

Extracellular dopamine and alterations on dopamine transporter are related to reserpine toxicity in *Caenorhabditis elegans*

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Abstract Reserpine is used as an animal model of parkinsonism. We hypothesized that the involuntary movements induced by reserpine in rodents are induced by dopaminergic toxicity caused by extracellular dopamine accumulation. The present study tested the effects of reserpine on the dopaminergic system in *Caenorhabditis elegans*. Reserpine was toxic to worms (decreased the survival, food intake, development and changed egg laying and defecation cycles). In addition, reserpine increased the worms' locomotor rate on food and decreased dopamine levels. Morphological evaluations of dopaminergic CEP neurons confirmed neurodegeneration characterized by decreased fluorescence intensity and the number of worms with intact CEP neurons, and increased number of shrunken somas per worm. These effects were unrelated to reserpine's effect on decreased expression of the dopamine transporter, *dat-1*. Interestingly, the locomotor rate on food and the neurodegenerative parameters fully recovered to basal conditions upon reserpine withdrawal. Furthermore, reserpine decreased survival in vesicular monoamine transporter and *dat-1* loss-of-function mutant worms. In addition, worms

pre-exposed to dopamine followed by exposure to reserpine had decreased survival. Reserpine activated *gst-4*, which controls a phase II detoxification enzymes downstream of nuclear factor (erythroid-derived-2)-like 2. Our findings establish that the dopamine transporter, *dat-1*, plays an important role in reserpine toxicity, likely by increasing extracellular dopamine concentrations.

Keywords Dopamine transporter · Neurodegeneration · Neurotoxicity · Parkinson's disease · Worm

Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, affecting ~1 % of population over the age of 55 (Lees et al. 2009). PD symptoms include bradykinesia, tremor, rigidity and abnormal postural reflexes. At the time of symptomatology, 80–90 % of the dopaminergic neurons in the substantia nigra pars compacta are lost, resulting in reduced dopamine synthesis and release from striatal nerve terminals (Lang and Lozano 1998). The mechanisms underlying neurological alterations leading to progressive motor symptoms are poorly understood. The majority of PD cases are idiopathic in nature, and only 10 % have a known genetic linkage (Lees et al. 2009).

Animal models have proven highly informative in deciphering mechanisms associated with the etiology of PD. Reserpine is a monoamine depletory and it has been used in modeling PD etiology (see Beal 2001). Reserpine inhibits the vesicular monoamine transporter (VMAT), leading to loss of monoamine storage capacity. This model was instrumental in first demonstrating the therapeutic efficacy of what still remains the gold-standard treatment for

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PD, L-DOPA (Carlsson et al. 1957). However, reserpine's effects on dopaminergic neurodegeneration have yet to be fully clarified.

Caenorhabditis elegans (*C. elegans*) is a powerful genetic model and has been extensively used to explore molecular mechanisms of xenobiotic-induced PD (Nass and Blakely 2003; Nass and Settivari 2008), such as 1-methyl-4-phenylpyridinium (MPP+) (Braungart et al. 2004), 6-hydroxydopamine (6-OHDA) (Nass et al. 2002), rotenone or paraquat (Ved et al. 2005). Several characteristics make *C. elegans* an optimal model for neurodegenerative studies. It has only 320 neurons, eight of which are dopaminergic. The dopaminergic system in worms plays a role in several behaviors, including basal motor activity, egg-laying, defecation, sensation/response to food sources and habituation to touch (for review, see McDonald et al. 2007). At the molecular level, *C. elegans* harbors all genes (most of them are orthologues to vertebrates) for dopamine synthesis, packaging, release, reuptake and signaling (McDonald et al. 2007). In addition, worms are transparent, thus green fluorescent protein (GFP) expression under the dopamine transporter-1 (*dat-1*) promoter allows for in vivo visualization of the dopaminergic neurons (Nass et al. 2002; Nass and Settivari 2008). Furthermore, several mutant strains are available (Kuwahara et al. 2008), allowing the screening of mechanisms associated with drug-induced dopaminergic neurodegeneration.

The present study investigated reserpine's effects on the dopaminergic system exploring *C. elegans* behavior, morphologic and genetic properties. We hypothesized that the physiopathology of reserpine-induced parkinsonism involves dopaminergic toxicity caused by excess of extracellular dopamine.

Methods

Reagents

Reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise indicated.

Caenorhabditis elegans strains and handling

The following *C. elegans* strains were used: wild-type (WT) N2 (+); BY200, *dat-1::GFP(vtIs1)* V; RM2702, *dat-1(ok157)* III; CB1111, *cat-1(e1111)* X; CB1112, *cat-2(e1112)* II; LX636, *dop-1(vs101)*; LX702, *dop-2(vs105)*; LX703, *dop-3(vs106)*; RB1254, *dop-4(ok1321)*; VP596 (dvIs19[pAF15(*gst-4::GFP::NLS*);vsls33[*dop-3::RFP*]). The VP596 strain was provided by Dr. Keith P. Choe (University of Florida, USA), and the other strains were obtained from the *Caenorhabditis* Genetics Center (CGC, University of Minnesota, Minneapolis, MN, USA).

Caenorhabditis elegans strains were handled and maintained at 20 °C as previously described (Brenner 1974). Gravid worms were maintained in 8P plates with NA22 bacteria and synchronized with hypochlorite solution (3.75 mL sterile water, 1 mL household bleach and 250 µL 10 N NaOH for 0.5 mL gravid worms). The remaining eggs were isolated with a 30 % sucrose gradient, washed with sterile water and resuspended in M9 buffer. Cultures were shaken at 24 rpm at 20 °C. After 13 h, synchronized L1 worms were used for the reserpine exposures, as described below.

Reserpine exposure

The reserpine exposure protocol was based on methodology described by Arya et al. (2009) and Srivastava et al. (2008). Reserpine stock solution (50 mM was prepared in acetic acid) was diluted in distilled water and poured over the surface of nematode growth medium (NGM) agar plates with *Escherichia coli* OP50 strain at final reserpine concentrations of 30 or 60 µM. Plates at 0.025 % acetic acid (reserpine vehicle) were used as control plates. Plates were allowed to dry and used 1 day after plating.

Synchronized L1 worms were transferred to control or reserpine (30 and 60 µM) plates. Adult worms were transferred each day to new plates. Reserpine exposure lasted for different days until the maximum of 8 days. Some reserpine-exposed animals were transferred to normal OP50-seeded NGM plates on day 4 of reserpine exposure for analysis. Furthermore, for several assays, worms were exposed to reserpine or vehicle from their L1 larval stage throughout their adulthood (3 days for control and the 30 µM reserpine groups and 4 days for the 60 µM reserpine group). This difference in the exposure times was driven by observations in the development assay, indicating in that worms exposed to 60 µM reserpine required an extra larval day to reach the adult stage. Worms were used for the following assays.

Survival assay

20–30 synchronized L1 worms [WT N2, BY200 (*dat-1::GFP(vtIs1)*) or *cat-1* (*cat-1(e1111)*), *cat-2* (*cat-2(e1112)*), *dat-1* (*dat-1(ok157)*), *dop-1* (*dop-1(vs101)*), *dop-2* (*dop-2(vs105)*), *dop-3* (*dop-3(vs106)*) and *dop-4* (*dop-4(ok1321)*) loss-of-function mutants] were transferred to individual 35 mm plates with vehicle or reserpine. Live worms were counted daily for 4 days. Each experiment was performed in triplicate (3 plates with 20–30 worms each) and in at least four independent worm preparations. Scores were normalized to percent of worms plated on day 0.

Developmental assay

Under normal conditions (when food is present and temperature is near 20 °C), worms proceed through a series of molts, entering the second, third and fourth larval stages (L2, L3, and L4, respectively), prior to becoming adults capable of laying their own eggs (Hope 1999). Twenty to thirty synchronized L1 WT N2 worms were exposed to reserpine or vehicle for 4 days in 35 mm plate. The larval stage was determined every 24 h by their morphology. Scores were normalized to the percent of worms on each plate. Each experiment was carried out in triplicate (three plates with 20–30 worms each) in six independent worm preparations ($n = 6$).

Pharyngeal pumping

The *C. elegans* feeding rate can be measured by the number of contraction–relaxation cycles (pumps) exhibited by the pharyngeal muscle, the worm's feeding organ (Avery 1993). Synchronized L1 WT N2 worms were exposed to reserpine or vehicle for 4 days. On day 3 and 4 of exposure, reserpine's effect on feeding rates on bacterial lawn was recorded according to Wang et al. (2008). Pharyngeal bulb contractions in each animal were counted in triplicate at 10-s intervals. Results were normalized per minute. Each experiment was carried out with 10 worms in four independent worm preparations ($n = 4$).

Defecation behavior

The interval between defecation cycles of synchronized L1 WT N2 worms exposed for 3 or 4 days to reserpine or vehicle was measured according to Wang et al. (2008). For each worm, defecation cycles were counted three times. Each experiment was carried out with 10 worms in four independent worm preparations ($n = 4$).

Egg laying and egg production

Egg laying and egg production were used to evaluate the reproductive state of the worms. Synchronized L1 WT N2 worms were exposed to reserpine or vehicle for 4 days. On day 3 and 4, individual gravid adults were transferred to NGM agar plates and allowed to lay eggs for 2 h (Schafer and Kenyon 1995; Wang et al. 2008). The next day, the progeny number was counted as a measure of number of eggs laid per worm. To assess the number of eggs inside the uterus (egg production), worms were burst with bleach solution and the number of released eggs counted. Egg laying and egg production experiments were carried out with 10 worms each in four independent worm preparations ($n = 4$).

Locomotor rate

Dopamine-containing neural circuit mediates locomotor rate of *C. elegans*. Accordingly, well-fed animals with normal dopamine contents move more slowly in the presence of bacteria than in the absence of bacteria (Sawin et al. 2000). Here this basal slowing response (or food-sensing response) was assayed as described (Sawin et al. 2000) in L1 synchronized WT N2 worms exposed to reserpine for 3, 4 or 8 days, or for 4 days followed by 4 days on normal NGM agar plates. *cat-2(e1112)* loss-of-function mutants, in which the tyrosine hydroxylase activity is abolished, resulting in the absence of dopamine synthesis, were used as positive controls. Worms were transferred to NGM agar plates in the presence or absence of an OP50 bacteria ring, and the number of body bends was counted during 20 s 5 min after transferred. Each experiment was carried out with five worms in six independent worm preparations ($n = 6$).

Dopamine content analysis

L1 synchronized WT N2 worms were exposed to reserpine or vehicle throughout their adulthood (3 days for control and the 30 μ M reserpine groups and 4 days for the 60 μ M reserpine group). Next, they were washed off the plates several times with M9. 200,000 worms per group were pelleted in a tube and the supernatant removed. Tubes were immediately frozen in liquid nitrogen and stored at -80 °C. Dopamine content was measured with high-performance liquid chromatography (HPLC) according to the method described by Benedetto et al. (2010). Each experiment was performed with 200,000 worms in four independent worm preparations ($n = 4$).

GFP fluorescence quantification

Here we used the BY200 strain, which expresses GFP under the control of the dopamine re-uptake transporter 1 promoter, *dat-1::GFP(vtIs1)*. L1 synchronized BY200 worms were exposed to vehicle or reserpine throughout adulthood (3 days for control and the 30 μ M reserpine groups and 4 days for the 60 μ M reserpine group). Ten to fifteen reserpine-exposed worms were mounted on 4 % agarose slides and were anaesthetized with 0.2 % tricaine/0.02 % tetramisole. GFP fluorescence in dopaminergic cephalic (CEP) neurons of five randomly chosen worms was captured with a Plan-Apochromat 20 \times objective on a LSM510 confocal microscope (Carl Zeiss MicroImaging, Inc) as previously described (Benedetto et al. 2010; Caito et al. 2013). Images were processed with the Zeiss LSM Image Browser. GFP fluorescence quantification was performed using ImageJ 1.36 software as previously described (Gavet and Pines 2010). Each experiment was performed

with five worms in seven independent worm preparations ($n = 7$).

Neurodegeneration assay

BY200 worms were exposed to vehicle or 30 or 60 μM reserpine for 3, 4 or 8 days, respectively. Worms were transferred to normal NGM agar plates with OP50 on day 4. For the test, fifteen worms from each group were mounted on 4 % agarose pads and anesthetized with 0.2 % tricaine/0.02 % tetramisole. Neuronal defects in the CEP dopaminergic neurons were evaluated with an epifluorescence microscope (Nikon Eclipse 801, Nikon Corporation, Tokyo, Japan) equipped with a Lambda LS Xenon lamp (Sutter Instrument Company) and Nikon Plan Fluor 20 \times dry and Nikon Plan Apo 60 \times 1.3 oil objectives. Worms were considered to have intact CEP neurons when they did not show any shrunken soma or loss of dendrites or somas in any one of the four CEP neurons. Shrunken somas in CEP neurons were also counted. Each experiment was performed with 15 worms in five independent worm preparations ($n = 5$). Confocal images acquired for illustration were captured through Plan-Apochromat 20 \times objective on a LSM510 confocal microscope (Carl Zeiss MicroImaging, Inc) scanning every 200 nm for XZ sections. Images were processed with the Zeiss LSM Image Browser.

RNA isolation and real-time polymerase chain reaction (PCR)

Synchronized L1 BY200 worms were exposed to reserpine for 8 days, or for 4 days followed by 4 days (recovery phase) on normal NGM agar plates. On day 4 and 8, about 200 worms per condition were washed with M9 and lysed in Trizol (Ambion) by freezing the worms in liquid nitrogen and thawing repeatedly four times at 37 °C. The various phases were separated with chloroform (EMDchemicals), and the top phase was removed into a microcentrifuge tube and precipitated in isopropanol at -20 °C for 1 h. Two μl glycogen (Ambion) was added before isopropanol to allow RNA pellet easy viewing. After precipitation, the RNA was washed in 75 % ethanol and resuspended in RNase-free water. RNA concentration was measured by NANODROP 2000 (Thermo Scientific) and an equal RNA amount was converted to cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

Real-time PCR was performed in CFX96 real-time system (Bio-Rad). cDNAs from the above were used as templates, and pre-designed TaqMan primer sets (Applied Biosystems) were used to detect *C. elegans ama-1* (Ce02462735_g1), *dat-1* (Ce02450891_g1) and *GFP* (Mr03989638_mr) expression. All the real-time PCRs were performed in a final volume of 20 μl 1X of the TaqMan

Universal PCR Master mix (Applied Biosystems). Each experiment was carried out with 200 worms in four independent worm preparations ($n = 4$).

Acute dopamine treatment

L1 synchronized N2 WT worms were acutely pretreated for 10 min with 10 mM dopamine or M9 (vehicle), according to Benedetto et al. (2010). Immediately thereafter, worms were transferred to NGM agar plates with vehicle or reserpine. Their survival was measured daily for 4 days. Each experiment was performed in triplicate (3 plates with 20–30 worms in each) in six independent worm preparations ($n = 6$).

Relative *Pgst-4::GFP* fluorescence intensity

For this experiment, we used the transgenic VP596 line, which expresses one fluorescent construct to monitor glutathione-S-transferases (GSTs) and another as a standard for worm number normalization (*Pgst-4::GFP* and *Pdop-3::RFP*, respectively). Worms were exposed to vehicle or reserpine from L1 larval stage throughout adulthood (3 days for control and 30 μM reserpine groups and 4 days for 60 μM reserpine group). Next, they were washed several times with M9 and S-buffer and transferred to a 384 microplate (200 worms per well, five wells per condition). GFP and RFP fluorescences were quantified according to Leung et al. (2011) (filters: GFP 485/20ex 528/20em; RFP 540/25ex 590/35em), and their ratio was calculated. Since *gst-4* is inducible by reactive oxygen species, its fluorescence intensity was taken as a marker for oxidative stress in the living worms (Kampkötter et al. 2007). Each experiment was performed with five wells with 200 worms per well, and repeated independently eight times.

Statistics

All statistical analyses were performed with Prism 6 (Graphpad software, La Jolla, CA, USA); three-way ANOVA was performed with Statistica 9 (Statsoft software, Tulsa, OK, USA). Data were analyzed by one, two or three-way ANOVA, followed by Tukey's multiple comparison test where appropriate. Results were considered statistically significant when $p < 0.05$.

Results

Reserpine decreases worm survival (Fig. 1)

Survival of WT N2 and BY200 worms exposed to reserpine was evaluated daily for 4 days. The results show a

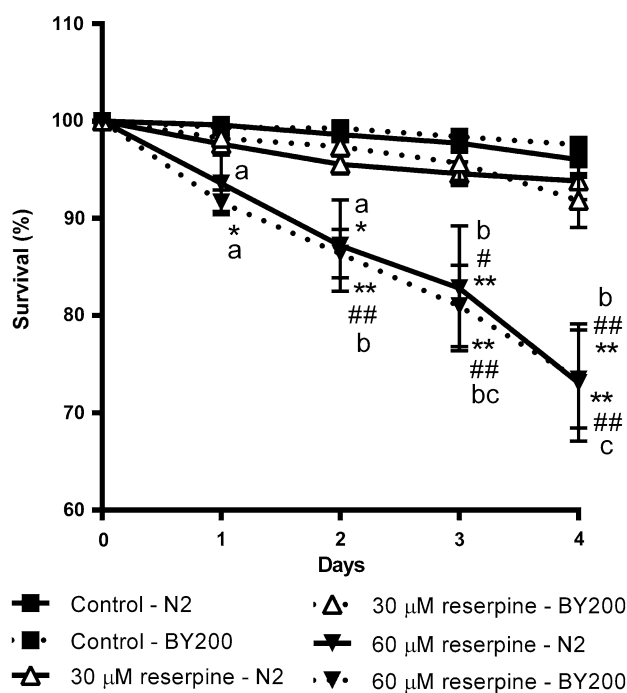


Fig. 1 Effect of reserpine on survival in WT N2 and BY200 worms. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Different letters show differences between the same strains on different days. *#Differences between groups on the same day: *Different of control group, $p < 0.05$; **Different of control group, $p < 0.001$; #Different of 30 μM reserpine group, $p < 0.05$; ##Different of 30 μM reserpine group, $p < 0.001$

significant interaction for the reserpine dose by exposure duration in WT N2 worms [$F(2, 90) = 21.77$, $p < 0.0001$; $F(4, 90) = 8.531$, $p < 0.0001$, respectively], as well as an interaction between reserpine and exposure duration [$F(8, 75) = 5.501$, $p < 0.0001$]. Sixty μM reserpine decreased the survival of BY200 worms relative to controls after 1 day exposure, and after 2 days in WT N2 worms, showing dose- and time-dependent effects. Sixty μM reserpine-exposed worms' survival on day 4 was around 73 % of the control values. No differences were found between different strains. BY200 was used as a control strain expressing GFP.

Reserpine causes developmental delay (Fig. 2)

The larval stage of WT N2 worms exposed to reserpine was evaluated daily for 4 days. We performed statistics comparing the larval stages of worms exposed to vehicle or 30 μM reserpine for 3 days or to 60 μM reserpine for 4 days. One-way ANOVA showed a significant effect of larval stage [$F(1, 30) = 95.93$, $p < 0.0001$], and post hoc analysis showed that, upon the described exposures, plates had a prevalence of adult worms in relation to other larval stages. Thus, while the control and 30 μM reserpine groups

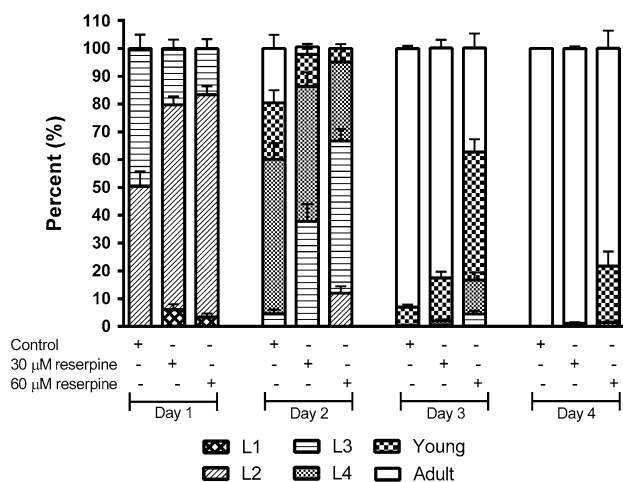


Fig. 2 Effect of reserpine on the development of WT N2 worms. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Worms got adults after exposure to vehicle or 30 μM reserpine for 3 days and 60 μM reserpine for 4 days

are in the adult stage on day 3, the 60 μM reserpine group reaches adulthood on day 4 of reserpine exposure.

Reserpine affects pharyngeal pumping, defecation and egg laying (Table 1)

Reserpine showed a significant effect on pharyngeal pumping [$F(2, 18) = 20.10$, $p < 0.0001$], reducing the rate on day 3 and 4 (30 or 60 μM). Moreover, both the reserpine dose and exposure duration showed a significant effect on defecation cycles [$F(2, 18) = 3.77$, $p = 0.043$; $F(1, 18) = 4.96$, $p = 0.038$, respectively]. Exposure duration showed a significant effect on egg production [$F(1, 18) = 4.61$, $p = 0.046$], and the dose showed a significant effect on egg laying [$F(2, 18) = 4.73$, $p = 0.022$]; however, no differences were found between experimental groups.

Reserpine decreases *C. elegans* locomotor rate on food, and this effect is not recapitulated after the termination of reserpine exposure (Fig. 3)

The number of body bends in reserpine-exposed worms was counted in the presence and absence of food on day 3, 4 or 8. No statistically significant effects of reserpine were found on locomotor rate when the test was performed in plates in the absence of bacteria (data not shown). However, in the presence of a food ring, reserpine showed a significant dose effect [$F(2, 45) = 92.59$, $p < 0.0001$], increasing the number of body bends compared with controls at each of the experimental doses and exposure days.

Next, we evaluated the effect of reserpine withdrawal on the locomotor rate on food. Two-way ANOVA showed a significant effect of the dose of reserpine [$F(2, 30) = 22.18$,

Table 1 Effects of reserpine on pharyngeal pumping, defecation time, egg production and egg laying of WT N2 worms

	Day 3			Day 4		
	Control	30 μ M reserpine	60 μ M reserpine	Control	30 μ M reserpine	60 μ M reserpine
Pharyngeal pumping/min	232.1 \pm 5.6	203.6 \pm 1.2*	199.1 \pm 6.1*	244.6 \pm 7.6	211.5 \pm 4.6*	203.0 \pm 9.2**
Defecation cycles (s)	54.9 \pm 0.6	58.1 \pm 2.3	52.0 \pm 1.8	64.8 \pm 4.7	64.6 \pm 4.3	53.7 \pm 3.9
Eggs/worm	16.2 \pm 0.1	15.5 \pm 1.3	13.2 \pm 0.7	15.3 \pm 1.7	18.1 \pm 1.3	18.3 \pm 1.8
Number of laid eggs/worm/2 h	14.7 \pm 1.2	11.9 \pm 1.1	9.5 \pm 1.3	12.4 \pm 1.5	10.8 \pm 1.1	10.0 \pm 0.8

Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. * Difference of control group in same day, $p < 0.05$; ** $p < 0.001$

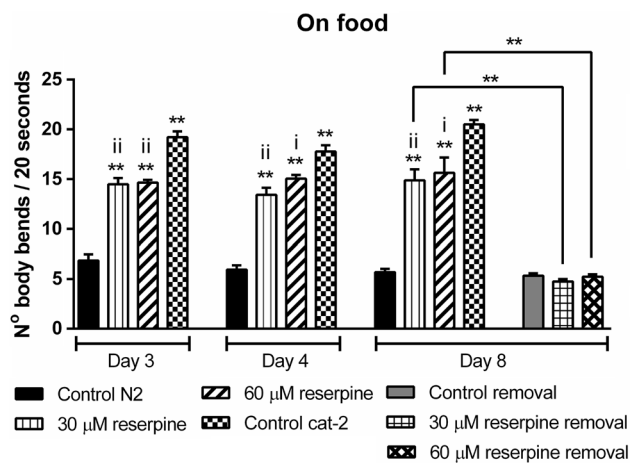


Fig. 3 Effect of reserpine on locomotor rate on food of WT N2 worms and *cat-2* loss-of-function mutants. Worms were exposed to reserpine from the L1 larval stage for 8 days. Some worms were removed from the reserpine-containing plates on day 4 and transferred to normal NGM plates. *Significant difference from control or indicated group on the same day; * $p < 0.05$; ** $p < 0.001$. ⁱDifference from *cat-2* group: ⁱ $p < 0.05$; ⁱⁱ $p < 0.001$

$p < 0.0001$], reserpine removal [$F(1, 30) = 112.8$, $p < 0.0001$] and an interaction between reserpine dose plus reserpine withdrawal [$F(2, 30) = 25.39$, $p < 0.0001$]. Reserpine (30 or 60 μ M) increased the locomotor rate of worms after 3, 4 or 8 days of reserpine exposure compared with controls. However, after 4 days of reserpine withdrawal, the reserpine-exposed worms recovered and their locomotor rate was indistinguishable from controls.

One-way ANOVA comparing WT N2 and *cat-2* loss-of-function mutants showed a significant strain effect on day 3, 4 or 8 of reserpine exposure [$F(3, 20) = 86$, $p < 0.0001$; $F(3, 20) = 85.47$, $p < 0.0001$; and $F(3, 20) = 39.59$, $p < 0.0001$, respectively]. *cat-2* loss-of-function mutants showed higher locomotor rate on food than WT N2 controls and reserpine-exposed worms for all days of analysis.

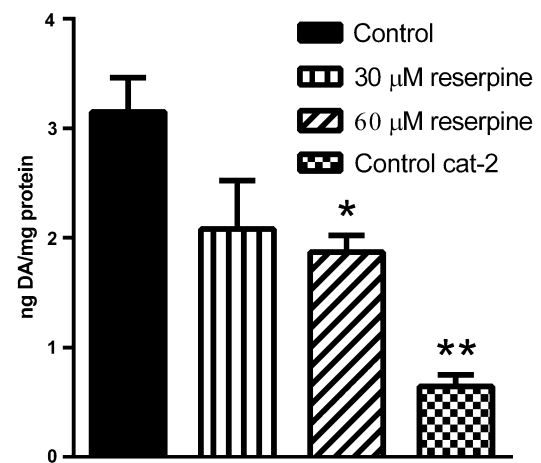


Fig. 4 Dopamine levels in WT N2 worms exposed to reserpine or vehicle throughout adulthood. *cat-2* loss-of-function mutants were exposed to control plates. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. *Significant difference of control group, $p < 0.05$; ** $p < 0.001$

Reserpine decreases dopamine levels (Fig. 4)

Dopamine content was analyzed by one-way ANOVA and showed decreased dopamine levels in WT N2 worms exposed for 4 days to 60 μ M reserpine versus controls [$F(3, 12) = 13.01$, $p < 0.0004$]. *cat-2* loss-of-function mutants also showed reduced dopamine levels compared to controls.

Reserpine decreases the fluorescence intensity of CEP dopaminergic neurons in BY200 worms (Fig. 5)

One-way ANOVA showed decreased [$F(2, 17) = 6.495$, $p < 0.05$] fluorescent intensity in CEP dopaminergic neurons in adult BY200 worms exposed to 60 μ M reserpine from the L1 larval stage versus controls.

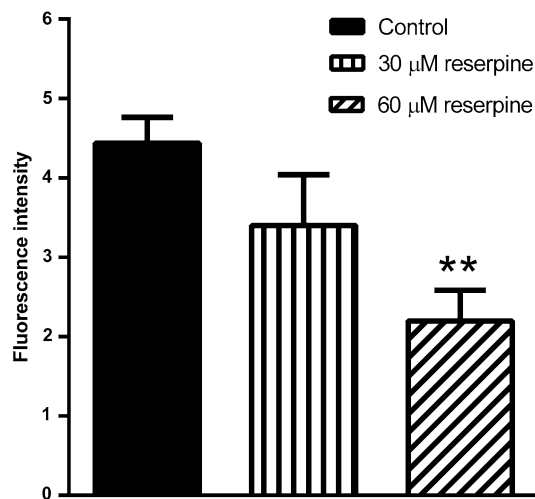


Fig. 5 Effect of reserpine on fluorescence intensity of CEP dopaminergic neurons in BY200 worms. BY200 worms were exposed to reserpine through adulthood (3 days of exposure for controls and 30 μ M reserpine groups, and 4 days of exposure for 60 μ M reserpine group). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. **Difference of control group, $p < 0.001$

Reserpine decreases the number of worms with intact CEP neurons and increases the number of shrunken soma in BY200 worm—worms fully recover from these effects upon reserpine withdrawal (Fig. 6)

CEP dopaminergic neuron morphology in BY200 worms was evaluated after 3, 4 or 8 consecutive days of reserpine exposure, as well as in worms exposed to reserpine for 4 days followed by 4 days of recovery (in absence of reserpine exposure). Two-way ANOVA showed a significant dose effect [$F(2, 31) = 63.72, p < 0.0001$] and no effect of exposure duration. Reserpine decreased the number of intact CEP neurons versus controls on days 3, 4 or 8 of reserpine exposure. In addition, two-way ANOVA showed a significant interaction between reserpine's dose and withdrawal of the drug [$F(2, 22) = 55.36, p < 0.0001$]. After 4 days of withdrawal, worms showed normal morphology in CEP neuron morphology that was indistinguishable from controls (Fig. 6b).

Reserpine also showed a significant effect on the number of shrunken somas per worm [$F(2, 32) = 64.07, p < 0.0001$], after 3, 4 or 8 day exposures. Two-way ANOVA showed a statistically significant interaction was noted for treatment by withdrawal [$F(2, 23) = 28.61, p < 0.0001$] after 8 days, with worms removed from reserpine on day 4 showing indistinguishable morphology on day 8 versus controls (Fig. 6d).

Reserpine increases *dat-1* gene and GFP expressions in BY200 worms (Fig. 7)

Two-way ANOVA showed a significant dose effect on *dat-1* gene expression [$F(2, 18) = 23.61, p < 0.0001$] in the absence of exposure duration effect. Reserpine increased *dat-1* gene expression in BY200 worms after 4 or 8 days of exposure versus controls. Furthermore, two-way ANOVA showed an interaction between reserpine dose and reserpine withdrawal from exposure [$F(2, 18) = 6.283, p < 0.00085$]. Four days after 4 days of reserpine exposure, worms showed *dat-1* gene expression levels indistinguishable from controls.

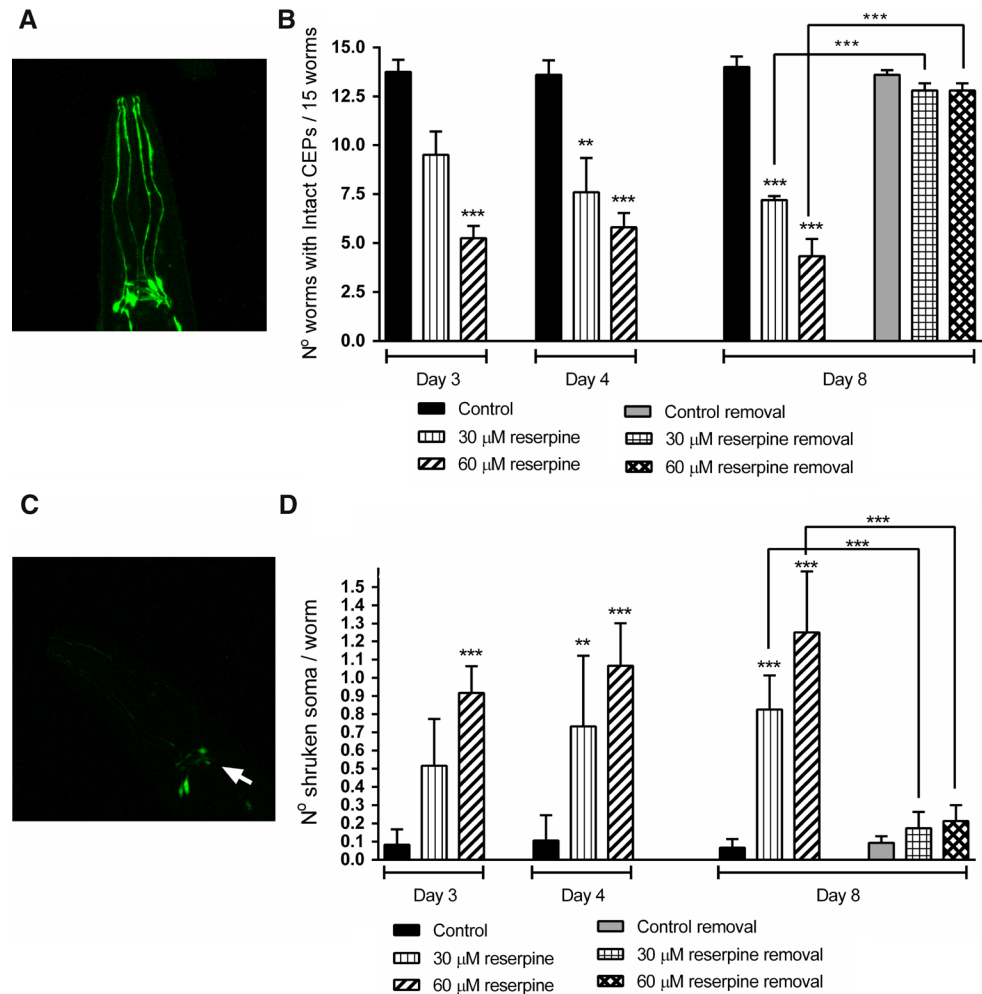
GFP expression showed analogous results for *dat-1* gene expression. Two-way ANOVA showed a significant dose effect [$F(2, 18) = 41.56, p < 0.0001$] on GFP expression in reserpine-exposed worms. BY200 worms showed increased GFP expression levels compared to controls after 4 or 8 of reserpine exposure. In addition, two-way ANOVA showed a statistically significant interaction was noted for the reserpine dose by withdrawal [$F(2, 18) = 13.18, p = 0.0003$]. Worms removed from reserpine and transferred to normal NGM plates on day 4 showed GFP expression levels that were indistinguishable from controls on day 8.

Loss-of-function mutants for vesicular monoamine transporter (*cat-1*) and for dopamine transporter (*dat-1*) are more sensitive to reserpine than WT N2 worms (Fig. 8)

Two-way ANOVA showed an interaction between time and strain [$F(32, 184) = 3.131, p < 0.0001$]. Exposure duration and strain showed a significant effect upon exposure to 30 μ M reserpine [$F(4, 173) = 1.25, p < 0.0001, F(7, 173) = 18.77, p < 0.0001$, respectively]. Exposure to 60 μ M reserpine showed an interaction between exposure duration and strain [$F(28, 155) = 2.61, p < 0.0001$]. Tukey's multiple comparison's test showed that reserpine (30 and 60 μ M) decreased the survival of *cat-1* and *dat-1* loss-of-function mutants compared to WT N2 worms (Fig. 8a). No differences were observed between WT N2 worms and *cat-2, dop-1, dop-2, dop-3* or *dop-4* loss-of-function mutants exposed to reserpine or vehicle.

Furthermore, statistical analyses were separately performed for *cat-1* and *dat-1* loss-of-function mutants. Two-way ANOVA was performed to assess survival in reserpine-exposed *cat-1* loss-of-function mutants assessing the reserpine dose and exposure duration as dependent variables. As result, two-way ANOVA showed a statistically significant interaction was noted for the reserpine dose by exposure duration [$F(8, 45) = 5.613, p < 0.0001$]. A significant interaction was also observed for the survival in *dat-1* loss-of-function mutants [$F(8, 70) = 4.696, p < 0.0001$]. Reserpine (30 and 60 μ M) decreased the survival of *cat-1*

Fig. 6 Effects of reserpine on CEP dopaminergic neuron morphology in BY200 worms. L1-synchronized worms were exposed to reserpine for 4 days followed by 4 days in normal NGM plates. **a** Shows a representative picture of four intact CEP neurons in a BY200 worm. **b** The number of worms with intact CEP neurons (without shrunken soma or lost) per 15 analyzed worms. **c** The arrow shows a representative shrunken soma. **d** The data of the number of shrunken somas per worm. **Difference of control or indicated group on the same day, ** $p < 0.001$; *** $p < 0.0001$



and *dat-1* loss-of-function mutants in relation to controls since the first day of exposure for 4 days (Fig. 8b).

Dopamine pretreatment sensitizes worms to reserpine exposure (Fig. 9)

Three-way ANOVA showed a significant interaction between the reserpine dose and pre-dopamine treatment [$F(2, 120) = 19.37, p < 0.0001$], reserpine dose and exposure duration [$F(6, 120) = 16.58, p < 0.0001$] and dopamine pretreatment and exposure duration [$F(3, 120) = 4.20, p = 0.0072$]. 60 μM reserpine-exposed worms showed lower survival rate versus controls on day 1, 2, 3 and 4; moreover, dopamine pretreatment further decreased the survival of these worms. In addition, 60 μM reserpine induced a time-dependent reduction in worms' survival.

Reserpine activates *gst-4* (Fig. 10)

One-way ANOVA showed a significant effect of the reserpine dose on relative *Pgst-4::GFP* fluorescence intensity in

VP596 worms [$F(2, 21) = 7.283, p = 0.004$]. 60 μM reserpine induced an increase in this parameter.

Discussion

The present study observed decreased survival, development and feeding, as well as alterations in reproductive and defecation characteristics in reserpine-exposed worms. Increased locomotor rate on food was accompanied by reduced dopamine levels in reserpine-exposed worms versus controls, analogous to *cat-2* worms, which lack tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis. Analysis of dopaminergic CEP neurons in reserpine-exposed BY200 worms showed reduced fluorescence, decreased number of worms with intact neurons and increased shrunken soma number per worm. Moreover, reserpine increased *dat-1* gene expression. Decreased survival of VMAT or *dat-1* loss-of-function mutants versus WT controls was observed upon reserpine exposure, as well as to worms pre-exposed to sublethal dose of

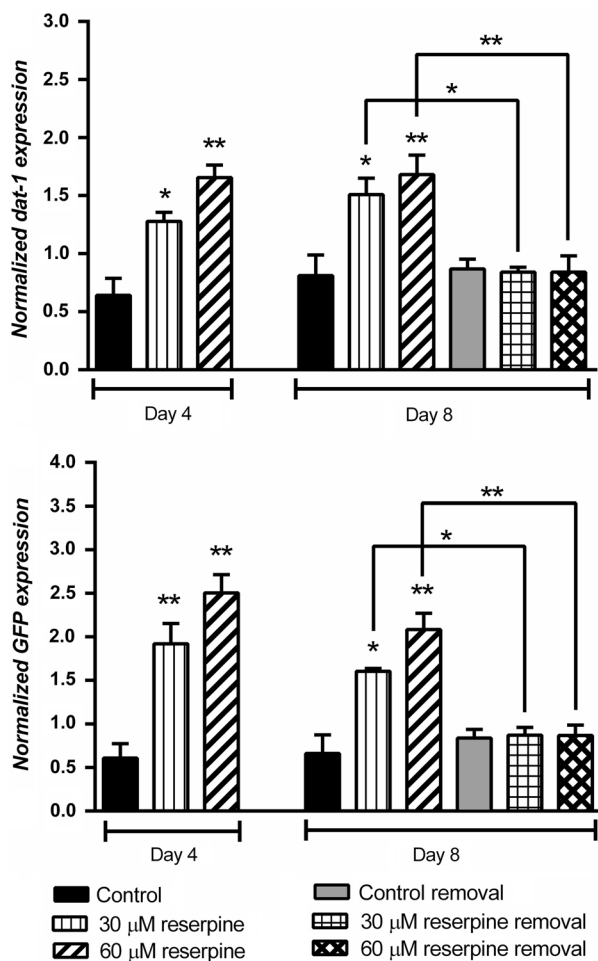


Fig. 7 Effect of reserpine on *dat-1* gene and GFP expressions in BY200 worms. Reserpine-induced effects were reversible when reserpine exposure was terminated on day 4. Data were analyzed by two-way ANOVA. *Significant difference from control or indicated group on same day $*p < 0.05$; $**p < 0.001$

dopamine. Reserpine also activated GSTs. Finally, dopaminergic neurons recovered their normal morphology upon reserpine removal, as well as locomotor rate on food and *dat-1* gene expression. These data provide novel understanding on reserpine's effects on the dopaminergic system, delineating mechanisms associated with reserpine-induced parkinsonism.

PD affects a large number of people around world, and animal models have been instrumental in deciphering PD pathophysiology. Reserpine recapitulates various aspects of PD, as it decreases dopamine levels and causes motor deficits (Duty and Jenner 2011). In addition, reserpine depletes all monoamines. It is noteworthy that in addition to dopamine (Jellinger 1991), other monoaminergic systems are affected in PD. The present study evaluated reserpine effects on the dopaminergic system in *C. elegans*, taking advantage of the nematode as a complimentary animal testing platform.

First, reserpine's general toxicity was assessed in worms. Reserpines dose- and time-dependently decreased survival in WT N2 and BY200 worms (Fig. 1), corroborating earlier observation (Arya et al. 2009; Saharia et al. 2012; Srivastava et al. 2008; Tauffenberger et al. 2013). Moreover, reserpine delayed the worms' development (Fig. 2), decreased their feeding rate, and altered defecation cycles and egg laying (Table 1). Our findings corroborate those by Srivastava et al. (2008), who reported that 30 μM reserpine exposure positively modulated development, pharyngeal pumping and broodsize in worms. Alterations in survival, development, defecation and egg laying may be a consequence of decreased feeding (Papaioannou et al. 2005), a complex behavior controlled by several neurotransmitters, such as serotonin, acetylcholine and glutamine (Papaioannou et al. 2005). However, it is not feasible to correlate the decreased survival just with decreased pharyngeal pumping since decreased pharyngeal pumping per se may increase worms' life span (Huang and Kornfeld 2004).

Decreased locomotor rate on food reflects the worm's ability to sense the presence of food and adjust its locomotor activity to allow for its consumption (Sawin et al. 2000). This behavior is mediated exclusively by a dopamine-mediated neural circuit, and the behavior is lost in *cat-2(e1112)*, a mutant strain deficient in tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine biosynthesis (Sawin et al. 2000). Reserpine-exposed worms increased their locomotor rate on food in comparison with controls (Fig. 3), consistent with Duerr et al. (1999), suggesting alterations in the dopaminergic system. Measurement of dopamine levels showed that 60 μM reserpine-exposed worms and *cat-2* loss-of-function mutants had reduced dopamine levels versus controls (Fig. 4), analogous to findings in reserpine-treated rodents (see review Duty and Jenner 2011). These reduced dopamine levels appear to be related to reserpine's inhibition of VMAT.

Caenorhabditis elegans dopamine neurons are sensitive to neurotoxins and pesticides, including 6-OHDA (Nass et al. 2002), MPP⁺ (Braungart et al. 2004) and rotenone or paraquat (Ved et al. 2005). Here, we report for the first time morphological alterations induced by reserpine in worm dopamine neurons. Reserpine decreased GFP fluorescence intensity of CEP dopaminergic neurons in BY200 worms (Fig. 5), consistent with Sulston et al. (1975). Furthermore, reserpine increased the number of shrunken soma and decreased the number of worms with intact CEP neurons (Fig. 6). These neurodegenerative effects were observed concomitant to increased *dat-1* gene expression (Fig. 7), confirming reserpine-induced neuronal damage.

Interestingly, morphological alterations induced by 4-day reserpine exposure in dopamine neurons were not observed when worms were removed from reserpine for the 4 subsequent days (Fig. 6). Thus, dopaminergic neurons

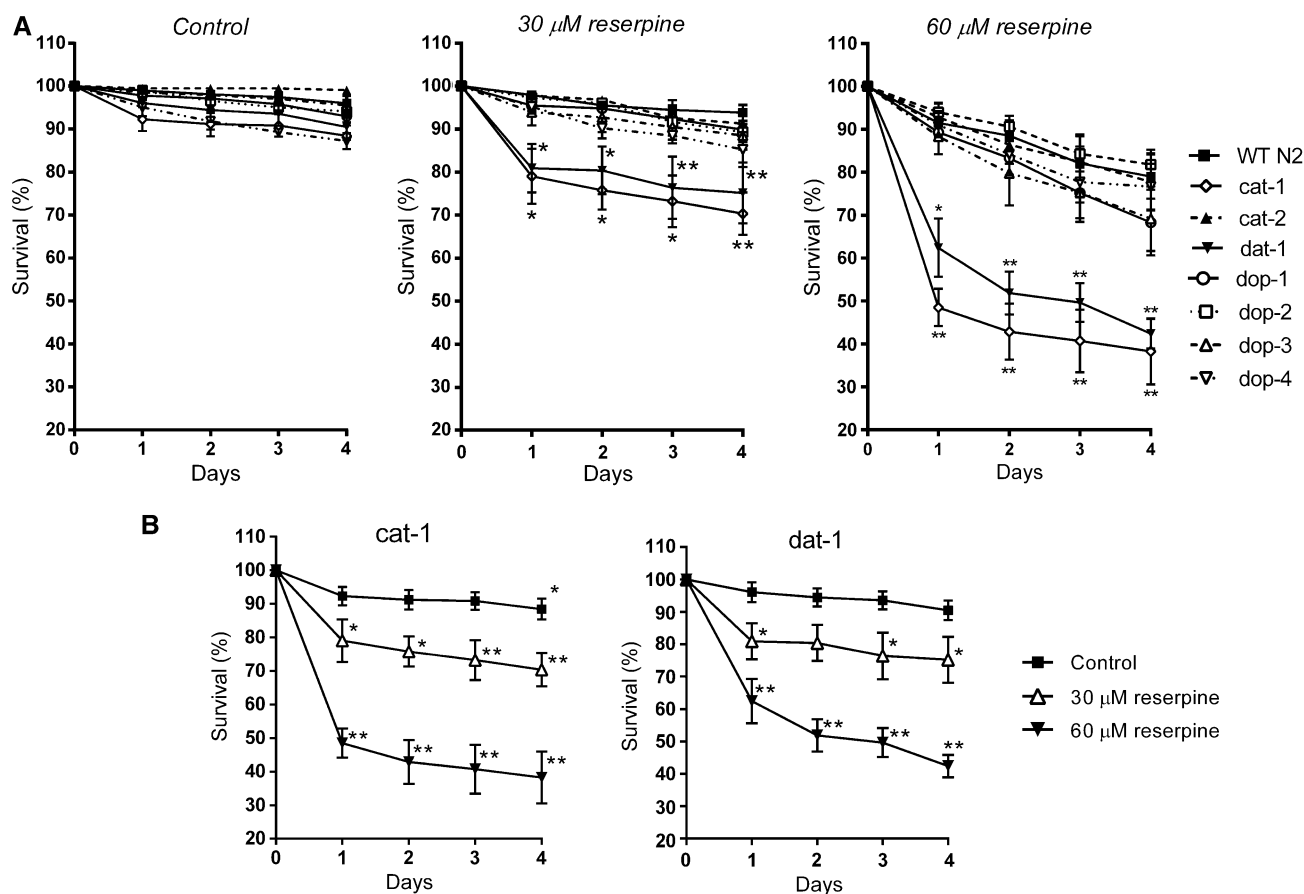


Fig. 8 Survival of dopaminergic protein loss-of-function mutants exposed to reserpine. *Cat-1* represents worms with loss-of-function of the vesicular monoamine transporter-2; *cat-2* of tyrosine hydroxylase; *dat-1* of dopamine transporter; *dop-1* and *dop-4* of dopaminergic like-D1 receptors; *dop-2* and *dop-3* of dopaminergic like-D2 receptors. **a** *Cat-1* and *dat-1* loss-of-function mutants are more sensitive to

reserpine exposure than WT N2 ones. *Express difference of WT N2 worms exposed to vehicle or reserpine for the same time (* $p < 0.05$; ** $p < 0.001$). **b** Reserpine decreases survival of *cat-1* and *dat-1* loss-of-function mutants after 1 day of exposure. *Different of day 0, $p < 0.05$; ** $p < 0.001$

recovered their normal morphology upon reserpine withdrawal. In agreement, although reserpine produces parkinsonian signs, its effects are transient and they fail to elicit dopamine neuronal death (see Duty and Jenner 2011). Notably, the neuronal morphology after reserpine withdrawal recovered, dopaminergic transmission was restored and worms showed normal locomotor rate on food (Fig. 3), as well as normal *dat-1* gene expression levels (Fig. 7).

Loss-of-function mutants of genes related to dopamine synthesis (*cat-2*), storage (*cat-1*), dopaminergic receptors (*dop-1*, *dop-2*, *dop-3* and *dop-4*) and dopamine transporter (*dat-1*) were exposed to reserpine and their survival evaluated. Reserpine decreased the survival of VMAT loss-of-function mutant (*cat-1* mutant) versus WT worms (Fig. 8), corroborating its monoamine depletory action via VMAT blockage. Moreover, *dat-1* loss-of-function mutant were more sensitive to reserpine versus WT worms (Fig. 8), showing that the dopamine transporter is also detrimental

to survival of reserpine-exposed worms. This observation demonstrates for the first time the ability of reserpine to interfere with dopamine transporter. It is noteworthy that PD brains showed a 50–70 % loss of the dopamine transporter (Seeman and Niznik 1990), consistent with experimental models of dopaminergic toxicity, including Mn (Benedetto et al. 2010) and antipsychotics (Fachinnetto et al. 2007a, b).

Dopamine transporter is responsible for dopamine clearance at the synapse, removing excessive extracellular dopamine into presynaptic dopaminergic termini (Cass et al. 1993; Kilty et al. 1991; Shimada et al. 1991). Its inhibition or loss-of-function leads to high extracellular dopamine levels and ensuing neurotoxicity (Huotari et al. 2002; McDonald et al. 2007). Reserpine decreased the survival of *dat-1* loss-of-function mutant. In addition, reserpine-exposed worms that were pretreated with exogenous dopamine showed lower survival versus worms that were not

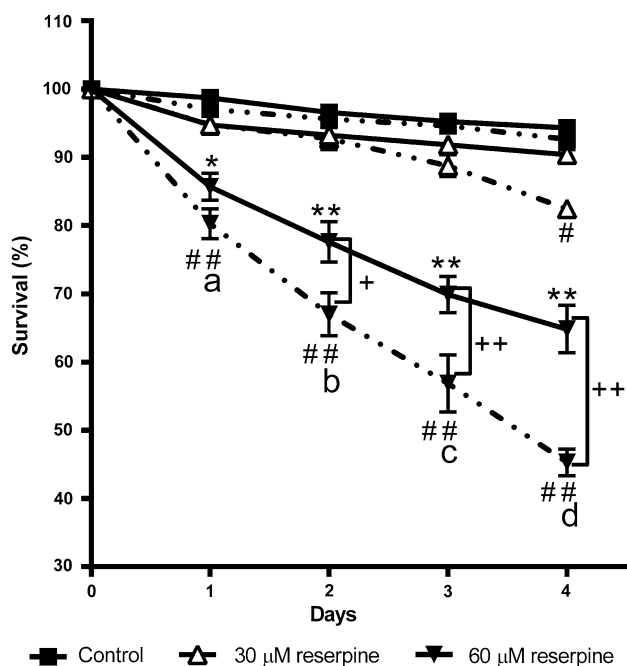


Fig. 9 Effect of dopamine pretreatment (10 mM/10 min) on survival of WT N2 worms exposed to reserpine. The *dashed lines* represent the dopamine pre-exposed worms. Data were analyzed by three-way ANOVA followed by Tukey's multiple comparison test. *Means different of control, $p < 0.05$; ** $p < 0.001$; #Different of dopamine control, $\#p < 0.05$, $\#\#p < 0.001$; +Difference between worms pretreated or no to dopamine followed by 60 μM reserpine exposure, $+p < 0.05$; $++p < 0.001$; *different letters* represent differences between days

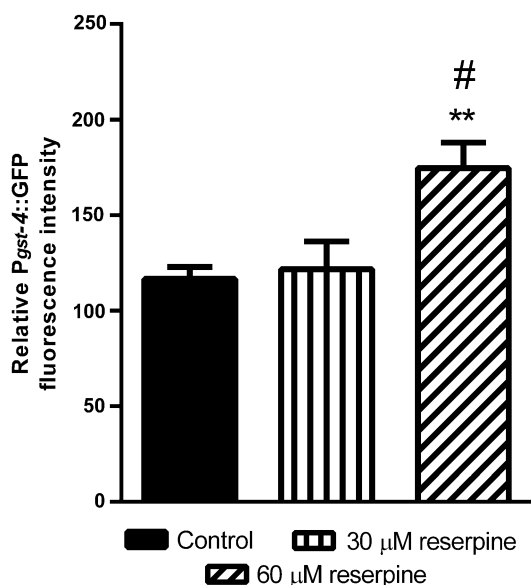


Fig. 10 Effect of reserpine on relative *Pgst-4::GFP* fluorescence in VP596 worms. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. **Difference of control group $p < 0.001$; #Difference of 30 μM reserpine group, $p < 0.05$

pretreated with dopamine (Fig. 9). This shows that extracellular dopamine is also involved in reserpine's neurotoxicity. Notably, Hossain et al. (2013) reported that increased extracellular dopamine levels may occur concomitant with loss-of-function of the VMT-2 in *cat-1* mutant worms. Accordingly, reserpine may cause extracellular dopamine accumulation and toxicity by interfering with dopamine transporter function (Eyerman and Yamamoto 2007; Park et al. 2002).

Excessive dopamine can oxidize dopamine to generate unstable quinones, which are highly reactive and cause lipid peroxidation (Aluf et al. 2011). Dopaminergic cell loss in PD patients and experimental PD models invokes excessive reactive oxygen species production (Cassarino et al. 1997; Lotharius et al. 1999; Pong et al. 2000; Wu et al. 2003; Yoo et al. 2003). Skn-1 is the *C. elegans* homologue of nuclear factor (erythroid-derived-2)-like 2 (Nrf2), and it is constitutively expressed in dopaminergic neurons where it regulates a number of phase II detoxification enzymes including GSTs (VanDuyn et al. 2010). Reserpine dose dependently increased the relative *Pgst-4::GFP* fluorescence intensity in VP596 worms (Fig. 10). This result indicates that reserpine induced an activation of GSTs, likely reflecting a defense mechanism to reactive species generated from reserpine exposure.

In conclusion, reserpine decreased dopamine levels in worms by its well-described effect on blocking VMAT activity. In addition, we demonstrated that reserpine caused damages to dopaminergic neuron morphology, which are probably related to alterations on dopamine transporter that lead to increased extracellular dopamine levels and consequent neurotoxicity. These results provide new insights to PD pathophysiology and should facilitate the search for new therapeutic modalities; however, additional studies must be carried out to systematically evaluate the specific effects of reserpine on dopamine transporter function.

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Conflict of interest None.

References

- Aluf Y, Vaya J, Khatib S, Finberg JPM (2011) Alterations in striatal oxidative stress level produced by pharmacological manipulation of dopamine as shown by a novel synthetic marker molecule. *Neuropharmacology* 61:87–94

- Arya U, Dwivedi H, Subramaniam JR (2009) Reserpine ameliorates A β toxicity in the Alzheimer's disease model in *Caenorhabditis elegans*. *Exp Gerontol* 44:462–466
- Avery L (1993) The genetics of feeding in *Caenorhabditis elegans*. *Genetics* 133:897–917
- Beal MF (2001) Experimental models of Parkinson's disease. *Nat Rev Neurosci* 2:325–334
- Benedetto A, Au C, Avila DS, Milatovic D, Aschner M (2010) Extracellular dopamine potentiates Mn-induced oxidative stress, lifespan reduction, and dopaminergic neurodegeneration in BLI-3-dependent manner in *Caenorhabditis elegans*. *PLoS Genet* 6:1–18
- Braungart E, Gerlach M, Riederer P, Baumeister R, Hoener MC (2004) *Caenorhabditis elegans* MPP+ model of Parkinson's disease for high-throughput drug screenings. *Neurodegener Dis* 1:175–183
- Brenner S (1974) The genetics of *Caenorhabditis elegans*. *Genetics* 77:71–94
- Caito SW, Valentine WM, Aschner M (2013) Dopaminergic neurotoxicity of *S*-ethyl *N*, *N*-dipropylthiocarbamate (EPTC), molinate, and *S*-methyl-*N*, *N*-diethylthiocarbamate (MeDETC) in *Caenorhabditis elegans*. *J Neurochem* 127:837–851
- Carlsson A, Lindqvist M, Magnusson T (1957) 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature* 180:1200
- Cass WA, Zahniser NR, Flach KA, Gerhardt GA (1993) Clearance of exogenous dopamine in rat dorsal striatum and nucleus accumbens: role of metabolism and effects of locally applied uptake inhibitors. *J Neurochem* 61:2269–2278
- Cassarino DS, Fall CP, Swerdlow RH, Smith TS, Halvorsen EM, Miller SW, Parks JP, Parker WD Jr, Bennett JP Jr (1997) Elevated reactive oxygen species and antioxidant enzyme activities in animal and cellular models of Parkinson's disease. *Biochim Biophys Acta* 1362:77–86
- Duerr JS, Frisby DL, Gaskin J, Duke A, Asermely K, Huddleston D, Eiden LE, Rand JB (1999) The cat-1 gene of *Caenorhabditis elegans* encodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors. *J Neurosci* 19:72–84
- Duty S, Jenner P (2011) Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. *Br J Pharmacol* 164:1357–1391
- Eyerman DJ, Yamamoto BK (2007) A rapid oxidation and persistent decrease in the vesicular monoamine transporter 2 after methamphetamine. *J Neurochem* 103:1219–1227
- Fachinetto R, Villarinho JG, Wagner C, Pereira RP, Avila DS, Burger ME, Calixto JB, Rocha JB, Ferreira J (2007a) *Valeriana officinalis* does not alter the orofacial dyskinesia induced by haloperidol in rats: role of dopamine transporter. *Prog Neuropsychopharmacol Biol Psychiatry* 31:1478–1486
- Fachinetto R, Villarinho JG, Wagner C, Pereira RP, Puntel RL, Paixão MW, Braga AL, Calixto JB, Rocha JB, Ferreira J (2007b) Diphenyl diselenide decreases the prevalence of vacuous chewing movements induced by fluphenazine in rats. *Psychopharmacology* 194:423–432
- Gavet O, Pines J (2010) Progressive activation of CyclinB1-Cdk1 coordinates entry to mitosis. *Dev Cell* 18:533–543
- Hope IA (1999) Background on *Caenorhabditis elegans*. In: Hope IA (ed) *C. elegans: a practical approach*. Oxford UP, New York, pp 1–15
- Hossain M, Wickramasekara RN, Carvelli L (2013) B-Phenylethylamine requires the dopamine transporter to increase extracellular dopamine in *Caenorhabditis elegans* dopaminergic neurons. *Neurochem Int* 73:27–31
- Huang CXC, Kornfeld K (2004) Measurements of age-related changes of physiological processes that predict lifespan of *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 101(21):8084–8089
- Huotari M, Santha M, Lucas LR, Karayiorgou M, Gogos JA, Männistö PT (2002) Effect of dopamine uptake inhibition on brain catecholamine levels and locomotion in catechol-*O*-methyltransferase-disrupted mice. *J Pharmacol Exp Ther* 303:1309–1316
- Jellinger KA (1991) Pathology of Parkinson's disease. Changes other than the nigrostriatal pathway. *Mol Chem Neuropathol* 14:153–197
- Kampkötter A, Nkwonkam CG, Zurawski RF, Timpel C, Chovolou Y, Wätjen W, Kahl R (2007) Effects of the flavonoids kaempferol and fisetin on thermotolerance, oxidative stress and foxO transcription factor DAF-16 in the model organism *Caenorhabditis elegans*. *Arch Toxicol* 81:849–858
- Kilty JE, Lorang D, Amara SG (1991) Cloning and expression of a cocaine sensitive rat dopamine transporter. *Science* 254:578–579
- Kuwahara T, Koyama A, Koyama S, Yoshina S, Ren CH, Kato T, Mitani S, Iwatsubo T (2008) A systematic RNAi screen reveals involvement of endocytic pathway in neuronal dysfunction in alpha-synuclein transgenic *C. elegans*. *Hum Mol Genet* 17:2997–3009
- Lang AE, Lozano AM (1998) Parkinson's disease. First of two parts. *N Engl J Med* 339:1044–1053
- Lees AJ, Hardy J, Revesz T (2009) Parkinson's disease. *Lancet* 373:2055–2066
- Leung CK, Deonarain A, Strange K, Choe KP (2011) High-throughput screening and biosensing with fluorescent *C. elegans* strains. *J Vis Exp* 51:1–5
- Lotharius J, Dugan LL, O'Malley KL (1999) Distinct mechanisms underlie neurotoxin-mediated cell death in cultured dopaminergic neurons. *J Neurosci* 19:1284–1293
- McDonald PW, Hardie SL, Jessen TN, Carvelli L, Matthies DS, Blakely RD (2007) Vigorous motor activity in *Caenorhabditis elegans* requires efficient clearance of dopamine mediated by synaptic localization of the dopamine transporter DAT-1. *J Neurosci* 27:14216–14227
- Nass R, Blakely RD (2003) The *Caenorhabditis elegans* dopaminergic system: opportunities for insights into dopamine transport and neurodegeneration. *Annu Rev Pharmacol Toxicol* 43:521–544
- Nass R, Settivari RS (2008) *Caenorhabditis elegans* models of Parkinson's disease: a robust genetic system to identify and characterize endogenous and environmental components involved in dopamine neuron degeneration. In: Nass R, Przedborski S (eds) *Parkinson's disease: molecular and therapeutic insights from model systems*. Elsevier Academic Press, New York, pp 347–360
- Nass R, Hall DH, Miller DM, Blakely RD (2002) Neurotoxin-induced degeneration of dopamine neurons in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 99:3264–3269
- Papaoiannou S, Marsden D, Franks CJ, Walker RJ, Holden-Dye L (2005) Role of a FMRF amide-like family of neuropeptides in the pharyngeal nervous system of *Caenorhabditis elegans*. *J Neurobiol* 65:304–319
- Park SU, Ferrer JV, Javitch JA, Kuhn DM (2002) Peroxynitrite inactivates the human dopamine transporter by modification of cysteine 342: potential mechanism of neurotoxicity in dopamine neurons. *J Neurosci* 22:4399–4405
- Pong K, Doctrow SR, Baudry M (2000) Prevention of 1-methyl-4-phenylpyridinium- and 6-hydroxydopamine-induced nitration of tyrosine hydroxylase and neurotoxicity by EUK-134, a superoxide dismutase and catalase mimetic, in cultured dopaminergic neurons. *Brain Res* 881:182–189
- Saharia K, Arya U, Kumar R, Sahu R, Das CK, Gupta K, Dwivedi H, Subramaniam JR (2012) Reserpine modulates neurotransmitter release to extend lifespan and alleviate age-dependent A β proteotoxicity in *Caenorhabditis elegans*. *Exp Gerontol* 47:188–197
- Sawin ER, Ranganathan R, Horvitz HR (2000) *C. elegans* locomotory rate is modulated by the environment through a dopaminergic

- pathway and by experience through a serotonergic pathway. *Neuron* 26:619–631
- Schafer WR, Kenyon CJ (1995) A calcium-channel homologue required for adaptation to dopamine and serotonin in *Caenorhabditis elegans*. *Nature* 375:73–78
- Seeman P, Niznik HB (1990) Dopamine receptors and transporters in Parkinson's disease and schizophrenia. *FASEB J* 4:2737–2744
- Shimada S, Kitayama S, Lin CL, Patel A, Nanthakumar E, Gregor P, Kuhar M, Uhl G (1991) Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. *Science* 254:576–578
- Srivastava D, Arya U, SoundaraRajan T, Dwivedi H, Kumar S, Subramaniam JR (2008) Reserpine can confer stress tolerance and lifespan extension in the nematode *C. elegans*. *Biogerontology* 9:309–316
- Sulston J, Dew M, Brenner S (1975) Dopaminergic neurons in the nematode *Caenorhabditis elegans*. *J Comp Neurol* 163:215–226
- Tauffmanberger A, Julien C, Parker JA (2013) Evaluation of longevity enhancing compounds against transactive response DNA-binding protein-43 neuronal toxicity. *Neurobiol Aging* 34:2175–2182
- VanDuyn N, Settivari R, Wong G, Nass R (2010) SKN-1/Nrf2 inhibits dopamine neuron degeneration in a *Caenorhabditis elegans* model of methylmercury toxicity. *Toxicol Sci* 118:613–624
- Ved R, Saha S, Westlund B, Perier C, Burnam L, Sluder A, Hoener M, Rodrigues CM, Alfonso A, Steer C, Liu L, Przedborski S, Wolozin B (2005) Similar patterns of mitochondrial vulnerability and rescue induced by genetic modification of alpha-synuclein, parkin, and DJ-1 in *Caenorhabditis elegans*. *J Biol Chem* 280:42655–42668
- Wang MC, Rourke EJ, Ruvkun G (2008) Fat metabolism links germline stem cells and longevity in *C. elegans*. *Science* 322:957–960
- Wu DC, Teismann P, Tieu K, Vila M, Jackson-Lewis V, Ischiropoulos H, Przedborski S (2003) NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *Proc Natl Acad Sci USA* 100:6145–6150
- Yoo MS, Chun HS, Son JJ, DeGiorgio LA, Kim DJ, Peng C, Son JH (2003) Oxidative stress regulated genes in nigral dopaminergic neuronal cells: correlation with the known pathology in Parkinson's disease. *Brain Res Mol Brain Res* 110:76–84