strategies were attempted, none proved suitable for performing TH immunohistochemistry.

In conclusion, our study is the first to demonstrate that dihydropyridines and resveratrol are unable to reverse motor deficits induced by paraquat in *Drosophila melanogaster*. Additionally, we demonstrated that two of the tested dihydropyridines (isradipine and nifedipine), resveratrol, and ethanol (vehicle of resveratrol) in the doses tested are responsible for locomotor impairments in flies, while nimodipine alone did not alter locomotor behavior in flies.

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